International Union of
Microbiological Societies
Congresses

July 27 – August 1, 2014

Union internationale des
sociétés de microbiologie
Congrès

27 juillet – 1er août 2014

IUMS 2014

MONTRÉAL, CANADA

XIVth International Congress of Bacteriology and Applied Microbiology | XIVth International Congress of Mycology and Eukaryotic Microbiology | XVIth International Congress of Virology

XIVe Congrès international de bactériologie and Applied Microbiology | XIVth Congrès international de mycologie et microbiologie eukaryote | XVIth Congrès international de virologie

ABSTRACTS
RÉSUMÉS

Organized with the Canadian Society of Microbiologists and the National Research Council of Canada
Organisé avec la Société canadienne des microbiologistes et le Conseil national de recherches du Canada

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PREFACE

This volume of the International Union of Microbiological Societies 2014 edition contains all IUMS 2014 Congresses submitted abstracts for:

Keynote Plenary Sessions
Bridging Sessions
Workshop Sessions and
Poster presentations.

The content of this book is available in electronic format only via the internet.

FOREWORD

The organizers of IUMS 2014 Congresses are proud to present this collection of abstracts during Congresses of Bacteriology and Applied Microbiology, Mycology and Eukaryotic Microbiology and Virology, held this year in the vibrant city of Montréal (Québec, Canada) from July 27 to August 1, 2014.

This electronic collection of abstracts contains all submissions accepted for oral or poster presentation at iUMS 2014 as of July 18, 2014. Abstracts received after this date, as well as those included here, can be accessed using ***myIUMS2014*** the congress mobile app. Abstracts are presented as they were submitted. The organizers take no responsibility for the content or grammatical mistakes and errors in the texts.

Pierre Belhumeur
MEM Scientific Program Vice-Chair

Charles Dozois
BAM Scientific Program Vice-Chair

Alain Lamarre
VIR Scientific Program Vice-Chair

Montreal, Canada

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Opening Ceremony and Scientific Lecture

PL01.01 - Living in a Microbial Universe

Julian Davies[1]
[1]*Life Sciences Institute, UBC, Vancouver, Canada*

In the years since the last IUMS in Sapporo, the science of Microbiology has undergone an exciting revolution as the result of radical changes in methodology. Exponential advances in genomic technologies have changed the biological concepts governing the living world. These powerful instrumental and computational methods have amply confirmed that all life is a part of the Microbial World. Deciphering the chemical interactions between microbes and other beings will contribute to the development of previously-unimagined applications: medical, agricultural, environmental, and industrial. The problem of antibiotic resistance is here to stay but novel genomic approaches to the discovery of new and specific antimicrobials are already showing promise. Even in the most complex biological systems, it is often possible to predict the roles of microbes without being able to culture them. However, it is essential to study living microbes in the laboratory and knowledge of new biochemical pathways will now make this more feasible. This is a great time to be a microbiologist and organizations such as IUMS must grasp the opportunity to enhance the teaching, development and applications of bacteriology, mycology, virology and systems biology.

Plenary Sessions

BAM-PL02.01 - Genome biology of the species *Listeria*: predicting virulence

Trinad Chakraborty[1]
[1] *Justus-Liebig University, Germany*

Listeria monocytogenes is a food-borne pathogen with a high mortality rate that has served as an invaluable model for intracellular parasitism. Comparative genome sequencing of representative genomes comprising all species for the genus Listeria as well as strains representing clonal lineages of the pathogenic species *L. monocytogenes* continues to provide valuable insight on the evolution of pathogenicity within this species. There is now clear evidence indicating that the various non-pathogenic species of *Listeria* have been derived by gene loss and/or mutational decay of virulence- and niche-adaptive factors from a progenitor strain that harboured many of the currently known virulence factors. There is currently great interest in predicting virulence from genome sequences for example, during outbreaks. Genome-based phylogeny has revealed four lineages of the pathogenic species *L. monocytogenes*, but an understanding of the basis of graded levels of virulence exhibited by these isolates is lacking. Comparative transcriptome analysis of intracellular growth has also uncovered additional levels of adaptive evolution in growth among the different lineages of *L. monocytogenes*. Analysis of the pan-genome of *L. monocytogenes* strains revealed an extensive, as yet unexplained gene-repertoire in these genomes, and provides evidence for the evolution of these strains by gathering genes from organisms in different environmental and host niches.

Plenary Sessions

BAM-PL02.02 - A quantitative systems biology study on a model bacterium

Luis Serrano Pubul[1]
[1]*Center For Genomic Regulation, Barcelona, Spain*

The goal of Systems Biology is to provide a quantitative and predictive description of a living system to the extent that it can be fully simulated in a computer. We have undertaken such Endeavour using as a model the small bacterium, M. pneumoniae. use Mycoplasma pneumoniae, a human pathogenic bacterium causing atypical pneumonia as model system for our study. Containing a reduced genome with only 690 ORFs, this bacterium is an ideal organism for exhaustive quantitative and systems-wide studies, avoiding technical limitations due to exceeding sample complexity, constrained by limitations in dynamic range and resolution of current generation mass spectrometers. Available data on the transcriptome, on protein complexes, as well as on metabolic pathways facilitate the integration of the data generated for this study into an organism-wide context. Additionally, M. pneumoniae represents a relevant organism to study stochastic noise in living systems. The cells are significantly smaller than other bacteria, such as Eschericha coli (0.05 mm3 and 1 mm3, respectively) resulting in principle in an increased susceptibility to abundance fluctuations of cellular molecules. Our analysis shows that even apparently simpler organisms have a large hidden layer of complexity and that for every question we have answered we have got two new ones. We are still far away to be get a full understanding of a cell.

Plenary Sessions

BAM-PL03.01 - Cell division with and without the FtsZ machinery

Romain Mercier[1], Yoshikazu Kawai[1], Jeff Errington[1]
[1]*Centre For Bacterial Cell Biology, Newcastle University, Newcastle, UK*

The peptidoglycan wall is a hallmark of bacterial cells and was probably present in their last common ancestor, at around the dawn of cellular life on earth. The last common ancestor also probably possessed a sophisticated division machine based around the widely conserved FtsZ protein. This tubulin-like protein assembles into a ring structure at the site of impending division and then recruits 10 or so well conserved proteins that help bring about cytokinesis in most bacteria. How cell wall synthesis is regulated during growth and division remains an important, largely unsolved problem in bacteria. We have recently approached this problem by studying cell wall deficient, or "L-form" bacteria. Using *B. subtilis* as a model we discovered that switching from the walled to the L-form state is surprisingly simple, requiring only one or two genetic changes. However, the L-forms are remarkably altered in terms of their growth and proliferation. The FtsZ-based division machine becomes completely dispensable, and the cells divide, instead, by a membrane blebbing or tubulation and fission mechanism. We have recently examined the formation of L-forms in a range of other bacteria, including the Gram negative *E. coli*, and find that their properties are largely similar to those of *B. subtilis*, including ability to dispense with the proteinaceous division machine. These studies of L-forms have wide implications, including mechanisms of antibiotic resistance, the origins of life and the generation of artificial cells. Key references Leaver M, Domínguez-Cuevas P, Coxhead JM, Daniel RA, Errington J. (2009) Life without a wall or division machine in Bacillus subtilis. Nature 457, 849-853. Mercier R, Kawai Y, Errington J. (2013) Excess membrane synthesis drives a primitive mode of cell proliferation. Cell 152, 997-1007. Errington J. (2013) L-form bacteria, cell walls and the origins of life. Open Biology 3, 120143. Kawai Y, Mercier R, Errington J. (2014) Bacterial cell morphogenesis does not require a preexisting template structure. Curr Biol. 24, 863-867.

Plenary Sessions

BAM-PL03.02 - Intercellular molecular transfer in the filaments of heterocyst-forming cyanobacteria

Enrique Flores[1]
[1]CSIC, Seville, Spain

Filamentous, heterocyst-forming cyanobacteria are multicellular organisms in which, under nitrogen-limiting conditions, growth requires the activity of two interdependent cell types: the vegetative cells that fix carbon dioxide performing oxygenic photosynthesis and the dinitrogen-fixing heterocysts. As studied in the model strain Anabaena sp. PCC 7120, heterocysts differentiate from vegetative cells in a process that involves the execution of a complex genetic program and the intercellular transfer of regulators including a PatS-related peptide, an inhibitor of heterocyst differentiation. In the diazotrophic filament, vegetative cells provide heterocysts with reduced carbon (mainly in the form of sucrose) and heterocysts provide the vegetative cells with fixed nitrogen. The heterocysts conspicuously accumulate cyanophycin, a polymer made of aspartate and arginine that is a dynamic reservoir of nitrogen. Cyanophycin is degraded in the heterocysts by cyanophycinase producing beta-aspartyl-arginine, which is hydrolyzed to aspartate and arginine by an isoaspartyl dipeptidase mainly present in the vegetative cells. This compartmentalized catabolism of cyanophycin implies that beta-aspartyl-arginine is transferred as a nitrogen vehicle from heterocysts to vegetative cells. The cyanobacteria are diderm bacteria, and the cyanobacterial filament consists of individual cells that are enclosed in a continuous outer membrane and share a continuous periplasm. The cells in the filament seem to be connected by septal junctions analogous to metazoan gap junctions. Integral membrane proteins that are located at the intercellular septa, SepJ, FraC and FraD, are likely components of the septal junctions and appear to have a dual function in binding and communicating adjacent cells in the filament. The communication function can be probed with fluorescent tracers such as calcein and 5-carboxyfluorescein, which have permitted to distinguish two different types of septal junctions, those containing SepJ and those containing FraC and FraD. Research with heterocyst-forming cyanobacteria has thus unraveled structures and mechanisms for intercellular communication that were previously unknown in bacteria.

Plenary Sessions

MEM-PL02.01 - Taxonomy and technology

Keith Seifert[1]
[1]*Agriculture & Agri-Food Canada, Ottawa, Canada*

Microbial taxonomy was born with the invention of the microscope and its development as a science is intimately associated with developments in technology. Distrust of automation and suspicions about over-reliance on machine generated data are persistent concerns in the history of microbiology. The importance of the methodical and sometimes plodding task of describing, naming and classifying species is often questioned. In part this is because it never seems to keep pace with our ability to generate increased quantities of data and new kinds of data. But it also reflects the tendency of taxonomists and ecologists to lock themselves in narrow towers, focusing on groups of a priori interest, and unwilling or unable to consider the broad spectrum of life. Now, with the advent of whole genome sequencing, environmental genomics, and concepts such as the human microbiome, the challenges facing the taxonomic process seem critical. Should microorganisms known only from DNA sequences be formally named? Should we enable automated, machine-run naming and classification of species? This presentation, given from the perspective of a taxonomist working with microfungi, will be a meditation on the nature of discovery, whether science is simply the process of generating and archiving digital data, and to what extent human intervention is required (or desirable) in the interpretation of Big Data.

Plenary Sessions

MEM-PL02.02 - Phytoplankton Diversity and the Global Climate

Alexandra Worden[1]
[1]*Monterey Bay Aquarium Research Institute, Moss Landing, USA*

Photosynthesis in the marine biosphere is responsible for half of the annual uptake of CO2 from earth's atmosphere. The organisms that mediate this uptake are tremendously diverse – their major commonality is simply that they can perform photosynthesis. This diversity presents a major challenge for efforts to model the global carbon cycle and how climate change will impact the marine biosphere and photosynthetic activities within it. Here, we will discuss some of the approaches and grand challenges behind studying newly discovered (but sometimes uncultivated) eukaryotic phytoplankton. More broadly, we will explore phytoplankton diversity in the context of ecological differentiation and the global carbon cycle.

Plenary Sessions

MEM-PL03.01 - From phenotypes to pathways: global exploration of cellular networks using yeast functional genomics

Brenda Andrews[1]
[1]*University of Toronto, Toronto, Canada*

The entire landscape of eukaryotic genetic research has been transformed by our ability to rapidly sequence genomes – while we can now map genomes efficiently, we do not yet know how to interpret genome variation to predict inherited phenotypes. Emerging evidence suggests that we must account for genetic interactions in order to relate genotype to important phenotypes in any eukaryotic system. To systematically explore genetic interactions, our group developed a unique functional genomics platform called 'synthetic genetic array' (SGA) analysis that automates yeast genetics and enables the systematic construction of double mutants. We developed two powerful pipelines which combine SGA and automated microscopy for systematic and quantitative cell biological screens or phenomics. Our first pipeline uses SGA to introduce fluorescent markers of key cellular compartments, along with sensitizing mutations, into yeast mutant collections. We then perform live cell imaging on the mutant arrays using HTP confocal microscopy to quantitatively assess the abundance and localization of our fluorescent reporters, providing cell biological readouts of specific pathways and cellular structures in response to thousands of genetic perturbations. Our second pipeline exploits the yeast GFP collection, a unique resource consisting of thousands of strains with different genes uniquely tagged with GFP. This remarkable collection has been arguably underutilized for systematic analysis of the proteome, largely due to the challenges associated with analysis of large sets of cell biological data. We addressed this challenge by adopting a high-content screening approach to measure protein abundance and localization changes in an automated fashion on a genome scale. Our general approach, in particular our network analysis and visualization methods, are readily extensible to other systems.

Plenary Sessions

MEM-PL03.02 - Septin heteropolymers and higher-order structures

Michelle Momany[1]
[1]*University of Georgia, Athens, USA*

Septins are highly conserved in animals and fungi and are increasingly recognized to play roles as important and diverse as those of actin and microtubules. Septin roles include acting as diffusion barriers and coordinating cytokinesis and nuclear division. Defects in septins have been associated with human diseases such as cancer and Alzheimer's. Like other cytoskeletal elements, septins associate into higher-order structures. Septins are classified into five orthologous groups. Monomers from different groups associate to form nonpolar heteropolymeric rods that in turn assemble into higher-order structures including rings and filaments that can be visualized by fluorescent microscopy of GFP-tagged septins. The mechanisms driving septin heteropolymer and higher-order structure assembly are only beginning to be understood. The filamentous fungus *Aspergillus nidulans* has one septin from each phylogenetic group. Four of the *A. nidulans* septins are orthologs of the core septins in *S. cerevisiae* and the fifth septin, AspE, is lacking in unicellular yeasts and appears to be ancestral. We have examined septins from defined stages of *A. nidulans* development using a variety of methods. Our results show that at least two distinct septin heteropolymer populations co-exist in *A. nidulans*, and that while AspE is not a subunit of either heteropolymer, it is required for assembly of septin higher-order structures found in multicellular development.

Plenary Sessions

VIR-PL02.01 - RNA virus replication assemblies

Cristina Risco[1]
[1]*Cell Structure Lab, Centro Nacional de Biotecnología, CNB-CSIC, Madrid, Spain*

Replication and assembly of many RNA viruses take place in specific intracellular compartments known as 'virus factories', 'viral inclusions' or 'viroplasms'. Our knowledge of the biogenesis and architecture of these unique structures has increased considerably in the last few years, due to technical advances in cellular, molecular and structural biology. Early in infection, viral polymerases and cofactors interact with cell membranes, where they build functional replication complexes (RC) and replication organelles. These structures recruit viral and cell factors to build a platform on which viral replication and morphogenesis are spatially connected. Factories are very dynamic, as their structure changes over time. A number of imaging technologies are helping us to understand how factories are built and work. 3D imaging shows the complex organization of virus factories, in particular the new interactions built between virus-induced membranous scaffolds, cytoskeleton and recruited organelles. A summary of recent findings on the structure of factories will be presented, including 3D reconstructions of serial sections, electron tomography and molecular mapping with antibodies and with the new clonable tag for electron microscopy based on the small metal-binding protein metallothionein (MT). Study of the intracellular distribution of MT-tagged viral replicase proteins revealed virus-induced cell structures not previously observed. Designing strategies to combine functional and mechanistic studies with 3D imaging will be necessary to understand the meaning of the structures built by viruses in the infected cell. Fernández de Castro, I., Volonte, I., Risco, C. Virus factories: biogenesis and structural design. Cell Microbiol, 2013. 15: 24-34. Risco, C., Fernández de Castro, I., Sanz-Sánchez, L., Narayan, K., Grandinetti, G., Subramaniam, S. 3D imaging of viral infections. Annu Rev Virol, 2014. 1 (in press). Funded by research grant BIO2012-33314 from the Spanish Ministry of Economy and Competitiveness and grant 200620F0024 from the PIF program of the Spanish National Research Council (CSIC).

Plenary Sessions

VIR-PL02.02 - Host cell factors and pathways promoting and restricting hepatitis C virus replication

Ralf Bartenschlager[1]
[1]*Department of Infectious Diseases, Molecular Virology, University of Heidelberg, Heidelberg, Germany*

Hepatitis C viruses (HCV) comprise a group of positive-strand RNA viruses belonging to the *Flaviviridae* family. As a major cause of acute and chronic liver disease worldwide, HCV has received much attention. With the advent of highly efficient and robust cell culture systems for HCV, the principles of the viral replication cycle have been unravelled. A surprisingly high number of molecules that are essential for or that promote HCV entry into hepatocytes has been identified. Moreover, important insights into the biogenesis and architecture of the membranous replication compartment induced upon viral infection have been gained and surprising parallels as well as differences to the Dengue virus that belongs to the same virus family have been discovered. Several host cell factors required for formation or activity of the HCV replication machinery, such as cyclophilin A and phosphatidyl inositol-4-kinase III-alpha, have been identified and they represent attractive targets for host factor-targeting antiviral therapy. In addition, we discovered a crucial role of non-structural protein 5A for the formation of the membranous HCV replication factory. This function appears to be targeted by highly potent NS5A-targeting drugs that are close to approval for clinical use. With respect to assembly and release of infectious HCV particles, apolipoproteins, most notably ApoE, are of central importance. This host cell factor is part of the virion and assumed to be required for the peculiar association of HCV particles with lipids. These results underscore the close link between HCV and its host cell and they will be discussed during my presentation.

Plenary Sessions

VIR-PL02.03 - Cauliflower mosaic virus CaMV hijacks the cell translational machinery and target-of-rapamycin TOR for translation of its pregenomic RNA

Lyubov Ryabova[1], Mikhail Schepetilnikov[1]
[1]Institut de Biologie Moléculaire des Plantes (IBMP), Strasbourg, France

Viruses lack a protein synthesis machinery encoded by the viral genome and utilize the host translation apparatus to synthesize viral proteins. Moreover, plant pararetroviruses have to modify the cell translation machinery to establish translation of several main open reading frames (ORFs) from the same pregenomic RNA—a process normally strictly prohibited in eukaryotes. Transactivation of repeated initiation events (reinitiation) on polycistronic pregenomic 35S RNA was studied intensively in Cauliflower mosaic virus (CaMV). A CaMV protein translational transactivator/viroplasmin (TAV) is sufficient to deregulate protein synthesis and overcome cellular barriers to reinitiation. TAV promotes retention on polyribosomes and re-use of eukaryotic translation initiation factors (eIFs), particulary eIF3 and the host factor RISP that are recruited at the stage of cap-dependent initiation to regenerate reinitiation-competent ribosomal complexes. The functional role of these factors would be to ensure recruitment of the initiator Met-tRNA and the 60S ribosomal subunit to the reinitiating 40S ribosomal subunit. The protein kinase TOR (target of rapamycin) is at the center of a conserved eukaryotic signaling pathway that perceives metabolic energy and regulates cell growth and survival. Its main target is the translation machinery, particulary eIFs, phosphorylation of which up-regulates protein synthesis. TAV constitutively up-regulates TOR and thus the translation machinery. TAV is an upstream TOR effector that interacts physically with TOR and triggers a cascade of TOR-responsive phosphorylation events in favor of viral replication, particularly phosphorylation of RISP and subunit h of eIF3. Phosphorylation of the latter proteins is required to promote repeated initiation events. Both TOR and eIF3h are required for successful infection and can be considered as resistance factors for CaMV. The mechanism of TOR activation by TAV is under investigation. According to our recent results, TAV recruits a small GTPase, ROP, into its complex with TOR, which can function in TOR activation. Thus the exceptions found in CaMV translation strategies can lead to deeper understanding of the cellular rules.

Plenary Sessions

VIR-PL02.04 - Analysis of alpha herpesvirus axon-cell spread

Lynn Enquist[1]
[1]*Department of Molecular Biology and the Princeton Neuroscience Institute Princeton University; Princeton, USA*

An essential process for the transmission of alphaherpesvirus infection from host to host is spread from axons of peripheral nervous system neurons to cells in peripheral epithelia after reactivation from the latent state in neurons. We have developed two methods to analyze axon to cell spread events using a compartmentalized neuronal culture system. The first method is called tri-color infection and uses HSV-1 and pseudorabies virus (PRV) recombinants that express one of three different fluorescent proteins. The fluorescence profiles of cells infected with the virus mixtures provides an assessment of the number of expressed viral genomes. Strikingly, epithelial cells infected by axon-cell spread express fewer than two viral genomes suggesting that a limited number of virions participate in individual axon-cell events. The second method uses live-cell fluorescence microscopy to image individual axon-cell spread events by tracking single capsids that leave axons to infect epithelial cells. We found that most axon-cell events involve a single capsid. Together, these complementary analyses reveal that axon-cell spread events are restricted to small numbers of viral particles, most often a single virion. Such a bottleneck process suggests that the diversity of the virus population that replicates in epithelial cell lesions and is available for spread to other hosts is limited.

Bridging Plenary Sessions

BR-01.01 - Ancient and modern leprosy bacilli

Stewart Cole[1]
[1]*EPFL, Lausanne, Switzerland*

Leprosy, which has afflicted human populations for millennia, results from infection with Mycobacterium leprae, an unculturable pathogen with an exceptionally long generation time. Considerable insight into the biology, evolution and drug resistance of the leprosy bacillus has been obtained from genomics. M. leprae has undergone reductive evolution and pseudogenes now occupy half of its 3.34 Mb genome. Comparative genomics of modern leprosy bacilli revealed 99.97% sequence identity, uncovered strong phylogeographic associations and generated useful epidemiological tools for outbreak investigation. Until the Middle Ages, leprosy was endemic in Europe but then disappeared. Whole genome sequencing of M. leprae present in skeletal remains suggests that the disappearance was not due to loss of virulence and allowed the mutation rate to be estimated. There is growing evidence for the emergence of a new leprosy bacillus, M. lepromatosis, that is associated with "Lucio's phenomenon", an invasive form of leprosy. The evolutionary relationship between M. leprae and M. lepromatosis will be presented.

Bridging Plenary Sessions

BR-01.02 - Global burden of human fungal diseases and their underlying diseases

David Denning[1]
[1]*University of Manchester, Manchester, UK*

Few estimates of the global burden of fungal disease have been made. In the Global Burden of Disease project 2010 update (Lancet 2012), fungal skin diseases were the fourth most common health problem affecting 985 miilion people after dental caries, tension-type headache and migraine. Other global estimates are 1 million with cryptococcal meningitis complicating AIDS, 4.8 million with allergic bronchopulmonary aspergillosis complicating asthma in adults (193 million adult of 334 million asthmatics total), 1.2 million patients with chronic pulmonary aspergillosis following pulmonary tuberculosis and about 75+ million women with vaginal candidiasis 4 or more times each year (5-8% of adult women under 50 years). Less robust estimates include 400,000 cases of candidaemia, 100,000 cases of Candida peritonitis, over 200,000 cases of invasive aspergillosis, over 400,000 cases of Pneumocystis pneumonia, and over a million cases of fungal keratitis annually. The fungal disease diagnostic market is China is conservatively estimated to be $1.9bn. The number of people suffering and dying from fungal infections has been estimated in 33 countries covering ~55% of the world's population (see www.life-worldwide.org/media-centre/news/). There is much variation between countries. Especially high incidence rates of Candida bloodstream infection in Brazil (15/100,000) and Spain (10.7/100,000), an extremely high rate of mucormycosis in India (170,000 cases annually, 13/100,000) related to the burgeoning epidemic of diabetes there, over 160,000 cases of invasive aspergillosis in China (11.9/100,000) partly related to COPD, over 38,000 cases of cryptococcal meningitis in AIDS in Uganda (110/100,000) and 75,000 and 18,000 cases of *Pneumocystis* pneumonia in Nigeria (48/100,000) and Brazil (39.6/100,000) respectively. Even more prevalent were allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS) complicating asthma. For example, an estimated 390,000 ABPA cases were estimated for Brazil (201/100,000), 491,000 cases for China (36.1/100,000) and at least 592,000 ABPA cases in India (47/100,000). Estimates of tinea capitis were very high in Africa, notably 15,580,000 cases in Nigeria (~50% of 155 million population are children) (1000/100,000) and 1,700,000 children affected in Kenya (4,300/100,000).

Bridging Plenary Sessions

BR-01.03 - Dynamics of Influenza Diversity

Elodie Ghedin[1]
[1]*New York University, Global Institute of Public Health, New York, USA*

Acute RNA viruses like influenza A virus are the principle agents of emerging disease. However, little is known about the extent, pattern and drivers of intra-host genetic diversity in these infectious agents. Understanding the dynamics of influenza A virus evolution within—and transmission between—hosts is critical in predicting newly emerging strains and modeling epidemics. Present molecular epidemiology studies rely on consensus genome sequences as representative of the virus population an infected individual carries and transmits, but they do not capture viral genetic diversity within the infected host. Deep sequence data of influenza virus populations show that multiple variants can be transmitted during an epidemic. By imposing selection pressure immune competence of the host and antivirals will greatly influence the extent and structure of intra-host viral diversity and the transmission potential of the strains. The short infections associated with influenza viruses, however, limit the window available for genetic diversity to be generated and probed within individual hosts. Studying patterns of viral genetic diversity in infected hosts of different immune status represents a unique opportunity to study viral evolutionary dynamics and to define how prior immunity, or other factors, such as virus subtype and strain, may affect transmission. I will present data from deep sequencing and single molecule sequencing experiments in the characterization of the mutational spectrum that can be found in influenza virus populations. One of our aims is to develop a model using intra-host genomic data of influenza diversity to reconstruct chains of transmission. In this manner we hope to answer important questions on transmission bottleneck, replication dynamics and fitness.

Poster Session

BAM-PM1001 - Highlights of our *Lactobacillus plantarum* toolbox; applications of inducible food-grade promoters and a versatile shuttle vector in a novel, plasmid cured strain

Silvia Heiss[1], Angelika Hoermann[1], Lukas Feuchtenhofer[1], Margot Sonnleitner[1], Stefan Heinl[1], Reingard Grabherr[1]
[1]*University of Natural Resources and Life Sciences, Vienna, Austria*

Lactic acid bacteria have been used worldwide for centuries to generate safe, storable food- and feedstuff, such as fermented dairy products, sourdough and forage. By creating silage of plant material, farmers gain storable feed due to acidification through organic acids and release of proteinogenic toxins. The strain used in this study was isolated from stable grass silage in Austria. *Lactobacillus plantarum* CD033 harbours one native plasmid which encodes, amongst a total of 8 open reading frames, a toxin/antitoxin system that leads to host-cell killing upon plasmid loss. Accordingly, plasmid curing was only achieved by introducing the antitoxin-gene on a helper plasmid and treatment with a curing agent, thereof generating a novel plasmid free strain. Moreover, plasmids were designed as shuttle vectors for *E. coli* for additional cloning and expression options. Notably, the strain is transformable with PCR-generated products and therefore we can introduce smaller constructs directly after PCR-amplification, without the need for accessory *E. coli* specific sequences. Interestingly, additional pUC19-backbone sequences influence expression levels in *L. plantarum*. Additionally, a palette of promoters was established, which is newly employed in *L. plantarum*. For instance, we successfully applied the T7 RNAP expression system and food-grade inducible promoters are also available. Expression of reporter gene mCherry was quantitatively measured in the BioLector® micro-fermentation system and was found to be capable of competing to existing systems, such as the constitutive synthetic P11-promoter. Moreover, expression cassettes were integrated into the *L. plantarum* 3NSH chromosome for achieving stable gene expression. In conclusion, the aim of our study is engineering of recombinant starter cultures for optimized silage processes. By combining food- grade and host-strain derived features, we are designing a toolbox for the plasmid free *Lactobacillus plantarum* strain 3NSH that is already regarded as safe, to improve its applicability in farming and biotechnology.

Poster Session

BAM-PM1003 - Characterization of Escherichia coli isolated from samples of different biological and environmental sources

Annabelle Ferrera Boza[1], Lourdes Enriquez de Madrid[2]

[1]School of Microbiology, Universidad Nacional Autónoma de Hondruas, Tegucigalpa, Honduras, [2]Master´s Program in Infectious and Zoonotic Diseases, School of Microbiology, Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras

The prevalence of diarrheagenic E. coli in childhood diarrhea and the role of contaminated water supplies and food products in disease transmission in Honduras have not been well- studied. The aim of this study was to identify E. coli pathotypes, including E. coli O157:H7, ETEC and EPEC. Escherichia coli from 5 different biological and environmental sources were isolated and characterized during a five-year span, starting 2007. A total of 266 samples, 117 from human feces, 52 ground beef, 8 river stream-water, 20 residual water and 69 household stored water containers were examined by conventional methods for E. coli and the isolated strains were subjected to multiplex PCR targeting genes for the thermostable and -labile toxins (STh, STp and LT) of ETEC, and for the eae and bfpA genes of EPEC; presence or absence of Shiga toxin genes (stx1 and stx2) characteristic of E. coli O157:H7 strains were targeted. Three (6%) ground beef and one (5%) residual water samples were positive for shiga-toxin producing E. coli, while 17 (22%) samples either from river stream-water or household water containers were characterized as ETEC (29% LT toxin, 18% STp toxin, 35% STh, 18% LT/STh)and 3 (4%) samples from the same sources as EPEC. ETEC was detected in 14 (12%) of the 117 studied children with episodes of diarrhea (64% LT; 29% STh, 7% LT/STh); another five (4%) episodes were identified as EPEC. Our data is the first molecular E. coli report that suggests the presence of E. coli pathotypes circulating in Honduras.

Poster Session

BAM-PM1005 - Identification of putative key amino acids involved in HtpB protein-folding independent functions

Karla Valenzuela[1], Gabriel Moreno-Hagelsieb[2], Rafael Garduno[3]
[1]Dalhousie University, Halifax, Canada, [2]Wilfrid Laurier University, Waterloo, Canada, [3]Dalhousie University, Halifax, Canada

Chaperonins (e.g. GroEL) are highly conserved housekeeping proteins that interact with co-chaperonins (e.g. GroES) to help other proteins to fold. However, bacterial chaperonins can also have protein-folding independent functions, and some act as proteases, toxins or adhesins. The Legionella pneumophila chaperonin (HtpB) has been implicated in host cell invasion, mitochondria recruitment, and cell signalling. We hypothesize that these unique HtpB functions are due to amino acid substitutions at key positions. 1374 HtpB orthologs were aligned using ClustalOmega and the Evolutionary Trace (ET) method was used to rank the amino acids by their relative evolutionary importance. A low rank indicates high conservation whereas less conserved amino acids are ranked high. Since E. coli GroEL is the most studied chaperonin, it was used as the reference chaperonin. The GroEl and HtpB sequences where compared and 142 different residues were found. Then, each amino acid substitution was scored based on the BLOSUM62 matrix and 41 less likely to occur substitutions (negative scores) were selected. Finally, a low ET rank and a negative BLOSUM62 score were used as criteria to select 5 residues for site directed mutagenesis: methionine 68 and 212, serine236, lysine 298 and asparagine 507. Also, a less conserved region composed of residues 471 to 475 was selected based on its negative BLOSUM62 score. Using bioinformatic tools we were able to predict the amino acids that could be involved in HtpB folding-independent functions.

Poster Session

BAM-PM1007 - Molecular genetics and systems biological analysis to the pathogenesis of drug-resistant type malaria

Koichi Shirakawa[1], Mitsuaki Nishibuchi[2]
[1]Department of Pathogenic Microbiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [2]Center for Southeast Asian Studies, Kyoto University, Kyoto, Japan

Chloroquine and Pyrimethamine are frequently used in areas where there are malaria patients. In addition, an increase in the frequency of use of placebo in these areas was reported by the United Nations Office on Drug and Crime. Many of anti-malarial drugs, the effect is well known. However, effects on the target molecule is complex and understanding is difficult. In our study, was discussed in molecular genetics approrch characteristics of resistant type from the functional aspect of transporters and channels in the membrane of phagosome. Therefor, in our analysis, we focused on the functional changes and mutation of Pyrimethamine resistance gene and Chloroquine resistance gene. In addition, we researched for metabolic and structural proteins to complement the functional decline.When we use a anti malaria drug, for example, we should moderate that we consider only a long-term continuous use as a main cause of the genetic mutation. Many sensitivity type in the blood absorbs a lot of molecules of a drug given. As a result, the quantity of the drug which a resistant type absorbs decreases. Anti-malaria drug molecules in blood decreased, and it was suggested that resistant type increased because the effect attenuated. Furthermore, in the treatment of malaria, it is important to use a drug measured exactly for patient and should concentrate on complex drugs for a short term.

Poster Session

BAM-PM1009 - Developing molecular biology and genomic tools for studying *Burkholderia contaminans*

Maria. Agustina López De Volder[1], Ruhi Bloodworth[2], Carrie Selin[2], Silvia Cardona[2], José Degrossi[1]
[1]Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina., [2]Department of Microbiology, University of Manitoba, Winnipeg, Canada.

Burkholderia contaminans is a Gran-negative bacterium that was recently defined as a specie of the *B. cepacia* complex (Bcc). It can be found in natural environments, as contaminant of industrial products and as opportunistic pathogen infecting the lung of cystic fibrosis (CF) patients. Although *B. contaminans* is the prevalent species in CF patients in Argentina, knowledge about its mechanism of pathogenesis is very limited when compared with other Bcc members like *B. cenocepacia* or *B. multivorans*, which are common in North America and Europe. In this work, we described phenotypic characteristics of argentinian isolates, determined the feasibility of applying genetic tools developed for other Bcc species and produced the draft genomes of five *B. contaminans* isolates. While most of *B. contaminans* isolates presented filament morphology, similar to *B. anthina*, some isolates exhibited short rod morphology, similar to *B. cenocepacia*. Filament morphology correlated with the presence of yellow-green pigment and 50% of the strains of *B. contaminans* produced β-Haemolisis. Standard transpososon mutagenesis techniques were successful using the antibiotic marker trimethoprim and gentamicin or ampicillin to select against donor and helper strains. Genomes were sequenced with an Illumina MiSeq sequencer, read assembly was performed with Velvet and the contigs were preliminarily annotated with the Rast server (http://rast.nmpdr.org/). Seven genes used in the *Burkholderia cepacia* complex Multilocus Sequence Typing (MLST) database (http://pubmlst.org/bcc/) were retrieved from the Rast server and compared against those deposited in the MLST database. This analysis confirmed that the sequenced genomes indeed corresponded to *B. contaminans*. However, all Argentinian isolates formed a new sequence type. Further analysis of the draft genomes indicated no evidence of genomic island BcenGI11, which is related to virulence in *B. cenocepacia* J2315. In summary, we have developed research tools for *B. contaminans* that will allow a better understanding of this emerging opportunistic pathogen.

Poster Session

BAM-PM1011 - Identification of NDM-1 and IMP Metallo β-Lactamase genes using real-time PCR

Taru Singh[1], Shukla Das[1], V.G Ramachandran[1], Dheeraj Shah[2], Rumpa Saha[1]
[1]Microbiology Department,University College of Medical Sciences & GTBH, Delhi, India, [2]Department of Paediatrics, University College of Medical Sciences, University of Delphi, New Delhi, India

Background- Metallo beta lactamase enzymes (MBL) are encoded by transferable genes, which appear to spread rapidly among gram-negative bacteria in developing countries. Objective: The objective of this study was to develop a syber green real-time PCR assay followed by a melt curve step for rapid detection and identification of NDM-1 & IMP genes on the amplicon melting peak. Materials and Methods: A total of 120, including 40 healthy controls E..coli isolates were taken from a Tertiary Care hospital of Delhi. These isolates were subjected to Real time PCR for *NDM-1 & IMP* genes using SYBR green dye. Results: Presence of NDM-1 & IMP gene was identified in less than 50% of the samples.Transciptome analysis was also done to see the expression of the genes. Conclusions: The rapid detection of MBL-producing isolates could be helpful for epidemiological purposes and for monitoring the emergence of MBL-producing isolates in clinical settings. The detection of such isolates could help rapidly establish standards for hospital infection control measures to minimize the spreading of these resistant determinants. The present study confirmed the presence of multiple genetic mechanisms for carbapenemases production among the clinical isolates of *E..coli* in north India.

Poster Session

BAM-PM1013 - Characterization of amoebal proteins present on multilamellar bodies in Dictyostelium discoideum.

Alix M. Denoncourt[1,2], Valérie E. Paquet[1,2], Myriam M. Ouellet[1,2], Stéphanie Dallaire-Dufresne[1,2], Steve J. Charette[1,2]
[1]Institut de biologie integrative et des systemes, Quebec, Canada, [2]Centre de recherche de l'institut universitaire de cardiologie et de pneumologie de Quebec, Quebec, Canada

Introduction: Amoebae are ubiquitous organisms that feed on bacteria by phagocytosis. However, some pathogenic bacteria can resist enzymatic degradation in amoeba's lysosomes and therefore be packaged in multilamellar bodies (MLBs). These MLBs containing bacteria are then secreted in the environment. The bacterial packaging process provides protection to pathogenic bacteria against various environmental stresses. Objectives: The objective of this study, using the amoeba model Dictyostelium discoideum, is to determine the bacterial packaging mechanism, which is still unknown. The protein and lipid composition of MLBs has been recently elucidated and three amoebal proteins specifically found on MLBs have been identified, including the protein Gp17. Methods: Expression vectors for these proteins in fusion with the green fluorescent protein (GFP) have been created. The analysis of the behaviour and localization of these proteins in the endocytic pathway of amoebae was undertaken by electron and fluorescence microscopy. Results: The expressed proteins accumulated in cellular compartments. The expression of Gp17 stimulated the formation of intra-lysosomal structures, suggesting that this protein could play a role in MLBs formation. Conclusions: A better understanding of the processes involved in MLB formation may ultimately help to evaluate the role of bacterial packaging in the transmission of respiratory diseases.

Poster Session

BAM-PM1015 - Prevotella modulate the Pseudomonas mediated host response in a Cystic Fibrosis derived bronchial epithelial cell line

Anne Bertelsen[1], Thamarai Schneiders[1], J Stuart Elborn[1]
[1]Centre for Infection and Immunity, Queens University, Belfast, UK

Anaerobic bacteria such as Prevotella spp have been isolated from the respiratory tracts of Cystic Fibrosis (CF) patients along with the common CF pathogen Pseudomonas aeruginosa. However, the contribution of anaerobes to disease pathogenesis in the CF respiratory tract remains poorly understood. We hypothesise that clinical CF Prevotella isolates utilise the Toll like receptor pathway to activate transcription of pro-inflammatory cytokines, whilst also modulating the host inflammatory response associated with P.aeruginosa. CF derived bronchial epithelial (CFBE) cells exposed to live P.aeruginosa alone elicit mRNA expression of the pro-inflammatory cytokines interleukin 8 (IL-8), tumour necrosis factor alpha (TNFα), chemokine ligand 2 (CXCL2), interleukin 6 (IL-6), C-reactive protein (CRP) and lipopolysaccharide binding protein (LBP). However when CFBE cells are exposed to P.aeruginosa prior to Prevotella nigrescens exposure there is a significant reduction in the pro-inflammatory response associated with P.aeruginosa. When mRNA levels in co-culture are compared to the mRNA levels obtained from P.aeruginosa exposed cells, expression of IL-8 is reduced 2.5 fold,TNFα-10.5 fold, CXCL2 -1.35 fold, IL-6-2 fold and there was no detectable mRNA for either CRP or LBP. This trend is observed with other Prevotella spp. To elucidate the mechanism associated with this reduced inflammatory response, we performed tissue culture and cytokine measurements after exposure to Prevotella -conditioned medium. The results demonstrate that the proliferation of P.aeruginosa is significantly inhibited by factors within the Prevotella conditioned medium. This suggests that secretory factors from Prevotella spp inhibit the growth of P.aeruginosa in co-culture, thereby likely reducing the host inflammatory response associated with P.aeruginosa.

Poster Session

BAM-PM1017 - Lactobacillus sakei probio-65 an ideal probiotic candidate for the treatment of atopic dermatitis and skin disorder

Yong-Ha Park[1], Irfan Ahmad Rather[1]
[1]*Department of Applied Microbiology and Biotechnology, Yeungnam University, Gyeongsan, Korea*

Atopic dermatitis (AD) is an inflammatory skin disease which affects a large percentage of the world's population. Probiotics have been found to modulate immune responses and thus are now being suggested as potential treatments for allergies. Lactobacillus sakei probio-65, isolated from Kimchi, exhibited probiotic properties and showed antibacterial activity, especially against Staphylococcus aureus. During in vitro analysis, probio-65 exhibited immuno-stimulating properties like increased production of nitric oxide and decreased histamine production. In vivo analysis in artificially inducing AD in NC/Nga mice showed that the oral administration of viable, or heat-inactivated probio 65 improved the condition of skin and reduced the scratching frequency. The serum levels of IgE, cutaneous T-cell attracting chemokine (CTACK) and IL-4 and IL-6 concentration were significantly decreased by this therapy. Expression of Thymus and activation-regulated chemokine and CTACK in AD-like skin lesions were inhibited by both live and dead probio 65. Inhibition of beta-hexosaminidase release and secretion of IL-4, TNF-ά and IL-6 from RBL-2h3 cells were also observed. Supplementation of probio 65 in children with atopic dermatitis was associated with a substantial clinical improvement and a significant decrease in chemokine levels. In a double-blind, placebo-controlled trail, children with minimum SCORing of Atopic Dermatitis (SCORAD) score of 25 were randomized and administered either daily probio 65 or daily placebo supplementation for 12 weeks. The SCORAD total scores adjusted by pretreatment values were lower after probio 65 treatment than placebo treatment. In addition, there was a 31% improvement in mean disease activity with probiotic strain probio 65 as compared with 13% improvement with placebo use. Compared with placebo, probiotic administration associated with the lower pretreatment-adjusted serum levels of CCL`7 and CCL27, which significantly correlates with SCORAD total score. These results suggest that probio-65 may represent a potential novel therapeutic agent in the treatment of atopic dermatitis and skin disorder.

Poster Session

BAM-PM1019 - The biological molecular factors that influence SMG-host symbiosis

Karissa Giraldi[1], Dawn Bowdish[1], Michael Surette[1]
[1]*McMaster University, Hamilton, Canada*

The Streptococcus Milleri/Anginosus group (SMG) is a group of three genetically related species: S. anginosus, S. constellatus, and S. intermedius. Members of the SMG are considered commensals, as they are found in about a third of the healthy population, colonizing diverse mucosal surfaces. They are, however, the most commonly isolated organism from pleural empyemas, a frequent isolate from abscesses, and a significant contributor to cystic fibrosis lung infection. Why do some individuals benefit from a commensal relationship with these bacteria while others suffer from invasive infection? I hypothesize that the host cell receptor toll-like receptor 2 (TLR2) is essential for mediation of macrophage-SMG interactions; the SMG is able to modulate immune detection and macrophage killing through expression of capsule and inhibition of apoptosis, respectively. I have two aims for my investigation: 1) determine whether SMG strains use capsule to prevent recognition by host cells via TLR2; 2) elucidate the molecular mechanism(s) by which the SMG avoid killing by macrophages. The HEK-Blue-hTLR2 cell line (HB2) was used to measure TLR2 activation with five reference SMG strains previously found to induce either a low, intermediate, or high release of pro-inflammatory cytokines. When relative TLR2 activation was calculated, there was no positive correlation between degree of immune response and TLR2 activation. These results lead to the investigation of whether capsule modifies the TLR2 response; to do this, capsule mutants will be screened using the HB2 cell line. To determine whether modulation of cell death is a component of SMG pathogenicity, SMG-stimulated primary macrophages will be stained with flow cytometry dyes propidium iodide and annexin V to visualize apoptosis and necrosis. If one could discern the factors involved in the transition from commensalism to pathogenicity, this may aid in understanding individual susceptibility to infection.

Poster Session

BAM-PM1021 - Response of the bacterial symbiont Holospora caryophila to different growth conditions of the host Paramecium

Michele Castelli[1], Olivia Lanzoni[1], Sergey I. Fokin[1], Martina Schrallhammer[2], Giulio Petroni[1]
[1]Department of Biology, Università di Pisa, Pisa, Italy, [2]Institute of Hydrobiology, Technische Universität Dresden, Dresden, Germany

Previous studies have shown that the associations between ciliates and their bacterial symbionts display various degrees of stability. Some symbionts can be maintained relatively well under standard laboratory conditions whereas others are frequently lost, especially when the host is growing with a high division rate. In this study, the variation of infection level by the symbiont Holospora caryophila within its host population Paramecium octaurelia was investigated in response to three feeding regimes, which differed in the relative amount of food provided at regular time intervals. The density of hosts and symbionts were measured as follows: Paramecium cells were directly counted in Bouin-fixed samples, and the approximate number of Holospora inside each host cell was estimated by fluorescence in situ hybridisations applying a specific probe. The response of the ciliates to each treatment was determined as a nearly exponential growth rate proportional to the amount of food received. After 24 days, the symbiont was maintained at an infection level close to 100% in all treatments. Even after a subsequent starvation phase of 20 days this finding remained valid, although at intermediate time-points in both phases some fluctuations were observed. These results show that H. caryophila is able to maintain efficiently its infection in the tested range of host growth conditions, thanks also to the possibility of an effective re-infection in case of loss. Thus, these observations can be the basis for more detailed investigations on the relationship between H. caryophila and its host. Moreover, since the growth conditions tested have been shown to be suitable to obtain massive growth of the obligate symbiont, they could be applied in studies that require large quantities of material, for example in genomic or proteomic analyses.

Poster Session

BAM-PM1023 - Engineered Salmonella typhimurium inhibits tumor growth in lung carcinoma mice

Catierine Werle[1], Guilherme Milanez[1], Juliana Pastorello[1], Bruna Leite[1], Marcelo Brocchi[1]
[1]State University of Campinas, Campinas, Brazil

The cancer is the second most frequent cause of death. The factors that affect cancer outcomes, such as disease recurrence, risk of second malignant neoplasms, and the late effects of cancer treatments, becomes more important. For almost 200 years has been known that bacteria have the ability to colonize solid tumors and induce tumor shrinkage. It has been know since the 1940s that anaerobic bacteria can selectively grow in hypoxic and necrotic areas of tumors. The bacteria Salmonella enterica Typhimurium are facultative anaerobes and has a particular promise as a cancer therapeutic because it can be manipulated and has been shown to preferentially accumulate in tumors compared with other organs after systemic injection. The utility and toxicity of these bacterial therapies can be enhanced by genetic manipulation. In additional, attenuated S. Typhimurium strains are safety for humans and other animals. This study aims to evaluate the anti-tumor potential of attenuated new mutants of S. Typhimurium in murine models. Six –week-old female C57Bl/6 mice were subcutaneously injected with 106 3LL (lung Carcinoma cells). After 12 days mice were injected intratumoral with 105 CFU/mL of S. enterica LGBM1, a attenuated null-mutant for a DNA-biding protein. To investigate the therapeutic effect of bacteria, tumor growth was monitored every two days using digital calipers. The volume of tumor was calculated using the formula V = L x W2 x 0.52 where L is the length and W the width. Our results showed a reduction in tumor growth in mice treated in compared to the mice treated with PBS (placebo). More tests are needed, but our preliminary data demonstrate a high potential for the use of attenuated mutants of S. Typhimurium here described as antitumor agents and may be an alternative to be explored in conjunction with conventional treatments in which tumors has shown resistance.

Poster Session

BAM-PM1025 - Preconditioning with low butyric acid concentrations reduces levels of cytokine induced chemoattractant (CINC)-1 production by small intestinal epithelial cells challenged with bacterial lipopolysaccharide but not viral double-stranded RNA

Padmaja Shastri[1], Julia M. Green-Johnson[1]
[1]*University of Ontario Institute of Technolgy, Oshawa, Canada*

Studies pertaining to the anti-inflammatory effects of the gut microbiota derived fermentation metabolite butyric acid have been conflicting. Elucidating the impact of this Short Chain Fatty Acid (SCFA) on intestinal epithelial cells (IEC) can provide insight into mechanisms of action of the gut microbiota and prebiotics. We examined effects of the SCFA butyric acid on TLR agonist-induced chemokine production using IEC-6, a non-transformed rat small intestinal cell line. Production of cytokine-induced chemoattractant-1 (CINC)-1 by IEC-6 was induced by treatment with either the TLR4 agonist lipopolysaccharide (LPS) purified from E. coli or the TLR3 agonist poly I:C, a synthetic variant of viral dsRNA. Effects on CINC-1 production were first evaluated by co-incubating IEC-6 cells with either 10mM or 25mM butyric acid and 20ng/ml of LPS or 40 ug/ml of poly I:C for 6 hrs. Additionally, we tested whether pre-incubation of IEC-6 cells with either 10mM or 25mM butyric acid, prior to challenge with LPS or poly I:C, differentially influenced CINC-1 production. CINC-1 levels were evaluated by Enzyme-Linked Immunosorbant Assay (ELISA) and IEC-6 viability was measured by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) dye reduction assay. Co-incubation with butyrate did not significantly alter CINC-1 production induced by either LPS or poly I:C. In contrast, levels of LPS-induced CINC-1 production were significantly decreased when IEC-6 cells were pre-incubated with 10mM butyric acid ($p=0.01$). Pre-treatment with 25mM butyric acid significantly ($p<0.05$) reduced IEC-6 viability. Poly I:C-induced CINC-1 production was unaffected by butyric acid pre-treatment. Furthermore, butyric acid (10mM for 6 hrs) did not alter TLR4 expression on LPS-treated IEC-6 cells as measured by flow cytometry. These results suggest that effects of butyric acid on TLR agonist-induced CINC-1 production by IEC are dependent on time of exposure relative to TLR agonist challenge, and also vary with the type of TLR agonist challenge.

Poster Session

BAM-PM1027 - Identifying the nature of the muralytic activity of resuscitation promoting factor B from Mycobacterium tuberculosis

Jonathan Samson[1], Dr. Anthony Clarke[1]
[1]Department of Molecular & Cellular Biology, University of Guelph, Guelph, Canada

Mycobacterium tuberculosis is the bacterium responsible for the second most deaths globally due to a single infectious agent. With one third of humans infected with M. tuberculosis the need for effective treatments of tuberculosis has never been higher. M. tuberculosis, similar to other Gram positive organisms, can enter a dormant state of growth characterized by extremely low metabolic activity. First identified in Micrococcus luteus, resuscitation promoting factors (Rpfs) were found to increase the speed with which dormant cells renewed normal metabolic activity and growth. Five Rpfs were soon found in M. tuberculosis and many studies sought to identify their importance and possible role in pathogenesis. RpfB, the most studied of the Rpfs, has been found to have muralytic activity. The nature of this activity has been the subject of much debate, and whether this enzyme acts similarly to a lytic transglycosylase or muramidase remains unknown. Composed of five domains, RpfB is the largest and most complex of the Rpfs. In order to understand the exact nature of the observed muralytic activity, three constructs of RpfB were engineered: the full length protein, the protein possessing the G5 domain and catalytic domain and the catalytic domain. These constructs were expressed andand the protein products were purified from Escherichia coli BL21. The nature ofIdentification of the muralytic reaction products was made using activity was assayed with various tools including high pH anion exchange chromatography of the reaction products and reverse phase high performance liquid chromatography coupled with tandem mass spectrometry.

Poster Session

BAM-PM1029 - Mapping of a protein interaction network required for enterobactin biosynthesis and secretion in E. coli

Paknoosh Pakarian[1], Peter Pawelek[1]
[1]Concordia University, Montreal, Canada

Ferric iron is essential for survival and growth of most bacteria. To obtain low-bioavailability ferric iron from the extracellular environment, most bacteria synthesize and secrete high-affinity iron chelators known as siderophores. By taking up iron-siderophore complexes, bacteria can survive and proliferate in low-iron environments. The E. coli catecholate siderophore enterobactin is synthesized in the cytoplasm by seven enzymes, EntCBDAEF and EntH, and then transported out of the cytoplasm by the inner-membrane protein EntS. Interactions between EntBDEF, that comprise a non-ribosomal peptide synthesis (NRPS) module, have already been reported. We hypothesize that the Ent NRPS enzymes, as well as the DHB biosynthetic machinery (EntCBA) and the EntS efflux transporter, all participate in a large multiprotein complex localized to the inner membrane. To investigate the extent of Ent protein interactions, we have employed complementary in vitro and in vivo techniques. A bacterial two-hybrid (BACTH) system was used to map Ent protein interactions in vivo. Using this assay, we have now identified previously unreported interactions in the Ent pathway. BACTH outcomes were verified by in vitro approaches, including pull-down and cross-linking assays. Concurrently, we have attempted co-localization of interacting Ent proteins in living E. coli cells via super-resolution fluorescence microscopy and confocal microscopy. Upon mapping of Ent interaction interfaces, complementation studies will be performed to study phenotypic effects of Ent protein interaction interface disruption.

Poster Session

BAM-PM1033 - Impacts of metal pollution from gold mining on water quality and selection of antimicrobial resistant bacteria

Janita Bosch[1], Cornelius Carlos Bezuidenhout[1]
[1]North-West University, Potchefstroom, South Africa

Gold mining pollution caused large amounts of metal to accumulated in the Wonderfonteinspruit (WFS) a tributary of the Mooi River (MR), South Africa. Such elevated levels may cause microbes to develop resistance to such metals. Resistance to metals may co-select for certain antibiotic resistance traits. The aims of the study were to determine (i) whether metal pollution in the WFS impacts on the water quality of the MR and (ii) whether this impact on selection of antimicrobial resistant bacterial populations in the two rivers. The Mooi River is the main source of water for Potchefstroom, a rural academic town. Physico- chemical parameters were measured using a portable multi-parameter instrument. Luria Bertani agar, supplemented with individual metals (up to 8.0 mM) or ampicillin (100 µg/ml), were used to isolate resistant bacteria. Metal resistant bacteria were tested for resistance to ampicillin (100 µg/mL), chloramphenicol (30 µg/mL), tetracycline (15 µg/mL) and streptomycin (50 µg/mL). The pH at was above 8 across all sites and the temperature low (10 to 17°C). Electrical conductivity, was above the South African target water quality range for irrigation in the WFS and in the MR after the convergence indicating potential impacts of WFS pn the MR. High amounts of metal resistant bacteria were isolated from the WFS and the MR after convergence. Levels of ampicillin resistant bacteria were higher at the MR site before the convergence. Elevated numbers of metal resistant bacteria were isolated at sites in the MR after the convergence. Results presented demonstrated that pollution had a deteriorating effect on the water quality of the WFS and MR after the convergence. The source of the high levels of antibiotic resistant bacteria before the convergence of the Wonderfonteinspruit and the Mooi River need to be established so that management options could be considered.

Poster Session

BAM-PM1035 - Common cell shape evolution of nasopharyngeal pathogens

Frederic Veyrier[1], Nicolas Biais[2], Nouria Belkacem[1], Cyril Guilhen[1], Sylvia Ranjeva[1], Jean-Yves Copé[1], Muhamed-Kheir Taha[1], Ivo Gomperts-Boneca[1]
[1]Institut Pasteur, Paris, France, [2]Brooklyn College, New York, USA

Respiratory infectious diseases are the third cause of worldwide death. The nasopharynx is the portal of entry and the ecological niche of many microorganisms of which, some are pathogenic to humans, such as Neisseria meningitidis (Nm) and Moraxella catarrhalis. These microbes possess several surface structures that interact with the actors of the innate immune system. In our attempt to understand the past evolution of these bacteria and their adaption to the nasopharynx, we first studied differences in cell wall structure, one of the strongest immune-modulator. We were able to show a modification of peptidoglycan composition (increased proportion of pentapeptides) and a cell shape change from rod to cocci selected along the past evolution of Nm. Using genomic comparison across species, we correlated the emergence of the new cell shape (cocci) with the deletion, from the genome of Nm ancestor, of only one gene: yacF. Moreover, the reconstruction of this genetic deletion in a bacterium harboring the ancestral version of the locus together with the analysis of the peptidoglycan structure, suggest that this gene is coordinating the transition from cell elongation to cell division. Accompanying the loss of yacF, the elongation machinery was also lost by some of the descendants leading to the change in the peptidoglycan structure observed in Nm. Finally, the same evolution was observed for the ancestor of M. catarrhalis. This suggests a strong selection of these genetics events during the colonization of the nasopharynx that may have been driven by the interaction with the immune system by reducing the cellular surface exposed to immune attacks without reducing the intracellular storage capacity.

Poster Session

BAM-PM1037 - Horizontal gene transfer and *Mycobacterium tuberculosis* pathogenesis

Joyce Wang[1], Francis Mann[2], Alex Dufort[1], Marcel A. Behr[1]
[1]McGill University, Montreal, Canada, [2]Winona State University, Winona, USA

Mycobacterium tuberculosis is the causative agent of human tuberculosis (TB), globally infecting over 2 billion people and responsible for more than 1 million deaths annually. In contrast, *Mycobacterium kansasii* is a closely-related environmental mycobacterium that occasionally gives rise to a non-transmissible, TB-like pulmonary disease. Comparative genomics studies between these organisms have detected 55 genes in the professional pathogen (*M. tuberculosis*) lacking from the environmental species (*M. kansasii*), with bioinformatic evidence supporting their acquisition by horizontal gene transfer (HGT). As HGT potentially modifies various adaptive attributes of the recipient strain, we set out to test whether genes putatively acquired by HGT had contributed to the pathogenesis of *M. tuberculosis*. We introduced a putative HGT locus, *Rv3377-8c* into *M. kansasii*; *Rv3377-8c* encode enzymes involved in the production of the diterpenoid isotuberculosinol (isoTB). Using gas chromatography-mass spectrometry, isoTB was detected in *M. kansasii:Rv3377-8c*, but not in *M. kansasii:empty vector*. In macrophage culture, *M. kansasii:Rv3377-8c* showed more replication within the first 24 hours of infection and further characterization of the intracellular behavior of *M. kansasii:Rv3377-8c* is ongoing. During *in vivo* infection, there was a trend showing increased bacterial burden in murine lungs 6 weeks after aerosol infection. Our data provide a proof-of-concept for using *M. kansasii* in gain-of-function screens to investigate the unique pathogenicity of *M. tuberculosis* and the role of *M. tuberculosis*-specific genes in host-pathogen interaction.

Poster Session

BAM-PM1039 - Investigating the conservation of metabolic function within prokaryotic species

Thomas Hemmy[1], Gabriel Moreno-Hagelsieb[1]
[1]*Wilfrid Laurier Univiersity, Waterloo, Canada*

A common assumption in microbial ecology is that bacteria of the same species will have highly similar, if not identical metabolic functions. This notion is contrasted by the prevalence of horizontal gene transfer amongst prokaryotes, and recent pan-genomic studies, which suggest that individual bacterium within a prokaryotic species can posses a wide range of genes, proteins, and, therefore, metabolic processes. When determining the species of a bacterium, the prokaryotic 16S ribosomal RNA genes are commonly analyzed, as they are highly conserved, and present within almost all bacteria, making them the ideal marker genes. To test the ability of prokaryotic 16s ribosomal RNA genes to accurately represent bacterial metabolic function, we have clustered the 16s ribosomal RNA sequences from prokaryotic species within the NCBI database together, placing sequences that share a >97% similarity into species-like groups known as operational taxonomic units. The metabolic function of each organism was then contrasted to the metabolic function of the other organisms within its operational taxonomic unit, allowing us to determine the degree of variation between the metabolic pathways of the species within each cluster. We found that although most operational taxonomic units contained only one species, the genetic variability suggest that a range of metabolic pathways could be present within each cluster, suggesting that metabolic function might also be variable within bacterial species.

Poster Session

BAM-PM1041 - Analysis of origins of replication of the two chromosomes in Rhodobacter sphaeroides 2.4.1

Madhusudan Choudhary[1], Bat-Erdene Myagmarjav[1]
[1]*Sam Houston State University, Huntsville, USA*

Rhodobacter sphaeroides 2.4.1 is a facultative anaerobic bacteria in α-3 subdivision of Proteobacteria. It has a complex genome, consisting of two chromosomes, chromosome I (CI) and chromosome II (CII) which are approximately 3Mb and 0.9Mb, respectively. The objective of this study was to identify the origins of replication of the two chromosomes and to further analyze them with respect to chromosomal or plasmid origin type. Using bioinformatics approaches, such as Z-curve analysis and GC-skew, three and five putative origin regions were found on CI and CII, respectively. The flanking regions of these putative regions were analyzed for the conservation of genes known to be located near confirmed replicative origins of other bacterial species. Each of the putative regions were amplified via PCR and ligated into a pLO1 vector, which contains a Kanamycin resistance gene and acts as a suicide vector in R. sphaeroides. These recombinant pLO1 plasmids were mobilized into R. sphaeroides using biparental mating of E. coli S17-1 and R. sphaeroides. R. sphaeroides' transconjugants were characterized for the autonomous replication of recombinant pLO1 plasmid in R. sphaeroides. Conservation of genes proximal to the replication origins as well as biological characterization of these putative origin sequences confirmed one origin of replication on each chromosome. Results also revealed that the replicative origin of the secondary chromosome is located near parA and parB genes as well as DnaA-like ORF, an arrangement shared by a number of megaplasmids in other bacteria.

Poster Session

BAM-PM1043 - EspI regulates the ESX-1 secretion system in response to ATP levels in Mycobacterium tuberculosis

Ming Zhang[1], Jeffrey Chen[1], Claudia Sala[1], Jan Rybniker[1], Neeraj Dhar[1], Stewart Cole[1]
[1]*Ecole Polytechnique Fédérale de Lausanne, Global Health Institute, Lausanne, Switzerland*

The function of EspI, a 70-kDa protein in Mycobacterium tuberculosis, has remained unclear. Although EspI is encoded by a gene within the esx-1 locus, in this study we show that it is not essential for ESX-1-mediated secretion or virulence in M. tuberculosis. We also provide evidence that reduction of cellular ATP levels in wild-type M. tuberculosis using the drug bedaquiline completely blocks ESX-1-mediated secretion. Remarkably, M. tuberculosis lacking EspI fails to exhibit this phenotype. Furthermore, mutagenesis of a highly conserved ATP-binding motif in EspI renders M. tuberculosis incapable of shutting down ESX-1-mediated secretion during ATP depletion. Collectively these results show that M. tuberculosis EspI negatively regulates the ESX-1 secretion system in response to low cellular ATP levels and this function requires the ATP-binding motif. In light of our results the potential significance of EspI in ESX-1 function during latent tuberculosis infection and reactivation is also discussed.

Poster Session

BAM-PM1045 - Green synthesis of silver nanoparticles is an newly emerging antimicrobials against multi drug resistant clinical isolates.

Saravanan Muthupandian[1], Jesu Arockiaraj[2], Anima Nanda[3]

[1]Institute of Biomedical Sciences, College of Health Sciences, Mekelle University, Mekelle, Ethiopia, [2]Department of Biotechnology, Faculty of Science and Humanities, SRM University, SRM Nagar, Kattankulathur, Chennai, India, [3]Department of Biomedical Engineering, Sathyabama University, Jeppiaar Nagar, India

Nanotechnology is an upcoming and fast developing field with potential applications for human welfare. Disease causing microorganisms that have become multi drug resistant are increasing and are the major cause for the public health problems. Therefore there is an urgent need for the development of new therapeutics for the treatment of multi drug resistant clinical pathogen. A green biosynthetic approach for metal nanoparticles has been suggested as promising eco-friendly newly emerging antimicrobials and alternatives to chemical methods. In this study, reported the green synthesis of silver nanoparticles (AgNPs) from aqueous floral extract of Abelmoschus esculentus L. In addition, the biosynthesized AgNPs were characterized using UV-vis spectroscopy, XRD, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The biosynthesized silver nanoparticles showed a maximum absorption in the visible region at 430 nm and the sizes ranged from 50-80 nm in spherical shape. Moreover the antimicrobial effect of green synthesized silver nanoparticles was evaluated against selected multi drug resistant gram positive and gram negative bacterial pathogens such as Pseudomonas aeuroginosa, Klebseilla pneumonia, Staphylococcus aureus and Bacillus subtilis. It reveals significantly higher antibacterial effect against Pseudomonas aeuroginosa, Klebsiella pneumonia and Staphylococcus aureus when compare with standard antibiotics. Furthermore, AgNPs analysed the selective toxicity of mouse embryo fibroblast cell line using MTT assay and it has shown there is no decrease in cellular viability in 50 µg/ml AgNPs concentration. Keywords: Green synthesis; Silver nanoparticles; Abelmoschus esculentus; Multi Drug Resistant; Antibacterial effect; cytotoxicity.

Poster Session

BAM-PM1047 - Identification and characterization of a novel Pantoea antibiotic produced by Pantoea ananatis BRT175

Alyssa Walterson[1], John Stavrinides[1]
[1]University of Regina, Regina, Canada

Pantoea is a Gram-negative, cross-kingdom pathogen that has been isolated from multiple environments, including plants, animals, humans, insects, and terrestrial and aquatic environments. Known to produce various antimicrobial agents, Pantoea is also an effective biological control agent with regards to Erwinia amylovora and fire blight. P. ananatis BRT175 produces a novel antibiotic, Pantoea Natural Product 1 (PNP-1), that specifically targets E. amylovora and other Pantoea strains. Previous research has determined there are seven genes involved in the production of this antibiotic, all of which have yet to be documented within the Pantoea genome. All seven genes appear to cluster together in a single genomic region, and are most closely related to regions in Pseudomonas fluorescens WH6 and Pseudomonas syringae pv. maculicola ES4326. An extensive survey of Pantoea strains shows that this particular antibiotic is not present outside of the BRT175 genome, suggesting that this is an entirely novel Pantoea antibiotic. The identification of a new Pantoea antibiotic highlights the diversity of natural products produced by Pantoea.

Poster Session

BAM-PM1049 - Evaluation of Antimicrobial Peptides (AMPs) as novel bactericidal agents for ex vivo stored human platelets

CD Atreya[1], KVK Mohan[1]
[1]US FDA, Center for biologics Evaluation and Research, Bethesda, USA

Bacterial contamination associated sepsis is still a complication in patients transfused with ex vivo stored platelets (PLTs) as they are stored at room temperature. A pathogen inactivation approach with ideally no side effects would help improve the safety of this life saving transfusion product. We have recently shown that synthetic AMPs named PD1-PD4 derived from the thrombin-induced human platelet-derived antimicrobial protein, and repeats of Arg-Trp (RW1-RW5) both individually and in combinations, demonstrate microbicidal activity against Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Bacillus cereus (Mohan et al, Transfusion 2010; Mohan et al, Clin. Microbiol. Infect. 2013). Further, we have also demonstrated that platelets treated with a mixture of RW3 and RW4 peptides maintain their in vitro properties similar to the untreated platelets during 7 days of storage (Bosch-Marce et al, Transfusion 2013). Preclinical evaluation of the peptides (PD1-4 or RW1-RW5) in rabbits did not elicit a significant humoral (antibody) response and human platelets treated with four selected peptides (PD3, PD4, and RW2-RW5) based on their best AMP activity demonstrated no adverse effect on the platelet recovery and survival in a SCID mouse model (Bosch-Marce et al Transfusion 2014). Overall, these results taken together, suggest that application of carefully selected AMPs, with demonstrated safety, as novel bactericidal agents is feasible toward addressing the bacterial contamination of ex vivo stored human platelets.

Poster Session

BAM-PM1053 - Plant peptides – the natural antibiotics

Emese Petra Balogh[1], Tímea Mosolygó[1], Adrienn Karai[1], Fanni Kerekes[1], Hilda Tiricz[3], Dezső Virók[2], Éva Kondorosi[3,4], Katalin Burián[1]

[1]Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary, [2]Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary, [3]Institute of Biochemistry, Biological Research Center, Szeged, Hungary, [4]Institut des Sciences du Végétal – CNRS, Gif-sur-Yvette, France

Members of the Chlamydiaceae family are obligate intracellular bacteria with a unique developmental cycle. Chlamydia trachomatis causes trachoma and genitourinary infections and is of great public health significance because of the possible late consequences (pelvic inflammatory disease, ectopic pregnancy, or infertility) of the untreated cases. To avoid these consequences of C. trachomatis infection, antibiotic strategies aim to eradicate the pathogen even in asymptomatic infections. Although first-line antibiotics have been proven to be successful in the treatment of C. trachomatis infections, treatment failures have been observed in several cases. Furthermore, the administration of antibiotics may blunt the development of natural immune responses to C. trachomatis. Development of new anti-microbial agents is required to overcome this problem. Antimicrobial Peptides (AMPs) that serve as host defense molecules are produced by various organisms across the evolutionary spectrum. To date, more than 800 AMPs have been discovered, including 270 from plants. Nodule-specific Cysteine Rich (NCR) peptides produced by symbiotic plants have AMP-like structure and activities. We tested the effect of NCR peptides on C. trachomatis and found that seven of the peptides tested exerted dose-dependent antibacterial activity. Significant anti-chlamydial activity was observed after a 15-min incubation period. During a liquid chromatography-tandem mass spectometry (LC-MSMS) test, the chlamydial ligand of the NCR247 peptide was revealed. Our results suggest that NCR peptides can be a useful option in the development of new, natural antimicrobial agents.

Poster Session

BAM-PM1055 - Antibacterial action of honey resembles that of cell wall-active antibiotics

Katrina Brudzynski[1], Calvin Sjaarda[1]
[1]Bee-Biomedicals Inc, St. Catherines, Canada

Buckwheat honeys indiscriminately kill methicilin-resistant and sensitive bacterial species but the underlying mechanism remains obscure. To facilitate discovery of cellular targets for honey antibacterial compounds, we employed the method of phenotypic profiling. Using ampicillin as a drug model, we directly compared morphological and functional changes in Escherichia coli induced by honey and ampicillin. Firstly, we demonstrated the purity of tested honeys from potential β-lactam contaminations using quantitative LC-ESI-MS. Exposure of log-phase E. coli to honey or ampicillin resulted in time-and concentration-dependent changes in cell shape with the appearance of filaments at sub-inhibitory concentrations and spheroplasts at bactericidal concentrations. Extent of bacterial survival depended on growth phase at which E. coli cells were exposed to honeys. Time-kill kinetics of log-phase E. coli revealed that the reduction of cell viability to >4log10 CFU/ml required honey concentrations of 2xMBC and 1 hr incubation. Image-based microscopy showed marked cell wall damage and cell lysis. Moreover, fluorescence-activated cell sorting indicated increased permeability of the lipopolysaccharide of outer membrane to more than 90% of E. coli cells treated by honey. Consistently with flow cytometry results, both honey and ampicillin caused endotoxin release in significantly higher amounts than untreated, control cells. In the model of ampicillin resistance, E. coli transformed with the ampicillin resistance gene remained sensitive to honey cytotoxicity and displayed morphological changes and endotoxin release as ampicillin-sensitive cells. β-Lactamase protected ampicillin-resistant E. coli from antibacterial action of ampicillin. In conclusion, the ability of honey and ampicillin to induce structural changes to the cell wall and LPS underlies antibacterial activity of both agents. Since the cell wall is critical for cell growth and survival, honey active compounds would be applicable for therapeutic purposes. Differences in the mode of actions between honey and ampicillin against ampicillin-resistant strains may provide advantage in treatment of β-lactam-resistant pathogens.

Poster Session

BAM-PM1057 - Antimicrobial activity of aqueous and methanol extracts from Pseudanabaena sp.

Margarita Pineda-Lopez[1], Oscar Rodas-Suarez[1,2], Sandra Ruiloba-De Leon F[1]
[1]IPN, Mexico City, Mexico, [2]Escuela Nacional de Ciencias Biológicas, Mexico City, Mexico

There are bacteria that behave as effective opportunistic pathogens, responsible for community and hospital-acquired infections. It is often highly problematic to choose the most appropriate antimicrobial substances since the aforementioned bacteria have a high resistance to multiple antibiotics, as well as an extraordinary ability to acquire new resistance mechanisms. Therefore it is recommended to find new sources of antimicrobial agents such as cyanobacteria. Pseudanabaena sp. (ES03) is a type of cyanobacteria of solitary strands. It creates very fine aggregates without ramifications, and can have rectangular or squared cells, with slight constrictions, always with walls longer than they are wide; and with gas vesicles grouped in aerotops, located at the end of the cells. To evaluate the antimicrobial activity of Pseudanabaena sp. (ES03), methanol extracts of the cyanobacteria biomass were made and tested in different concentrations by agar diffusion method against Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 8739 and Salmonella typhi ATCC 6534. The methanol extract was obtained by subjecting the biomass to methanol and acetic acid treatment for 24 hours. Afterwards, it was evaporated until dry and resuspended in methanol. The microorganisms were cultivated in nutrient broth supplemented with yeast extract. For agar diffusions, tests were adjusted to 1 nephelometer tube and seeded by diffusion in Mueller Hinton plates. Extracts were impregnated (0.1 mL) in paper discs 0.6 cm diameter. The plates were incubated at 37 ° C for 24 hours and then the zones of inhibition around each disc were measured. With the results, analysis Dunnett one- way ANOVA was performed. The aqueous extracts showed antimicrobial activity against some of the strains.used, whereas the methanol extracts showed antimicrobial activity against various microorganisms, especially against Pseudomonas aeruginosa.

Poster Session

BAM-PM1059 - The comparison of nanoaerosolized fluoroquinolones to traditional therapeutic delivery methods in murine pulmonary Francisella infections

Crystal Lindblad[1], Albert Nwabueze[1], Rachel Pepin[1], Victor Morozov[2], Monique van Hoek[1]
[1]National Center for Biodefense and Infectious Diseases, Manassas, USA, [2]Institute of Theoretical and Experimental Biophysics, Moscow, Russia

Aerosolized therapeutics improve upon traditional delivery methods in cases of pulmonary infection due to their ease of administration, access to the large lung surface area, limited systemic distribution, and appropriate localization deep in the lung. New technology in the production of nanoaerosols allows for further improvement of these treatments due to their potential for deeper penetration, enhanced deposition, and efficacy of a lesser dose. Aerosol exposures to biological threat agents are particularly lethal and the nefarious use of this method poses a threat to homeland security. The development of a nanoaerosol-based delivery method for therapies can counteract the aforementioned threat as well as have positive future implications in the private sector regarding other lung diseases and drug delivery. To address the biological threat of intentionally released aerosolized Francisella and the increased need for new and improved treatment options within both the public and private sectors, nanoaerosol technology will be applied to treat pulmonary Francisella infections in mice. Treatment utilizing nanoaerosolized fluoroquinolones will be juxtaposed to traditional delivery methods to evaluate their comparative effectiveness against pulmonary tularemia infections.

Poster Session

BAM-PM1061 - Characterization of an antimicrobially active Microbacterium sp. strain D3N3

Jarvis Li[1], Hai Xu[1], Julian Davies[1]
[1]*Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada*

The discovery of antimicrobials from novel environmental bacteria is a promising route to combating the continuing threat of antibiotic resistant pathogens. The potential of novel secondary metabolite biosynthesis are repeated illustrated in the multitude of metagenomic studies of variety of environments. We have isolated
Microbacterium sp. strain D3N3 from a 13,000 year old sediment sample on the UBC campus. *Microbacterium* D3N3 shares 98.7% or higher sequence identity with the 16s rDNA sequences of seven known *Microbacterium* species. Culture extracts of D3N3 display broad-spectrum inhibitory activity against pathogenic bacteria including *Staphylococcus aureus* and *Acinetobacter baumannii*. Fractionation of an ethyl acetate extract of D3N3 culture by thin layer chromatography reveals a single fraction responsible for the inhibitory activity. The genome of strain D3N3 was sequenced using the Illumina HiSeq2000 platform. Reads were assembled to generate 36 scaffolds with a combined size of 3.2Mb. Examination of the draft genome sequence suggests the presence of terpene and type 3 polyketide biosynthesis modules.

Poster Session

BAM-PM1063 - Genomic-era methods for antibiotic discovery

Tomas Gverzdys[1], Justin Nodwell[1]

[1]*Department of Biochemistry, University of Toronto, Toronto, Canada*

Streptomyces are a genus of soil-dwelling Bacteria that are most well-known for their ability to produce a wide variety of secondary metabolites. Such molecules have found considerable use in the clinic as drugs with a variety of different activities, though of particular importance are those with anti-bacterial activity. After the 'golden age of antibiotic discovery' (1940 to 1960), the rate of antibiotic discovery from *Streptomyces* slowed to a trickle. Coupled with the constant rise in antibiotic resistance, it has been postulated that we are headed toward a post-antibiotic era. Indeed, to avoid this, a reinvigoration in antibiotic discovery is desperately needed. WAC04657 is a wild-isolate *Streptomyces* strain that produces antibiotic activity against several human pathogens. Given this activity's potential relevance to the clinic, we purified the molecule and elucidated its structure by nuclear magnetic resonance (NMR). It was was revealed to be a tetracyclic molecule with a tetronate ring. In an attempt to make an overproducer strain, we sequenced the genome of WAC04657 and assembled it into approximately 500 contigs. Based on the structure of the molecule, we were able to predict the enzymes involved in the biosynthesis and in this way locate the gene cluster responsible for producing the molecule. An overexpression and disruption strain built to confirm the involvement of these genes also made obvious the presence of several other related molecules. Future work is focused on characterizing the structures and activities of all these molecules, as well as assessing their toxicity to mammalian cells. The work described here establishes modern, genomic-era methods for drug discovery.

Poster Session

BAM-PM1065 - Methicillin resistant Staphylococcus aureus (MRSA) from mothers and children admitted to an Algiers hospital (April 2010-September 2011): predominance of the European virulent clone

Djoudi Ferhat[1], Benallaoua Said[1], Touati Abdelaziz[1], Celestino Bonura[2], Aleo Aurora[2], Mammina Caterina[2]

[1]Laboratoire de Microbiologie Appliquée, Faculté des Sciences de la Nature et de la Vie, Université A/MIRA, Bejaia, Algérie, [2]Department of Sciences for Health Promotion "G. D'Alessandro", Section of Hygiene, University, Via del Vespro, Palermo, Italy

Abstract Background.– In recent years, an alarming increase of infections with methicillin-resistant Staphylococcus aureus has been reported around the world. However, genetic and epidemiological characteristics, specific to each region and type of population, have been reported. The aim of this study is an epidemiological investigation of infections with Methicillin-Resistant Staphylococcus aureus among mothers and children in Algiers hospital. Methods.– all infections by Methicillin Resistant Staphylococcus aureus among mothers, newborns and children during a period of 18 months were studied. Strains were typed by molecular methods to determine the types of Staphylococcal Cassette Chromosome mec, amplification of Panton-Valentine Leukocidin genes and Multi Locus Sequence typing. Results. – A total of 29 infections cases by Methicillin Resistant Staphylococcus aureus have been recorded, including 25 patients under 15 years, 19 cases caused by Hospital acquired Methicillin Resistant Staphylococcus aureus. 28 strains carried a cassette chromosome Staphylococcal Cassette Chromosome mec type IVc, and belonged to the sequence type 80, and 26 isolates were producing Panton-Valentine Leukocidin. Conclusion. – the virulent Methicillin Resistant Staphylococcus aureus European clone (Sequence type 80) is dominant among mothers and children in an Algiers hospital.

Poster Session

BAM-PM1067 - The iron homeostasis regulator RyhB sRNA is involved in the virulence of the uropathogenic Escherichia coli strain CFT073

Gaëlle Porcheron[1,2], Rima Habib[1], Sébastien Houle[1,2], Mélissa Caza[1], François Lépine[1], Charles Dozois[1,2,3]
[1]INRS-Institut Armand Frappier, Laval, Canada, [2]Centre de Recherche en Infectiologie Porcine et Aviaire (CRIPA), Université de Montréal, Faculté de Médecine Vétérinaire, Saint-Hyacinthe, Canada, [3]Groupe de Recherche sur les Maladies Infectieuses du Porc (GREMIP), Université de Montréal, Faculté de Médecine Vétérinaire, Saint-Hyacinthe, Canada

Iron is both essential and potentially toxic for most living organisms, making the precise maintenance of iron homeostasis necessary for survival. In E. coli, iron acquisition and storage control is mediated by the global ferric uptake regulator (Fur) and the small regulatory non-coding RNA RyhB. While the role of these regulators in iron homeostasis has been well studied in a non-pathogenic E. coli strain, their impact on the production of virulence-associated factors is not currently known in a pathogenic E. coli strain. We thus investigated the role of Fur and RyhB in iron homeostasis and in the virulence of the pathogenic E. coli strain CFT073 involved in urinary tract infections in humans. We observed by mass-spectrometry analyses that the ∆fur mutant produced more of the siderophores (iron acquisition molecules) enterobactin, salmochelins and aerobactin than the wild type strain. By contrast, the ∆ryhB mutant produced less siderophores. We demonstrated by qRT-PCR that shiA, encoding for a permease of shikimate (involved in enterobactin and salmochelins synthesis), entB, implicated in enterobactin synthesis, and iucD, implicated in aerobactin synthesis, were repressed in the ∆ryhB mutant grown in minimal medium and in human urine. The role of Fur and RyhB in the virulence of CFT073 strain was investigated in a murine model of urinary tract infection. In co-infection experiments with the wild-type strain and either a single (∆fur or ∆ryhB mutant) or a double mutant (∆fur ∆ryhB mutant), all of the mutant strains were outcompeted by the wild-type strain, particularly in the kidneys. In a single-strain infection model, the ∆fur ∆ryhB and the ∆ryhB mutants were attenuated in the bladder. We have thus demonstrated for the first time the role of the RyhB sRNA on expression of iron acquisition systems and colonization of the urinary tract by pathogenic E. coli in the murine infection model.

Poster Session

BAM-PM1069 - Bacterial pathogens associated with wound infections in University of Calabar Teaching Hospital, Calabar, Nigeria.

Ofonime Ogba[1], Oluwayemisi Olorode[2], Baki Mandor[3], Gloria Adie[3], Lydia Abia-Bassey[1], Helena Omang[3]
[1]Department of Medical Laboratory Science, University of Calabar, Calabar, Nigeria., [2]Department of Pharmaceutical Microbiology, Faculty of pharmacy, Niger Delta University, Bayelsa, [3]Department of Medical Microbiology/Parasitology, University of Calabar Teaching Hospital, Calabr, Nigeria.

Wound infections, which could be accidental, pathological or post-operative are important causes of morbidity and mortality among patients and can delay healing and cause wound breakdown. Multiple antibiotic resistance among bacterial populations is a great challenge in the effective management of wound infections. This prospective cross-sectional study was carried out at the University of Calabar Teahing Hospital, Calabar, between April 2012 to June 2013 to identify the bacterial pathogens associated with wound infections and their antibiotic suceptibility profile. Wound swabs and pus were collected from the 251 patients included in the study. Subjects were aged 6-87 years, with a mean age of 36.98± 15.59 and a male to female ratio of 0.6: 0.4. Specimens were inoculated on appropriate media and cultures were incubated at 37°C aerobically. Bacterial isolates were Gram stained and microscopically examined. Pathogens were identified by biochemical test. The Kirby-Bauer disk diffusion method was used for antibiotic susceptibility testing. The prevalence of wound infection among subjects was 207(82.5%), with 132(52.8%) single bacterial growth and 75(30.0%) mixed bacterial infections. Most bacterial isolates were Gram negative bacilli. Staphylococcus aureus was the most prevalent 91(32.04%) pathogen followed by Pseudomonas aeruginosa 51(17.96%). Males 161(56.7%) were more susceptible than females 123(43.3%), but there was no influence of gender on the rates of infection p=0.29. Subjects aged 21-40 years had the highest prevalence 98(48.0%) of wound infections. The sensitivity profile of isolates to commonly used antibiotics including those used as pre-operative prophylactics ranged between 11.1% to 100%, the range for ceftriaxones was 13.3% to 100% while the fluoroquinolones had a range of 25.0% to 100%. Most of the isolates were resistant to ampicillin and amoxicillin. The high rates of antibiotic resistance calls for monitoring and optimization of antimicrobial use, multidisciplinary approach to wound management and routine microbiological surveillance of wound infections.

Poster Session

BAM-PM1071 - The Geh lipase in Staphylococcus aureus USA300 is required for hydrolysis of trilinolein

Brigitte Cadieux[1], Vithooshan Vijayakumaran[1], Mark A. Bernards[1], Martin J. McGavin[1], David E. Heinrichs[1]
[1]*University of Western Ontario, London, Canada*

Staphylococcus aureus causes various bacterial infections including abscesses, septicaemia, and endocarditis. In the past, methicillin resistant S. aureus (MRSA) was limited to hospital environments, but recently, there has been an increase in the number of community-acquired MRSA strains affecting healthy individuals. Moreover, the MRSA strains are resistant to multiple antibiotic classes making it extremely difficult to treat. Part of the innate immune response in the human host involves secretion of bactericidal lipids by the sebaceous glands in the skin and delivery of triglycerides into abscesses to control invading pathogens. Two lipases were identified in the MRSA strain USA300, which presumably, catalyze the hydrolysis of lipids as a means to counteract this facet of host innate immunity. Lipases are, as yet, only predicted virulence factors, and little information exists regarding their role in the pathogenesis of S. aureus. We constructed a deletion mutant of the Geh lipase in the USA300 background and characterized its growth in presence of the triglyceride, trilinolein. The wild-type strain exhibited a delayed growth while the Δgeh mutant grew as well as in absence of trilinolein suggesting that the majority of the lipase activity in culture supernatants was dependent on Geh. We have also shown that the pro-form of Geh is proteolytically processed by the metalloprotease aureolysin to yield the mature enzyme. A His-tagged version of Geh has been constructed. The pure protein had readily detectable lipase activity as detected by a colorimetric enzyme assay. Furthermore, using gas chromatography, we have demonstrated that pure Geh hydrolysed trilinolein to linoleic acid. Interestingly, proGeh and mature Geh had similar enzymatic activity. An active site point mutant (S->A) was generated and the enzyme was rendered catalytically inactive. Overall, these results confirm that Geh hydrolyses triglycerides and could play a role in the pathogenesis of S. aureus.

Poster Session

BAM-PM1073 - The role of type III effetor during the mucosal pathogenic bacteria infection

Hanako Morikawa[1], Keiko Ono[1], Ai Himeno[1], Sasakawa Chihiro[1], Minsoo Kim[1]
[1]*Division of Bacterial Infection Biology, Institute of Medical Science, University of Tokyo,Tokyo, Japan*

The rapid turnover and exfoliation of mucosal epithelial cells provides an innate defense system against bacterial infection Nevertheless, many pathogenic bacteria, including Shigella, are able to surmount exfoliation, and colonize the epithelium efficiently. We reported that the Shigella effector OspE, localizes focal adhesions and binds to integrin-linked kinase (ILK). OspE-ILK interaction enhances cell adhesion and promotes bacterial colonization. Shigella sustain their infectious foothold by employing special tactics to prevent detachment of infected cells. Importantly, OspE cognate genes are highly conserved among many enteropathogenic bacteria, including Shigella, enteropathogenic Escherichia coli (EPEC), enterohemorrhagic E. coli (EHEC), Citrobacter rodentium, and Salmonella, suggesting that OspE plays a general role in bacterial infection. We show here that the role of OspE homolog during Citrobacter rodentium infection. Citrobacter rodentium OspE reinforces host cell adherence to the basement membrane by interacting with ILK. The number of focal adhesions is increased by ILK-OspE binding dependent manner. Using Citrobacter rodentium in vivo infection mouse model, we elucidates the in vivo role of Citrobacter rodentium OspE. We finds that wild type Citrobacter rodentium infection mouse shows the severe inflammation, internal hemorrhaging and diarrhea, whereas these pathogenic features were not prominent after ospE deletion mutant strain infection. OspE homologs play a key role in the establishment of bacterial infections, and we need to further elucidate the specific molecular mechanisms in this process.

Poster Session

BAM-PM1075 - EU-OPENSCREEN: chemical tools to study bacterial physiology and virulence

Bahne Stechmann[1]
[1]*Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany*

There has been a growing interest in recent years in using small molecules as 'tool compounds' (i.e. chemical inhibitors or activators of biological components) to study bacterial pathogens due to their unique advantages: as small molecules have an immediate, reversible and titratable effect on cellular functions, they are highly useful in studies of slowly or non-dividing bacteria such as Mycobacterium tuberculosis (i.e. under conditions when protein and RNA half-lives are prolonged and the effect of RNA interference is low); they allow for molecular studies of physiology and pathogenesis of previously intractable or genetically challenging bacterial pathogens (e.g. Chlamydia); they were instrumental in identifying and characterizing essential genes, which are, for instance, involved in cytoskeleton dynamics and bacterial cell wall, and represent novel targets for antibiotics; and finally, host-pathogen interactions can be addressed in vivo during infection by the conditional use of chemical probes. EU-OPENSCREEN is a European open-access research infrastructure on the European ESFRI roadmap (European Strategy Forum on Research Infrastructures) with the aim to develop novel research 'tool compounds' for all areas of the Life Sciences (incl. microbiology; molecular, cell, and plant biology; synthetic and medicinal chemistry; pharmacology and early drug discovery etc.). EU-OPENSCREEN supports all stages of a tool development project, including high-throughput screening, assay adaptation and chemical optimization of 'hit' compounds. EU-OPENSCREEN is expected to start operations in late 2015 EU-OPENSCREEN offers to external researchers open access to its shared resources, including the latest screening technologies, medicinal chemistry services and a unique compound collection composed of commercial and proprietary compounds. Microbiologists are invited who have a robust and suitable assay and are interested in collaboratively developing chemical 'tool compounds' for their target-of-interest.

Poster Session

BAM-PM1077 - Proteomic analysis of Burkholderia cenocepacia K56-2 grown in synthetic cystic fibrosis sputum medium shows upregulation of virulence factor flagellin and increased motility

Brijesh Kumar[1], Harry Yudistira[1], Silvia Cardona[1,2]
[1]Department of Microbiology, University of Manitoba, Winnipeg, Canada, [2]Departmet of Medical Microbiology and Infectious Disease, University of Manitoba, Winnipeg

Background: Burkholderia cenocepacia is an opportunistic pathogen, member of Burkholderia cepacia complex (Bcc), which has been identified from cystic fibrosis (CF) patients. Its large genome is comprised of three chromosomes, which increases its genomic diversity and occurrence in various environmental niches. CF isolates of Bcc strains are intrinsically resistant to a large number of antibiotics, which makes difficult to treat the infection. An amino acid rich defined medium that nutritionally mimics CF sputum is called synthetic CF medium (SCFM). Our goal is to identify potential virulence factors by analysing proteomic changes in B.cenocepacia grown in the SCFM, which may be due to nutritional cues present in the medium. Methodology: B.cenocepacia J2315 strain, a CF clinical isolate was grown in SCFM and MOPS-Glucose media (used a control condition). A proteomic analysis was performed using iTRAQ. The expressions of differentially expressed proteins in SCFM were further confirmed by Western blot, phenotypic and enzymatic analysis. Results: In total 1144 proteins were identified by iTRAQ, which represent approximately 15 % of the total proteome. About 20 proteins were significantly upregulated and these proteins were from the COG categories, such as translation, carbohydrate and amino acid transport and metabolism, biogenesis, energy production and virulence factors. The upregulation of two them, flagellin and a peptidase were confirmed using Western Blot. In accordance with the upregulation of flagellin, bacteria were more motile in SCFM than in the control media. Conclusion: The results show that the nutritional environment, rich in amino acids, of the CF lung induces differential expression of virulence factors. We are currently elucidating how the amino acids present in SCFM induce the overexpression of flagellin.

Poster Session

BAM-PM1079 - Essential genes for the survival of Legionella pneumophila in water

Laam Li[1], Paulina Dudyk[1], Sebastien Faucher[1]
[1]*McGill University, Montreal, Canada*

Background: Legionella pneumophila is the causative agent of the potentially fatal pneumonia, Legionnaires' disease. This bacterium inhabits natural freshwater environments and man-made water systems. Human infection occurs through inhalation of aerosols originating from Legionella-contaminated sources, so the survival of L. pneumophila in water system is essential for propagation to the human host. Objective: The objective of this study is to identify and characterize the genes that are essential for Legionella pneumophila to survive in water. Methodology: Microarray analysis was conducted to detect the changes in gene expression of L. pneumophila upon exposure to water. Deletion mutants of bdhA and ppk were constructed, and their survival in water was monitored by CFU count and compared to wild-type (WT) to determine the importance of these genes. A green fluorescence protein (GFP) reporter assay was conducted to determine if bdhA and ppk are regulated by rpoS, which is a known stress response regulator. Results: While L. pneumophila is unable to replicate in water, which is a nutrient-limited condition, it survives well for up to a month without significant mortality. Our transcriptomic analysis showed that replication, transcription and translation are shut down in water, while some metabolic genes are turned on. The CFU of Δbdha and Δppk decreased to undetectable level much earlier than WT, suggesting that bdhA and ppk are important for survival in water. Interestingly, the result of GFP reporter assay showed that bdhA is regulated by rpoS. Conclusion: The genes bdhA and ppk were found to be important for the survival of L. pneumophila in water, and the regulation of bdhA by rpoS was demonstrated. Other genes will be further characterized. Understanding the genes that are important for L. pneumophila to survive in water may provide some insights on preventing the outbreak of Legionnaires' disease.

Poster Session

BAM-PM1081 - Microbial persistence through a subversive mechanism that uncouples bacterial clearance from inflammation

Tomoki Maekawa[1], Jennifer Krauss[2], Kavita Hosur[1], John Lambris[1], George Hajishengallis[1]
[1]University of Pennsylvania, Philadelphia, USA, [2]University of Louisville, Louisville, USA

Porphyromonas gingivalis (Pg) is a “keystone” pathogen that enhances the pathogenicity of the multispecies periodontal microbial community through the disruption of host–microbe homeostasis. In so doing, Pg manipulates select host signaling pathways to impair host immunity without significantly affecting the host inflammatory response, which serves the nutritional needs of the bacteria (inflammatory tissue breakdown products and hemin-derived iron). However, the molecular mechanism(s) by which Pg can selectively inhibit immune elimination without blocking inflammation have remained obscure. Our recent findings support a model according to which Pg can disarm and disassociate a host-protective TLR2-MyD88 pathway from a TLR2-PI3K proinflammatory pathway that promotes Pg survival. Specifically, we have shown that Pg causes proteasomal degradation of MyD88 in a C5a receptor- and TLR2-dependent manner, resulting in decreased killing of Pg by neutrophils. Moreover, the same Pg-induced C5aR-TLR2 crosstalk induces PI3K signaling which inhibits Pg phagocytosis by neutrophils and promotes the induction of a non-host protective proinflammatory response. This subtle manipulation of host signaling pathways is a matter of life and death for Pg and co-habiting species. If Pg caused generalized immunosuppression, this would inhibit bacterial killing, but would also deprive the bacteria of critical nutrients derived from inflammatory tissue breakdown. These findings indicate that periodontal bacteria have evolved to evade host immunity in an inflammatory environment that is essential to their persistence.

Poster Session

BAM-PM1083 - EscC associates preferentially with membrane constituents of enterohemorrhagic E. coli as revealed by electrophoresis mobility

Ching-Nan Lin[1], Yen-Chia Liang[1], Yi-Shun Gao[1], Syuan-Shao Lu[1], Wan-Jr Syu[1]
[1]*Institute of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan*

Background: EHEC O157:H7 is one of the pathogenic bacteria that transmit to human through contaminated foods. On the bacterial chromosome, there is a pathogenic island encoding a type III secretion (T3S) system that delivers virulence factors to the host cells. Gene escC is within this island and presumably codes for a secretin that oliogomerizes in the outer membrane to assist secretion. Methods: To characterize the importance of escC, we created an escC-deleted mutant of EHEC. We examined the bacterial phenotypes and compared the biochemical properties of EscC expressed from different host bacteria. Results: We have found that T3S capability of EHEC was abolished when escC was deleted. By complementation with a plasmid-expressed EscC, which was hexa-histidine-tagged, the phenotype of EHEC T3S was restored. When checked with SDS polyacrylamide gel electrophoresis (PAGE) using samples from bacterial total lysates, EscC expressed in EHEC migrating distinctly from that expressed in a K-12 strain. By fractionation, EscC was found in both membrane and cytoplasm fractions. EscC in the membrane fraction of EHEC preserved the slow SDS-PAGE migration pattern, but not so with that in the cytoplasmic fractions. To examine the compositional effect, we disrupted the bacteria physically and prepared the membrane fractions from both EHEC and K-12 strains. By adding strain-specific membrane fractions to the nickel-ion column-purified EscC, the SDS-PAGE pattern was re-examined. Increasing amounts of the membrane fraction from EHEC was found to affect the EscC migration pattern in SDS-PAGE, an observation not seen with additions of the K-12 membrane fraction. Furthermore, when the so-prepared membrane fraction from EHEC was first heated and then added to the purified EscC, the distinct SDS-PAGE migration pattern diminished. Conclusions: The membrane components of EHEC must differ from those of K-12 to some extent and there must be heat-sensitive membrane constituents/structures in EHEC preferentially interact with EscC.

Poster Session

BAM-PM1085 - OmpT mediated cleavage of the antimicrobial peptide LL-37 by uropathogenic Escherichia coli cystitis clinical isolates

Isabelle Desloges[1], Samantha Gruenheid[1], Hervé Le Moual[1]
[1]McGill University, Montreal, Canada

Uropathogenic Escherichia coli (UPEC) causes approximately 80% of community acquired urinary tract infections (UTIs), which results in diseases ranging from asymptomatic bacteriuria to urosepsis. Despite appropriate antibiotic treatment, approximately 25% of patients experience recurrent UTIs. New treatments approaches are required to reduce recurrent UTIs incidence. Antimicrobial peptides (AMPs) are components of innate immunity with both antimicrobial and immunomodulatory properties. It has been shown that human cathelicidin LL-37 protects the urinary tract against UPEC infection. Both epithelium- and neutrophil-derived LL-37 contribute to the protection of the urinary tract at different stages of infection. A number of studies have identified ompT as an important virulence gene present in 85-97% of UPEC isolates. In this study, we examined the OmpT proteolytic activity of 58 UPEC clinical isolates. Clinical isolates were divided into five groups of increasing clinical severity (fecal, asymptomatic bacteriuria, cystitis, pyelonephritis and urosepsis). In agreement with previous studies, ompT was present in 83-100% of the isolates responsible for symptomatic UTIs but present in only 65-70% of the asymptomatic isolates. All isolates were tested for OmpT activity using a Fluorescence Resonance Energy Transfer (FRET) substrate. In all groups, heterogeneity of OmpT activity was observed. Most isolates responsible for symptomatic UTIs and containing ompT exhibited increased OmpT activity compared to asymptomatic isolates. The group causing cystitis was further analyzed for LL-37 cleavage. Notably, OmpT activity correlated with the amount of OmpT protein produced by the different isolates and with the ability of the isolates to cleave human cathelicidin LL-37. However, one isolate (Cys-6) cleaved the FRET substrate but was unable to degrade LL-37. Together, these data show that OmpT protease activity is heterogeneous amongst UPEC clinical isolates. Furthermore this suggests that the ability of UPEC to cleave and inactivate LL-37 during bacterial colonization may vary depending on the isolate.

Poster Session

BAM-PM1087 - The Pseudomonas aeruginosa lectin LecB subverts Wnt signalling

Catherine Cott[1], Roland Thuenauer[1], Anne Imberty[2], Winfried Roemer[1]
[1]BIOSS Centre for Biological Signalling Studies, Freiburg, Germany, [2]CERMAV-CNRS Centre de Recherches sur les Macromolécules Végétales, Grenoble, France

Five to ten percent of all nosocomial infections in Europe and the United States are caused by Pseudomonas aeruginosa. The bacterium exhibits a large number of virulence factors to promote its pathogenicity. Among these, Pseudomonas lectins LecA and LecB are known to mediate adhesion to host tissues. In our work we investigated if LecB has functions beyond simple adhesion. For this, we analysed the influence of purified LecB on the migratory and proliferative potential of a lung epithelial cell line (H1299) using scratch assays and an EdU system, respectively. Furthermore, we investigated the impact of LecB stimulation on migration- and proliferation-related signalling pathways by polyacrylamide gel electrophoresis and Western blotting. These experiments showed that LecB strongly inhibits the migration and proliferation of H1299 cells. In addition, we observed a significant manipulation of the Wnt signalling. In particular, LecB was able to prevent nuclear translocation of ß-catenin during Wnt3a stimulation and directly influenced key player of this pathway. Pseudomonas aeruginosa has a propensity to infect and colonize wounds. Therefore, we propose the hypothesis that LecB, in addition to its function as an adhesin, facilitates Pseudomonas aeruginosa infections of injured tissue by manipulating the Wnt pathway in order to inhibit wound healing.

Poster Session

BAM-PM1091 - Human atypical enteropathogenic Escherichia coli cause type three secretion system-dependent changes in goblet cells in the rabbit ileal mucosa

Monica Vieira[1], Antonio Ferreira[2], Terezinha Knobl[2], Claudete Ferreira[2], Tania Gomes[1]
[1]Federal University of Sao Paulo, Sao Paulo, Brazil, [2]University of Sao Paulo, Sao Paulo, Brazil

Atypical Enteropathogenic Escherichia coli (atypical EPEC) are emerging pathogens that cause diarrhea in children and adults. This group of bacteria presents the locus of enterocyte effacement (LEE), responsible for the attaching and effacing lesion (AE) in enterocytes, and lacks the EAF (EPEC adherence factor) plasmid and the Shiga toxin genes. The AE lesion results from the interaction of the adhesin intimin and its translocated receptor (Tir), which is injected by a type 3 secretion system (T3SS) along with several other proteins into the host cells, subverting several of their functions. In previous studies we had demonstrated that two atypical EPEC strains isolated from diarrheic children with no other known enteropathogens had the ability of causing mucus hyper production when tested in the ligated rabbit ileal loop model. In addition, the two strains upregulated mucin production in HT29-MTX cultured cells by altering the MUC genes expression. Goblet cells synthesize and store mucins in the goblet cell theca region, which can be visualized after the PAS (periodic acid-Schiff) staining . In order to evaluate the changes in the rabbit intestinal epithelium caused by one of these atypical EPEC strains, bacterial suspensions of strain 3991-1 and its isogenic mutant deficient in the T3SS (3991-1 ΔescN) were inoculated in rabbit ileal loops. After 18 hours of interaction, we examined histological preparations of the intestinal sections that were stained with PAS, and observed a prominent increase in the intensity of the theca region in the goblet cells, which reflects an accumulation of glycoproteins. These changes were not seen in loops inoculated with the mutant nor with sterile Luria Broth. These results indicate that the atypical EPEC strain 3991-1 promotes glycoprotein accumulation in the intestinal goblet cells through a phenomenon involving the T3SS system.

Poster Session

BAM-PM1093 - Aprotinin inhibits the Citrobacter rodentium outer membrane protease CroP

John R. Brannon[1], Jenny-Lee Thomassin[1], Samantha Gruenheid[1,2], Hervé Le Moual[1,2,3]
[1]Department of Microbiology & Immunology, McGill University, Montreal, Canada, [2]Microbiome and Disease Tolerance Centre, McGill University, Montreal, Canada, [3]Faculty of Dentistry, McGill University, Montreal, Canada

The outer membrane proteases of Gram-negative bacteria play important roles during host-pathogen interactions. One such protease family is omptins, which are integral-membrane proteases involved in pathogenicity. Omptins are found mostly in pathogenic members of Enterobacteriaceae; for example, Escherichia coli (OmpT), Yersinia pestis (Pla), Salmonella enterica (PgtE), Shigella flexeneri (IcsP), and Citrobacter rodentium (CroP). Omptin substrates range from large proteins such as plasminogen to the small antimicrobial peptides (AMPs) of the innate immune system. We previously described the ability of OmpT in enterohemorrhagic, enteropathogenic, and uropathogenic E. coli to cleave the human AMP, LL-37. So far, an effective inhibitor of the omptin family remains to be identified. In this study, we characterized the ability of CroP to cleave AMPs and screened for potential inhibitors. We were able to purify native CroP to homogeneity. After the incubation of pure CroP with LL-37, analysis by mass spectroscopy revealed that CroP cleaves at dibasic motifs similar to OmpT. Purified CroP was capable of cleaving a synthetic fluorescence resonance energy transfer substrate containing the dibasic motif RK. Using this synthetic peptide and pure CroP, we were able to screen for CroP inhibitors. Interestingly, our results revealed that aprotinin was able to inhibit CroP at micromolar concentrations. As previously observed with serine proteases, our kinetics analyses revealed that aprotinin inhibited purified CroP in a competitive manner. We found CroP expressed from wild-type C. rodentium cells was inhibited by aprotinin. More importantly, aprotinin inhibited Pla of Y. pestis and to a lesser extent OmpT of enterohemorrhagic E. coli. Aprotinin is an optimal candidate for serving as the basis for new therapeutics targeting omptins.

Poster Session

BAM-PM1095 - Channel size and oligomeric structure determination of the B. burgdorferi integral outer membrane protein, P66.

Mari Bonde[1], Iván Bárcena-Uribarri[2], Ingela Nilsson[1], Linda Sandblad[1], Roland Benz[2], Sven Bergström[1]
[1]Department of Molecular Biology, Umeå University, Umeå, Sweden, [2]School of Engineering and Science, Jacobs University, Bremen, Germany

P66 is an integral outer membrane protein found in the Lyme disease spirochete Borrelia burgdorferi. The protein has a dual function in working both as an adhesin, binding to integrin and as a water filled channel called a porin. In artificial lipid bilayers the protein forms pores with an extremely high single channel conductance of 11 nS in 1M KCl. Black lipid bilayer experiments in the presence of nonelectrolytes of known hydrodynamic radii has been performed to investigate the actual diameter of the P66 channel. From these results the P66 channel was predicted to be ≤ 1.9 nm. When analyzing the complex formed by P66 with PEG 400, PEG 600 or maltohexaose, blockage of one P66 single-channel conductance unit was found to occur in about eight subconductance states. This indicates that the P66 channel could be an oligomer complex with eight individual channels. Blue Native PAGE and immunoblot analysis revealed a protein complex of approximately 460 kDa and confirmed this possible octameric organization of P66. Second dimension SDS PAGE showed that P66 is the only component of this pore-forming protein complex. Many characteristics of P66 has been possible to study by the black lipid bilayer assay and BN-PAGE but the structure of this big protein complex is still unknown. Ongoing studies therefore aim to resolve the structure by using protein purified from a P66 overexpressing B31 A3 B. burgdorferi strain for crystal structure analysis. In addition, structural studies will be attempted by visualizing purified P66 by electron microscopy.

Poster Session

BAM-PM1097 - Microbial colonization of tracheostomy tubes with emphasis on yeasts

Agnieszka Gniadek[1], Paweł Krzyściak[2], Janusz Warda[3], Dorota Ochońska[4], Tomasz Brzostek[1]
[1]Department of Medical and Environmental Nursing, Faculty of Health Sciences, Jagiellonian University Medical College, Kraków, Poland, [2]Department of Mycology, Chair of Microbiology, Jagiellonian University Medical College, Kraków, Poland, [3]Dutchmed Pl., Bydgoszcz, Poland, [4]Department of Epidemiology of Infection, Chair of Microbiology, Jagiellonian University Medical College, Kraków, Poland

Microbial colonisation of biomaterials implanted in the body of immunocompromised patients differs as far as its range is concerned. The research was aimed at evaluation of microbial colonisation, especially by yeast-like fungi, of tracheostomy tubes collected from patients diagnosed with larynx cancer. The object of the research were tracheostomy tubes collected within the period of 8 months in 2012/2013 from 21 patients diagnosed with larynx cancer and hospitalised in the Department and Clinic of Otolaryngology, Jagiellonian University Medical College in Cracow. The total of 33 tracheostomy tubes was made up of 21 Portex tubes and 12 metal ones. The identification of microbes detected on the tubes was carried out following the routine rules of microbiological diagnostic. The biofilm formation of yeasts was evaluated by crystal violet staining after incubation on microtiter plates. The cultures grown from the tubes included 30 bacterial isolates belonging to 6 genera and 26 fungal isolates belonging to 3 genera. The most frequent bacterial isolates were Staphylococcus epidermidis and Streptococcus viridians, whereas Candida albicans was the most frequent fungal isolate. As far as fungal isolates were concerned no difference has been observed between the two types of tubes ($\chi 2$ test, $p=0.4879$). All non-albicans Candida produced a big quantity of biofilm (OD = 3.00 to 4.00), whereas in case of C. albicans strains the production of biofilm was moderate (OD = 0.87 to 1.5). There has been no difference in the production of biofilm by C. albicans strains between the two types of tubes plastic and aluminium ones (U-Mann Whitney test, $p=0.8904$). Tracheostomy tubes, regardless the material they are produced of, are usually colonised by yeast-like fungi belonging to Candida species. Candida non-albicans produce more biofilm than fungi belonging to C. albicans species.

Poster Session

BAM-PM1099 - Applications of a murine model of Shigella flexneri infection

Angela Daurie[1], Lei Zhu[2], Samantha Gruenheid[2], John Rohde[1]
[1]Dalhousie, Halifax, Canada, [2]McGill, Montreal, Canada

Shigella flexineri is the causative agent of dysentery that kills thousands of people each year. It mainly affects children in the developing world where there is poor sanitation and lack of access to clean drinking water. An oral murine infection model, that uses BALB/c mice treated with the broad-spectrum antibiotic streptomycin, has been established and displays several hallmarks of Shigella infection in humans. Importantly, the streptomycin treated model of infection using wild-type Shigella does not result in mortality. We have used this model to investigate the role of several mutant Shigella strains during infection. We have identified a Shigella mutant (ΔospG) that causes 30% mortality in BALB/c mice. The mechanism behind this increase in mortality is under investigation. We have also infected several different strains of mice with wild-type Shigella. We find that infecting with Shigella results in a range of disease, from severe with incidence of mortality to colonization with no signs of clinical illness. We have identified a susceptible strain of mouse that has increased mortality compared to the published BALB/c model, providing a possible system to identify genes that control susceptibility to infection. In conclusion, the streptomycin treated mouse model of shigellosis provides an opportunity to understand the genetic basis of Shigella pathogenesis in the bacteria and the host.

Poster Session

BAM-PM1101 - H. pylori-positive patients colonized by vacA d1 strains are at increased risk of gastric cancer and duodenal ulcer

Dulciene Queiroz[1], Adriana Gomes[1], Charles Anacleto[1], Rafael Silva[1], Sérgio Batista[1], Sílvia Giannattasio[1], Anderson Rocha[1], Andreia Rocha[1], Gifone Rocha[1]

[1]Laboratory of Research in Bacteriology, Faculdade de Medicina, UFMG, Belo Horizonte, Brazil

Helicobacter pylori is a risk factor for duodenal ulcer (DU) and distal gastric cancer (GC). Vacuolating cytotoxin (VacA), one of the major H. pylori virulence factors, is encoded by the vacA, a polymorphic gene with variations in the signal (s1/s2), intermediate (i1/i2) and middle (m1/m2) regions of the gene. Recently, a new polymorphism, located downstream i region, characterized by an 81-bp deletion (d2) or without the deletion (d1) was described and was seen to be, amongst the vacA polymorphisms, the best marker of pre-malignant gastric lesions (Yamaoka et al, 2009). The d1 sequence consists of STTSQ motif followed by an almost perfect repetitive motif (AKNDKNES), where is the cleavage site that results in p33 and p55 peptides and VacA activation. Because, we are unaware of studies evaluating the vacA d polymorphism as a risk for H. pylori-associated diseases and the vacA d pattern of our strains, we aimed to sequence the vacA d and i regions of H. pylori strains from patients with GC (n=90), DU (n=89) and chronic gastritis (CG) (n=172). Mixed infection (n=10) was not included in the analyses. Most of our strains were d1, but differently from that observed by Yamaoka, most of them (60.1%-205/341) had only one AKNDKNES motif (two repetitive motifs were seen in 41 cases, most of them in GC (P=0.005). We also observed a new d1 variant in 11 samples. 97.7%(333/241) of the vacA i1 and i2 variants were also d1 and d2, respectively ($p<0.001$). The d1 and i1 genotypes were more frequently found ($p<0.001$) in DU (89.5% and 88.4%, respectively) and GC (91.8% and 88.3%, respectively) than in CG (60.7% and 61.4%, respectively). In logistic models adjusting for age and gender, both d1 and i1 remained associated with GC (d1,OR=4.14, 95%CI=1.95-8.81 and i1,OR=3.33, 95%CI=1.84-6.33) and DU (d1,OR=4.66; 95%CI=2.12-10.21 and i1,OR=1.03, 95%CI=1.01-1.04).CNPq/FAPEMIG.

Poster Session

BAM-PM1103 - Periodontitis mainly increases osteoclast formation via enhancing the differentiation of quiescent osteoclast precursors into osteoclasts without proliferation

Dong-Eun Lee[1], Ji-Hye Kim[2], Jeong-Heon Cha[3], Eun-Jung Bak[4], Yun-Jung Yoo[5]
[1]Yonsei University College of Dentistry, Seoul, Korea

Periodontitis is inflammatory disease caused by bacteria and characterized by both inflammation and bone loss and stimulate the osteoclastogenesis. Tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoclasts are formed in sequential steps: proliferation and differentiation of hematopoietic progenitors into quiescent osteoclast precursors (QOPs), followed by fusion of QOPs. In this study, we investigated whether enhancement of osteoclast formation by periodontitis is derived from the stimulation of hematopoietic progenitor proliferation or from the fusion of QOPs. Ligatures were placed around the first molars in the left mandibles of rats. The rats received drinking water containing bromodeoxyuridine (BrdU) after ligation. The number of inflammatory cells in the distal area was counted. Alveolar bone loss was histologically estimated by measuring the distance from the cementoenamel junction to the alveolar bone crest in the distal area and determining the percentage of periodontal ligament area in the furcation. The number of osteoclasts and the percentage of BrdU+ osteoclast nuclei were counted after TRAP and BrdU double labeling. The number of polymorphonuclear cells increased at day 1, then rapidly decreased. The number of mononuclear cells increased in a time-dependent manner up to day 3 and remained until day 10. Alveolar bone loss of increased in a time-dependent manner. The number of osteoclasts peaked at day 3 then gradually decreased. At peak, the BrdU+ osteoclast nuclei percentages were 10.6% in both area. These results indicate that most of the osteoclasts formed after periodontitis induction are derived from preformed QOPs, suggesting that enhancement of osteoclast formation by periodontitis might be mainly caused by the stimulation of QOPs fusion. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2012R1A1A2041214). The authors have declared that there are no conflicts of interest.

Poster Session

BAM-PM1105 - Phenotypic characterization of *Salmonella enteric* Typhimurium mutant strains to genes *ihfA* or *ihfB* responsible to encode IHF (*Integration host factor*)

Meiriele Neves[1], Guilherme Mendes[1], Aline Oliveira[1], Gerson Nakasato[2], Marina Gilioli[1], Camila Carmo[1], Marcelo Brocchi[1]

[1]University of Campinas, Campinas, Brazil, [2]Londrina State University, Londrina, Brazil

Salmonella enterica, a facultative anaerobic bacterium, is a common cause of acute food-borne diseases worldwide, causing self-limited gastroenteritis and/or systemic infections. It is estimated that *S. enterica* isolates causes 93.8 million human infections and 155,000 deaths annually worldwide. To minimize the impact of salmonelosis, it is important to monitor this bacteria distribution in many countries and implement control measures throughout the food production chain. Therefore, the deep characterization of the *S. enterica* virulence factors and how they are regulated is mandatory to improve the control of this pathogen. Recent studies of our group have indicated that null mutants of *S. enterica* to *ihfA* or *ihfB*, genes responsible to encode subunits A and B of IHF (*Integration host factor*), respectively, show virulence attenuation. Based on these results, the objective of this work was to investigate the biological role of IHF in *S. enterica* Typhimurium using phenotypic assays such as growth *in vitro*, motility and survival in the presence of 0,85% bile salts. The results indicated that absence of *ihfA* or *ihfB* did not affect the rate of bacterial growth. Was also observed that WT (wild type) and mutant strains showed the same growth rates in LB medium or LB medium with 0.85% bile salts. However, in the motility assay, differences in bacterial motility were observed in LB (0.35%) agar plates. The mutant strains exhibited a lower motility when compared to the WT strains, suggesting an effect in flagellar biogenesis and/or chemotaxis. These results suggested that attenuation of *ΔihfA* or *ΔihfB* mutants could be explained in part by low motility. However, growth in vitro and resistance to bile salts were not affected. In fact, It is important to bacteria preserve these characteristics, since they are necessary attributes to host infections. Additional studies are necessary to further evaluate *S. enterica* Typhimurium *Δihf* phenotypes.

Poster Session

BAM-PM1107 - Effect of Molybdenum on Nodulation, Nitrogenase Activity, Nitrate Reductase Activity and Rhizobial Diversity of Hairy Vetch (*Vicia villosa* Roth) in Soil

Faridul Alam[1], Tae Young Kim[1], Pil Joo Kim[1], Yong Bok Lee[2]

[1]*Division of Applied Life Science (BK21 Plus), Gyeongsang National University, South Korea.*, [2]*Research Institute of Life Science, Gyeongsang National University, South Korea.*

Hairy vetch (*Vicia villosa* Roth) is a leguminous cover crop used with barley as green manure, the most popular practice in South Korea for reducing use of chemical fertilizer and maintaining soil fertility. In root nodules of Hairy vetch (HV), Rhizobia (R) bacteria require Molybdenum (Mo) to fix atmospheric or molecular nitrogen (N). The symbiotic association (R–HV) induces important physiological changes in each partner that lead to reciprocal benefits, mainly in nutrient supply like improved N_2fixation. A pot experiment was conducted in green house of College of Agriculture and Life Science, Gyeongsang National University, South Korea in 2012 to study the effect of Mo on nodulation, nitrogenase and nitrate reductase (NR) enzyme activity that influence on N_2fixation and biomass production. For this study, Mo was applied 0, 0.25, 0.5, 1.0, 2.0 kg ha^{-1} before seed sowing and native soil rhizobia performed nodulation. To know the effective N_2fixation, nitrogenase activity was measured by acetylene reduction assay of HV root. Mo application 1kg ha^{-1} produced highest plant nodule number (60 nodule $plant^{-1}$) nodule weight (52.3 mg $plant^{-1}$), root biomass (0.35 g $plant^{-1}$) and above ground biomass (5 g $plant^{-1}$), nitrogenase activity (1797.5 µmol $C_2H_4$$plant^{-1}$ $hour^{-1}$) and NR activity (2.11 µmol NO_2^- g^{-1} fresh wt hr^{-1} in leaf, 1.80 µmol NO_2^- g^{-1} fresh wt hr^{-1} in root) compared to native control soil. Immunofluorescence microscopy results indicated that cell growth and functions of nodules were optimum in 1 kg Mo ha^{-1} applied plants. Rhizobial 16S rRNA gene cloning and sequencing results indicated that a wide range of rhizobia bacteria belongs to different clusters that regulate in N_2fixation. Therefore, optimum Mo application (1kg ha^{-1}) was probably the best appropriate dose which showed better performance than that of native soil in respect of nodulation, nitrogen fixation and biomass production of Hairy vetch.

Poster Session

BAM-PM1109 - WITHDRAWN - Functional characterization of non-rhizobial bacteria isolated from root nodules of clitoria ternatea linn. (aparajita)

Abhinav Aeron[1,2], Dinesh Kumar Maheshwari[2]
[1]DAV (PG) College, Muzaffarnagar, India, [2]Department of Botany and Microbiology, Faculty of Life Sciences, Gurukula Kangri University, Haridwar, India

Bacteria belonging to class β, γ proteobacteria and Sphingobacteria, eight species and five genera including Enterobacter turicensis RCT5 (HM805112), E. cloacae RCT8 (HM805113), E. hormaechei RCT10 (HM771693), Pseudomonas geniculata RCT2 (HM805109), P. alcaliphila RCT11 (HM805114), Achromobacter xylosoxidans RCT3 (HM771692), A. xylosoxidans RCT4 (HM805110), A. xylosoxidans RCT7 (HM805111), Strenotrophomonas maltophila RCT30 (HM805115), S. maltophila RCT31 (HM771691), Sphingobacterium thalpophilum RCT1 (HM771694) were identified on the basis of partial 16S rRNA sequencing from root nodules of a multipurpose legume C. ternatea L. These were further analysed for various functional attributes such as solubilization of inorganic phosphate, organic phosphate, zinc and potassium, production of phytohormones and siderophores. Their ability to antagonize pathogenic fungi such as Macrophomina phaseolina, Fusarium udum, F. oxysporum, F. solani, Colletotrichum spp., Rhizoctania solani, Sclerotinia sclerotiorum was studied in dual culture, cell free culture filtrate and volatiles along with the factors that may be responsible for in vivo biocontrol such as lytic enzymes chitinase, glucanases, proteases, lipases, cellulases apart from degradation of oxalic acid and fusaric acid. These non-nodulating isolates showed exciting plant growth promoting characters of varying degree. E. turicensis RCT5 was foremost in all isolates able to solubilise tri-calcium phosphate, di-calcium phosphate and zinc phosphate from Pikovskaya`s agar, confirmed further by spotting it on NBRIP media and Rhizospheric P solubilisation media with three inorganic P substrates. E. turicensis RCT5 and S. maltophila RCT31 showed a zone of solubilization of potassium in Aleksandrov`s medium supplemented with potassiumaluminiumsilicate and exhibited a strong halo on Tris-Minimal media supplemented with Zinc phosphate but failed to solubilize other zinc salts. Production of β-1, 3 and β-1, 4 glucanases, Siderophore, IAA was evident all nodule isolates but none produced chitinase and protease. Antagonism varied from strong to average to weak and was strongest in S. maltophila RCT31 which inhibited all pathogenic fungi.

Poster Session

BAM-PM1111 - Evaluation of rhizobacteria for biological control of replant disease pathogens

Tristan Watson[1], Louise Nelson[1]
[1]*The University of British Columbia Okanagan Campus, Kelowna, Canada*

Alternative management strategies to the use of broad spectrum chemical fumigants have the capacity to lessen the environmental risks associated with commercial tree fruit production. There is currently a lack of alternatives for effective control of replant disease, a problem associated with decreased plant growth of newly planted fruit varieties on sites previously used for tree fruit production. In this study, rhizobacteria isolated from pea, lentil, and chickpea were evaluated for their ability to control fungal pathogens associated with replant disease. Twenty-six bacterial isolates were screened for suppression of Cylindrocarpon CYL1, Fusarium FUS2, Rhizoctonia RHZ1, and Phytophthora 797 using a dual culture inhibition assay. Pseudomonas fluorescens 2-28 and Pantoea agglomerans 6-20 suppressed all 4 fungal pathogens in vitro and were selected for greenhouse trials using 'Ambrosia' apple seedlings to evaluate the extent of biocontrol and root colonization. Only Cylindrocarpon CYL1 and Phytophthora 797 were found to significantly decrease shoot and root dry weight of apple seedlings. Both P. fluorescens 2-28 and P. agglomerans 6-20 significantly increased shoot and root dry weight of apple seedlings grown in soil inoculated with Cylindrocarpon CYL1, however neither isolate provided effective plant growth promotion of seedlings grown in soil inoculated with Phytophthora 797. P. fluorescens 2-28 was observed to significantly decrease root dry weight in comparison to control seedlings that did not receive any inoculation. Colonization experiments showed that P. fluorescens 2-28 and P. agglomerans 6-20 were present on the root system of seedlings at concentrations of 5 log CFU/g and 3 log CFU/g, respectively, 6 weeks after inoculation. Overall, P. agglomerans 6-20 shows the most potential for further development as a part of a combined alternative management strategy to mitigate the impact of replant disease.

Poster Session

BAM-PM1113 - Actinorhizal and mycorrhizal symbioses in alder shrubs exposed to fine tailings from the gold mining industry

Marie-Michelle Corbeil[1], Damase Khasa[2], Charles Greer[3], Sébastien Roy[1]
[1]Université de Sherbrooke, Sherbrooke, Canada, [2]Centre d'étude de la forêt et Institut de biologie intégrative et des systèmes, Ste-Foy, Canada, [3]National Research Council Canada-Energy, Mining and Environment, Montreal, Canada

In gold mines, most waste is in the form of a slurry left to sediment in vast areas termed tailings ponds. To reduce the environmental footprint of mining operations, a common practice is to attempt revegetation. Alder shrubs are good candidate species as they form nitrogen-fixing symbioses with *Frankia*, as well as with arbuscular and ecto-mycorrhizae. These associations help alders establish in nutrient-poor conditions. To enhance revegetation field trials on the Sigma mine site in Val-d'Or (Quebec), we performed an experiment in sterile conditions to study the effect of each symbiont on alder survival and development when exposed to different concentrations of tailings. Axenic speckled alders and green alders were inoculated with combinations of *Frankia* sp.,
Rhizophagus irregulare, and/or *Paxillus involutus* in order to characterize the impact of tailings on the development of each symbiosis. Plant survival rates, biomass, symbiosis establishment and extent, as well as nitrogen-fixing activities were measured. Following a 5-month growth trial, results showed that both speckled alder and green alder can survive in the presence of such tailings. *Rhizophagus irregulare* and *Paxillus involutus* had little or no impact on plants exposed to tailings, and we observed that *Frankia* could establish symbiosis in a mix of sand and tailings but was inhibited in pure tailings. In pure tailings, survival of green alder was better than that of speckled alder. The actinorhizal symbiosis enhanced biomass production in alder in the presence of tailings, and this response was best in green alder. These observations are important to tailor revegetation techniques to specific sites, and they confirm that the actinorhizal symbiosis can improve the development of alders colonizing mining wastes. Additional aerial biomass is a key element for the rehabilitation of these sites since nitrogen-rich leaf litter is the main route of soil conditioning by these early successional species.

Poster Session

BAM-PM1115 - Characterization of microbial communities associated with wetland plants along a metal contamination gradient around Sudbury, Ontario

Cagdas Kera Yucel[1], Peter Ryser[1], Nadia C.S. Mykytczuk[1]
[1]Laurentian University, Sudbury, Canada

The abundance and diversity of soil microorganisms is an important component of freshwater wetlands. Wetlands represent a transition from terrestrial to aquatic ecosystems and their microbial community influences valuable internal processes such as the cycling of nutrients and carbon. It is therefore critical that we understand how rhizosphere communities are influenced by pollution, and what effects this might have on wetlands on recovering lakes. It is important to understand how the microbial community varies between plant species and between sites. This study aims to explore differences in species composition and diversity of microbial communities associated with wetland plant rhizospheres along a gradient of environmental disturbance around Sudbury. Characterization of the microbial community included 16S/18S rRNA pyrosequencing to identify microbial diversity, and enzymatic analyses (hydrolases and lignases) to characterize microbial community function. Microbial communities were compared within the rhizobiome of two wetland plants, Chamaedaphne calyculata and Eriophorum vaginatum, across four sites (3 with medium-to-high Ni/Cu contamination and one low), which show a range of environmental disturbance including metal contamination and acidity relative to distance from industrial smelters. 16S/18S rRNA pyrosequencing results showed that the most dominant fungal genus was Oidiodendron (7.1 - 52.9 % of sequence reads), and Acidobacteriaceae (9.0 - 46.9 % of sequence reads) was the most common bacterial family. Some genera were only found in intermediate-high contaminated sites. These included the fungal genera Phialocephala, Sebacina and Gregarine, and the bacterial genus Afipia and unknown taxa belonging to the Nitrosomonadaceae, and Hyphomonadaceae. Understanding specific plant-rhizobiome trends will also shed light on factors contributing to plant community survival and productivity in metal contaminated sites. Any findings that suggest the microbial community displays specificity to plant species will expand our understanding of plant-microbe interactions and will aid in determining their importance and functional role in wetland plant ecological strategies.

Poster Session

BAM-PM1117 - Three distinct dihydrolipoamide dehydrogenases (LPDs) in Sinorhizobium meliloti

Martina Solano Bielen[1], David Meek[1], Brian Driscoll[1]
[1]McGill University, Sainte-Anne-de-Bellevue, Canada

Sinorhizobium meliloti has three genes with high homology to the Escherichia coli lpdA gene. The E. coli lpdA gene encodes the E3 subunit of three multi-enzyme complexes; pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase (OGD) and a branched-chained α-keto acid dehydrogenase (BKD). In S. meliloti, the lpdA1, lpdA2 and lpdA3 are proximal to the genes encoding for the E1 and E2 subunits of the PDH, OGD and BKD complexes, respectively. In our lab the lpdA1 and lpdA2 genes are of special interest due to their association with the TCA cycle, which generates energy to support symbiotic N2-fixation within root nodules of host plants such as Medicago sativa (alfalfa). To further our studies of these genes and to determine if the E3 subunits from these complexes are interchangeable, lpdA1 (Rm30368) and lpdA2 (Rm30360) mutants were isolated. Rm30368 was unable to grow in a defined medium with glucose as sole carbon source, while Rm30360 was unable to grow with glutamate as sole carbon source. Enzyme assays revealed significant decreases of PDH activity only in extracts of Rm30368, and of OGD activity only in extracts of Rm30360. Alfalfa plants grown on nitrogen-free substrate inoculated with either Rm30368 or Rm30360 were small and chlorotic, with small white pseudonodules, indicative of the failure of these cells to fix N2 during symbiosis. These results indicate that lpdA1 and lpdA2 are specific for PDH and OGD, respectively, and the necessity of a completely functional TCA cycle during symbiotic N2-fixation.

Poster Session

BAM-PM1119 - Metaorganism synergy: Salix purpurea-microbe partnerships leading to petroleum hydrocarbon bioremediation in soils

Antoine Pagé[1], Etienne Yergeau[1], Charles Greer[1]
[1]*National Research Council Canada, Montreal, Canada*

Millennia of co-evolution have united many microorganisms with plants into mutualistic super entities, or metaorganisms. As they collaboratively carry key biogeochemical processes, these consortia possess a large potential for the development of applications enabling improved natural resource management. When used in bioremediation, plant metaorganisms can considerably speed up the recovery of soils contaminated by industrial activity. This way, Yergeau et al., (2013) demonstrated that Salix purpurea fish creek significantly stimulates microbial genetic potential associated with the degradation of petroleum hydrocarbons (PHC) in its own rhizosphere. To specifically determine which PHC degradation pathways of which microorganisms are stimulated, we searched metatranscriptomic datasets for a series of functional genes, and assigned taxonomy to identified transcripts. Statistical analyses of normalized gene counts confirmed that the expression levels of various alkane, aromatic, and polyaromatic degradation pathways are higher in the rhizosphere of S. purpurea than in bulk contaminated soil. Several bacterial groups appear as the most responsive to the hypothesized plant cues that generated this microbial response (e.g. Burkholderiales, Caulobacterales, Actinomycetales, Xanthomonadales). The latter results provide early targets for work aimed at optimizing plant-microbe partnerships for bioremediation applications using Salix spp.
Reference cited: Yergeau E, Sanschagrin S, Maynard C, St-Arnaud M, Greer CW (2014) Microbial expression profiles in the rhizosphere of willows depend on soil contamination. ISME Journal 8:344-358.

Poster Session

BAM-PM1121 - Transcriptomic analysis of Sinorhizobium meliloti 1021 focussing on TCA cycle, nitrogen fixation, and carbon metabolism pathways

Patricia Goerner-Potvin[1], Brian Driscoll[1]
[1]*McGill University, Sainte-Anne-de-Bellevue, Canada*

Sinorhizobium meliloti 1021 is a nitrogen-fixing symbiont of legume plants Medicago, Melilotus and Trigonella. This bacterium is used as a model organism for tricarboxylic acid (TCA) cycle, and other genetic studies. In efforts to understand genome implications for symbiotic association with Medicago sativa, the complete S. meliloti 1021 bacteroid transcriptome was sequenced and was compared to the complete sequenced transcriptome of free-living cells grown on malate a as sole carbon source. A total of 1514 genes were downregulated a minimum of 2 folds, of which twenty-seven were downregulated more than 20 folds when compared to the control treatment malate. Most of these downregulated genes were of unknown function or part of the carbohydrate transport and metabolism COG group. A majority of the glycolysis pathway genes were also downregulated in bacteroids. A total of 1790 genes were upregulated a minimum of 2 folds. Genes involved in the TCA cycle were mostly upregulated in bacteroids, including a 10-fold transcription increase of the mdh-sucCDAB operon. Nitrogen fixation nif and fix genes underwent the highest transcription FC increase with values reaching 3000 folds. qRT-PCR experiments were also conducted on the mdh-sucCDAB operon in various carbon treatments, in addition to complete transcriptome sequencing of those carbon treatments.

Poster Session

BAM-PM1123 - Carotenoid biosynthesis and its regulation of Pantoea ananatis PA13, causing sheath rot and grain discoloration of rice

Okhee Choi[1], Yongsang Lee[1], Inyoung Han[1], Okyon Choi[2], Jinwoo Kim[1,2]
[1]Division of Applied Life Science (BK21Plus), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea

Carotenoids are yellow, red, and purple pigments widely distributed in plant and animal tissues. Carotenoids can be produced by plants, algae, some bacteria, and some fungi, but generally cannot be synthesized by animal so animals obtain carotenoids in their diets. Carotenoids are efficient free radical scavengers, and they enhance the vertebrate immune system. In some pathogenic bacteria, carotenoid is a virulence factor with an anti-oxidant action that helps the bacteria evade death by reactive oxygen attack. Pantoea ananatis strain PA13, the causal agent of sheath rot and grain discoloration of rice, produces yellow carotenoid pigments. Genome sequences revealed that strain PA13 harbors carotenoid biosynthetic genes, consisting of crtE, crtX, crtY, crtI, crtB, and crtZ. Reverse transcription polymerase chain reaction (RT-PCR) analysis revealed crtEXYIB genes are polycistronic and make an operon. We mutagenized the crtEXYIB genes of strain PA13 by gene replacement. The mutants no longer produced yellow pigments. Using mariner-based transposon mutagenesis, we also screened non-pigmented mutants of strain PA13. The transposon insertion sites were mainly mapped to the crtEXYIB and crtZ genes. We found that EanI/EanR quorum sensing (QS) system mediated by N-acyl-L-homoserine lactones (AHL) controls carotenoid pigmentation. EanI mutant no longer produced detectable levels of the QS signal molecules, leading to a loss of carotenoid production, which was restored by addition of N-(3-oxohexanoyl)-L-homoserine lactone or by complementation with eanI. In addition, we also found that the stationary-phase sigma factor RpoS is responsible for the regulation of carotenoid production.

Poster Session

BAM-PM1125 - Quorum sensing does not regulate in planta biofilm formation by Pectobacterium carotovorum subsp brasiliense (Pcb)

Lucy Moleleki[1,2], Rudi Pretorius[1,2], Gabolwelwe Mosina[1,2], Collins Tanui[1,2], Jacques Theron[1]
[1]Forestry, Biotechnology and Agriculture Institute, University of Pretoria, Pretoria, South Africa, [2]Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa

In a previous study, we showed that Pectobacterium carotovorum subsp brasiliense (Pcb) 1692 colonises susceptible potato plant stems by forming 'biofim-like' aggregates on xylem tissues. Over time, these aggregates tend to occlude the xylem leading to wilting of infected potato plant stems. We hypothesised that quorum sensing, as an important global regulator of virulence, is involved in in planta Pcb biofilm formation. To test this hypothesis, a quorum sensing defective mutant strain, PcbΔexpI-, was generated. As expected, the PcbΔexpI- mutant strain was impaired in the production of acyl homoserine lactones (AHL) compared to the wild type (WT) strain. In vitro and in planta growth analysis indicated that there was no significant difference in the growth rate of the mutant compared to the WT strain. Like other Pectobacterium spp quorum sensing-defective mutants, the PcbΔexpI- strain had reduced production of major plant cell wall degrading enzymes (PCWDE), subsequently, it was impaired in virulence on potato tubers and stems. Surprisingly, the ability of PcbΔexpI-_gfp mutant to colonise and form biofilm-like aggregates on potato plant stems did not differ significantly from that observed for the wild type WTPcb_gfp strain. The Tad/Flp-pilus was recently shown to be present in many phytopathogenic bacteria and it is believed that it plays an important role in virulence, possibly through formation of biofilms. Furthermore, it has been suggested that the Tad/Flp-pili of Pectobacterium spp are regulated by a novel regulator rather than the global regulator expI. Hence, we used a combination of transcription profiling and Transmission Electron Microscopy (TEM) to investigate the expression and presence of the Tad pilus in the mutant vs wild type strains under different growth conditions. Our results revealed the presence of both flagella and pili in the wild type while the mutant strain did not have any visible flagella but pili could be clearly visualised.

Poster Session

BAM-PM1127 - The role of polysaccharides in pH tolerance in Sinorhizobium meliloti

Justin Hawkins[1], Barney Geddes[2], Ivan Oresnik[1]
[1]University of Manitoba, Winnipeg, Canada, [2]University of Oxford, Oxford, UK

Sinorhizobium meliloti is a Gram-negative α-proteobacteria capable of entering into a symbiotic relationship with *Medicago sativa*. The establishment of symbiosis by *S. meliloti* is dependent on the production of low molecular weight (LMW) fractions of the exopolysaccharides (EPS) succinoglycan (EPS-I) or galactoglucan (EPS-II).
S. meliloti also produces two other symbiotically relevant polysaccharides; capsular polysaccharides (KPS) and cyclic β (1-2) glucans. Tolerance to high saline concentrations has been shown to be correlated with increased EPS production. In addition pH has also been observed to play a role in EPS production. Previous work has shown that the *exo* gene cluster involved in synthesis of EPS-I is up-regulated during growth in acidic conditions, though a direct role of EPS for pH tolerance has not been demonstrated. Mutants defective in EPS-I production were investigated for their ability to tolerate acidic conditions. We have shown strains carrying a mutation abolishing EPS-I (*exoY*) production are slightly sensitive to acidic pH. Interestingly, a mutation allowing only high molecular weight (HMW) EPS-I production shows dramatic sensitivity to acidic conditions. Consistent with a role for LMW EPS-I in tolerating acidic conditions, an endoglycanase responsible for the synthesis of LMW EPS-I shows increased expression under acidic conditions. Based on the slight sensitivity of the *exoY* mutant to acidity, it was hypothesized that other polysaccharides may be compensating for the lack of EPS-I production. Therefore, the role of KPS and cyclic β (1-2) glucans was also investigated. Production of cyclic β (1-2) glucans was not influenced by the loss of EPS-I, however its production was decreased in acidic media. The production of KPS was observed to be increased in the absence of EPS-I. Taken together, this data suggests a potential role for LMW EPS-I and KPS in tolerating acidic conditions.

Poster Session

BAM-PM1129 - Proteomics of the secretome of Streptomyces albus

Fatin Alsalmi[1], Louai Ajab Noor[1], Zaenab Aljassim[1], Mazen Saleh[1]
[1]Laurentian University, Sudbury, Canada

Most known species of Streptomyces are saprophytes but a few are recognized as pathogens. The information available on the pathogenesis of these species is scarce. Due to the phylogenetic relationship between Streptomyces and Mycobacteria, researchers are currently investigating the extent of this relationship and ask whether or not the relationship extends beyond phylogeny. This interest is further driven by the potential use of Streptomyces as hosts for heterologous expression of Mycobacterial proteins and the premise that Streptomyces could be used as a vaccine against mycobacterial infections, particularly Tuberculosis. Our laboratory has been characterizing the physiological relationship between these related pathogens using enzymatic and immunological markers. Our approach combines biochemical assays and proteomics to characterize the secretome of a clinical isolate related to Streptomyces albus and comparing it to that of Mycobcterium bovis BCG. Our data shows that indeed both share a number of secreted enzymes, including those implicated in playing a role in the virulence of Mycobacteria.

Poster Session

BAM-PM1131 - Biological and immunological characterizations of an novel vaccine candidate against C. difficile

Pele Chong[1], Jui-Hsin Huang[1], Zhe-Qing Shen[1], Shu-Pei Lien[1], Kuang-Nan Hsiao Hsiao[1], Chi-Chang Chen[1]
[1]Vaccine R&D Center, National Health Research Institutes, Zhunan Town, Taiwan

Clostridium difficile (Cd) is an emerging pathogen of opportunistic infection in hospitals worldwide and the main causative of antibiotic-associated pseudo- membranous colitis and diarrhea in human. The hospitalized patients treated with antibiotics have high risk of Cd infection (CDI), especially in children and elderly above 65 years old. Because of the intoxication of two large clostridial toxins, toxin A (tcdA) and toxin B (tcdB), that work synergistically. The beginning of CDI, the clostridial toxins specifically bind to unknown glycoprotein(s) on the surface of epithelial cells in the host intestine and then quickly enter into the cell through the receptor-mediated endocytosis. Disrupted intestinal barrier will ultimately lead to acute inflammation and diarrhea. Interestingly, the C-terminal receptor binding domain (RBD), responsible for initially binding to the host glycoproteins, is predicted and identified to have 35-38 homological short-term repetitive peptides which may involve in 7 potential receptor-binding sites. The specific roles and functions of these 7 putative binding regions are unclear. In this study, we designed three fragments which were located on N-terminal, middle, and C-terminal parts of tcdA. The results indicated that three regions possessed variable biological and immunological functions. The immunological activities are deeply correlated with their biological function. The N-terminal F1 and C-terminal F3 are very potent and can serve as Cd vaccine components.

Poster Session

BAM-PM1133 - Protective immune response induced by immunisation with pGP3 and pGP4 chlamydial plasmid proteins is mediated by CD4+ cells

Tímea Mosolygó[1], Ildikó Faludi[1], Emese P. Balogh[1], Ágnes M. Szabó[1], Dezső P. Virók[2], Valéria Endrész[1], Katalin Burián[1]

[1]Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary, [2]Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary

Urogenital tract infection with Chlamydia trachomatis is a leading cause of sexually transmitted infections. Although antibiotics are effective in treating chlamydial infections, due to the lack of obvious symptoms, many infections remain untreated, which potentially leads to complications. The most effective means to prevent C.trachomatis-induced complications would be vaccination. The highly conserved plasmid of Chlamydiae has been considered a virulence factor and the plasmid proteins have important role in the Chlamydia-specific immune response. This study aimed to evaluate the efficacy of vaccination with plasmid proteins in the prevention of Chlamydia muridarum lung infection using a mouse model. C57BL/6N mice were immunised with recombinant pGP3 or pGP4 proteins and infected with C.muridarum. The number of recoverable C.muridarum was detected with indirect immunofluorescence test. To evaluate the inflammation, cytokines were measured in the lungs of mice by ELISA. Spleen cells from immunised mice were depleted of CD4+ and CD8+ cells and administrated to naive mice. After C.muridarum infection the bacterial burden was determined. Naive mice were infected with C.muridarum, treated with sera of immunised mice in vitro or were treated in vivo with the immune sera during C.muridarum infection. Immunisation of the mice with pGP3 or pGP4 protein resulted in a significantly lower chlamydial burden, the lower IFN-g level indicated reduced inflammation in the lungs. In vitro or in vivo neutralisation of C.muridarum with sera obtained from immunised mice did not reduce the number of viable C.muridarum in the lungs of mice. Adoptive transfer of the CD4+ spleen cells isolated from the immunised mice resulted in significantly reduced bacterial burden. On the basis of our results, not the pGP3- and pGP4-specific antibodies, but the CD4+ cells are responsible for the protective effect of the immune response to plasmid proteins. This work was supported by OTKA National Research Fund PD100442, TÁMOP-4.2.2.A-11-1-KONV-2012-0035

Poster Session

BAM-PM1135 - Genetically engineered Yersinia pseudotuberculosis enhances immunogenicity against pneumonic plague

Wei Sun[1], Shilpa Sanapala[1], Roy Curtiss 3rd[1]
[1]*Arizona State University, Tempe, USA*

We constructed a Y. pseudotuberculosis mutant strain with yopK and yopJ double deletions to attenuate it. Then, we inserted the asd mutation into this construction to form χ10069 [Δasd-206 pYV-ψ2 (ΔyopJ315ΔyopK108)] for adapting with a balanced-lethal Asd+ plasmid to facilitate antigen synthesis. A hybrid protein composed of YopE (1-138aa) fused with full-length LcrV (YopENt138-LcrV) was synthesized in χ10069 harboring an Asd+ plasmid (pYA5199, yopENt138-lcrV) and could be secreted through type III secretion system (T3SS) in vitro and vivo. Challenge studies indicated that mice orally immunized with χ10069(pYA5199) strain were afforded most complete protection against with high dose of Y. pestis KIM6+ (pCD1Ap) (~500 LD50, 5.0 x 104 CFU) by intranasal challenge. Very few of mice immunized with χ10057(pYA3332, empty plasmid) and none of mice immunized with BSG were protected. Currently, we are measuring humoral and cellular immune responses primed in mice immunized with different strains. Our preliminary results suggest it is possible to use an attenuated Y. pseudotuberculosis strain delivering the LcrV antigen via T3SS as a potential live vaccine candidate against pneumonic plague.

Poster Session

BAM-PM1137 - Sublingual administration a P1 protein-based vaccine formulation reduces *in vivo Streptococcus mutans* oral colonization

Milene Tavares Batista[1], Ewerton Lucena Ferreira[1], Giuliana Stephani Oliveira[1], Luis Carlos de Souza Ferreira[1], Rita de Cássia Café Ferreira[1]

[1]*Vaccine Development Laboratory, Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*

Even in the 21st century dental caries remains a disease with worldwide distribution and high prevalence. The development of the disease is dependent on the presence of microorganisms capable of binding to the tooth surface and to produce large amounts of lactic acid, features presented by *Streptococcus mutans* , the main etiological agent of dental caries. The P1 protein of *S. mutans* is directly related to the oral colonization and considered a main vaccine antigen target. In our previous studies, we have demonstrated that a recombinant fragment derived from the N-terminal region of the P1 protein, termed $P1_{39-512}$, produced in a recombinant *Bacillus subtilis* strain preserves immunological features of the native protein as evaluated after parenteral (s.c.) administration. The aim of the present study was to evaluate the ability of a vaccine formulation containing $P1_{39-512}$ and a mucosal adjuvant (a non-toxic derivative of the heat-labile toxin of *E. coli*) to induce systemic and mucosal antibody response and in vivo protective immunity after sublingual administration. Our results have shown that administration of $P1_{39-512}$, either alone or combined with the adjuvant, induce high levels of systemic antibodies, but higher amounts were detected in the presence of the adjuvant. Additionally, antibodies raised in mice immunized with the adjuvanted vaccine formulation displayed more efficient S.mutans neutralization effects, based on an in vivo murine antibody-neutralization model. In addition, mice immunized with the antigen admixed with the adjuvant had significantly lower rate of oral colonization by *S. mutans* when challenged, compared to other immunized groups. In summary, our data suggest that sublingual administration of the recombinant $P1_{39-512}$ represents a new mucosal vaccine strategy capable to prevent *S. mutans* colonization and, therefore, caries development.

Poster Session

BAM-PM1139 - Tick prevalence and detection of spotted fever group rickettsiae in Kyoto city, Japan

Azusa Someya[1], Saki Kozono[1], Aki Ito[1], Natsumi Okamoto[1], Mitsuhiro Ikenaga[2], Akihiko Maeda[1]
[1]Kyoto Sangyo University, Kyoto, Japan, [2]Kyoto City Institute of Health and Environmental Sciences, Kyoto, Japan

Spotted fever group rickettsiae are the causative agents of rickettsiosis such as Rocky Mountain spotted fever. Tick has been identified as the vector of these pathogens. In Japan, over 100 patients of Japanese spotted fever caused by Rickettsia japonica have been reported every year. The expanded distribution of the disease has raised concerns for the public health. Therefore, the prevalence of ticks and their harboring rickettsia were tested in Kyoto city, Japan. Between April 2013 and January 2014, ticks were collected every week using flagging method from 5 mountainous points in Kyoto city, Japan. Four species of ticks belonging to genus Haemaphysalis (H.) including H. longicornis, H. flava, and H. kitaokai were captured. A small number of genus Ixodes and Amblyomma ticks were also captured. The dominant species was H. longicornis and H. flava in summer and in winter, respectively. Selected adult and nymph ticks of H. longicornis and H. flava were investigated for the presence of the Rickettsia 17-kDa genus-common antigen gene using PCR method. Rickettsial DNA was detected in more than 60% of H. longicornis. Approximately 10% of H. flava were also infected with rickettsiae. The segment of 17-kDa antigen gene was amplified in both tick species using the species specific primer pair for R. japonica. Selected positive PCR amplicons were sequenced and the consensus sequences were compared with those of previously characterized rickettsiae. They appeared closely related to R. japonica. These results suggest that ticks may appear in mountainous points near human living area all year round and spotted fever group rickettsiae frequently infect to these ticks in Kyoto city although their pathogenicity is still unclear.

Poster Session

BAM-PM1143 - Evolutionary dynamics revealed by time-course sequencing of freshwater cyanobacterial communities

Nicolas Tromas[1], Nathalie Fortin[2], David F. Bird[3], Charles W. Greer[2], B. Jesse Shapiro[1]
[1]Université de Montréal, Montreal, Canada, [2]National Research Council Biotechnology Research Institute, Montreal, Canada, [3]Université du Québec á Montreal, Montreal, Canada

Proliferation of harmful cyanobacteria is an expanding problem in many freshwater ecosystems, particularly in eutrophic lakes. The cyanobacterial genus Microcystis forms frequent summer blooms in eutrophic lakes, and some subspecies, like M. aeruginosa, produce toxic compounds called microcystins that pose an important public health threat. Predicting toxin production and bloom severity is still an unsolved problem, due in part to our lack of knowledge about population diversity among cyanobacteria taxa, particularly M. aeruginosa, which is often the dominant member of the bloom. Evolutionary dynamics of M. aeruginosa populations are poorly understood – specifically how genetic diversity is selected and maintained by natural selection in the population according to environmental and ecological factors. Here, we describe progress toward filling this gap using a 'reverse ecology' approach. We analyzed microbial community diversity from a time-course of approximately 100 water samples taken between 2006 and 2013 in Lake Champlain. At each time-point, we extracted total DNA and performed high-throughput illumina sequencing of the 16S V4 region as a taxonomic marker. Preliminary results of 16S sequencing showed a specific seasonal pattern, with a massive increase of Microcystis during summer, along with a sharp decrease in taxonomic diversity (low Shannon diversity) – a probably side effect of the bloom. Microbial diversity was also coupled to environmental factors such as nitrogen, phosphate and water temperature, in order to identify factors that might predict bloom dynamics from year to year. Finally, to measure the level of genomic variation present within the dominant Microcystis population, we are in the process of performing shotgun metagenomic sequencing of bloom time points. This will allow us to identify targets of natural selection (mutations, genes or strains of Microcystis) that might correlate with bloom severity or other environmental data.

Poster Session

BAM-PM1145 - Pyrosequencing analysis of early childhood caries from Chinese She and Han race

Hui Chen[1], Wen Jiang[1], Zongxin Ling[1], Jie Zhang[1], Xiaolong Lin[1], Yadong Chen[1], Jinjin Yu[1], Charlie Xiang[2]
[1]Department of Conservative Dentistry and Periodontics, Affiliated Hospital of Stomatology, College of Medicine, Zhejiang University, China, [2]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, College of Medicine, Zhejiang University, China

Dental caries is the most common chronic childhood disease in the world. However, there has been few knowledge about the oral microbiota of childhood from different races. The aim of the study is to explore the microbiota in dental plaque from chinese Han and She children by pyrosequencing anlysis .Method: Supragingival plaque were collected from 66 She and 60 Han children with or without dental caries. The V1–V3 hypervariable regions of bacterial 16S rDNA genes were amplified by multiply primers and analyzed by high-throughput pyrosequencing. Result : A total of 716,972 reads passed the quality control, belonging to eighteen phyla and 102 genera. Fifteen genera including *Actinomyces, Corynebacterium, Derxia, Leptotrichia, Neisseria, Streptococcus, Veillonella, Capnocytophaga, Prevotella, Granulicatella, Fusobacterium, Johnsonella, Kingella, Porphyromonas, Abiotrophia* were shared by 97% She and Han children with or without dental caries. These genera constituted 86.2-88.9% of the total microbiota of dental plaque in all subjects. However the quantity of dental plaque microbiota composition from She and Han subjects were various. Thirty genera of that such as *Actinomyces, Campylobacter, Neisseria, Leptotrichia, Prevotella, Porphyromonas, Capnocytophaga* showed significantly different between the Han and She subjects. Moreover three genera including *Gemella, Bergeyella, Granulicatella* were associated with dental caries in She group, whereas other nine genera including *Streptococcus, Actinomyces* were associated with dental caries in Han group. Conclusion: the framework of normal plaque microbiota from She and Han race was studid. The microbiota disparities are present in different racial groups and the potential cariogenic microbes in She and Han children was at a certain extent variable .

Poster Session

BAM-PM1000 - A novel thermostable xylanase derived from biocompost samples-direct cloning, characterization and applications.

Sonia Sharma[1], Amandeep Kaur[1], Ramesh Chander Kuhad[1]
[1]*University of Delhi South Campus, New Delhi, India*

A novel thermostable xylanase derived from biocompost samples-direct cloning, characterization and applications. Sonia Sharma, Amandeep Kaur and R.C. Kuhad Lignocellulose Biotechnology Laboratory, Department of Microbiology, University of Delhi South Campus, New Delhi, India Metagenomic approaches provide access to environmental genetic diversity without the limitation of the culture bias. It allows the direct screening of novel biocatalysts directly from the environmental samples such as soil and water. Discovery of new glycoside hydrolases with efficient biocatalytic properties for the efficient conversion of lignocellulosic material to bio fuels is a critical challenge for both developed and developing countries. In order to mine novel xylanases, metagenomic DNA libraries were constructed from biocompost samples collected from a sewage treatment plant and were screened for genes conferring xylanase activity. Function-driven screening identified a novel xylanase belonging to glycoside hydrolase family 43. The molecular mass of the purified xylanase was estimated to be 54 KDa. The enzyme was optimally active at pH 7.0 and 50°C temperature. Moreover, the enzyme was highly stable to temperature changes, retaining atleast 60% activity at temperature ranging from 20°C to 80°C. The potential of the enzyme to be used in the conversion of lignocellulosic biomass to fermentable sugars was examined. Among the various pretreated lignocellulosic substrates tested, maximum sugar release (227 mg/g of dry substrate) was observed in corn stover. The enzymatic hydrolysate of corncob obtained was further fermented with Pichia stipitis for bioethanol production.
Keywords – Metagenome, biocompost, xylanase, thermostable, enzymatic saccharification, biofuel.

Poster Session

BAM-PM1002 - Development and optimization of DNA transfer system in B. subtilis by using heterogeneous conjugal transfer

Takahiro Yokoi[1], Mitsuhiro Itaya[2], Hirotada Mori[3], Masakazu Kataoka[1]
[1]Graduate School of Science and Technology, Shinshu University, Nagano City, Japan, [2]Institute for Advanced Bioscience, Keio University, Tsuruoka City, Japan, [3]Department of Bioscience, Nara Institute of Science and Technology, Ikoma City, Japan

Bacillus subtilis is one of the most widely used bacteria in the industrial biotechnology. It is well known that *Bacillus subtilis* and the closely related species show non pathogenic and endotoxin free. Their fermentation method has been well optimized for producing various enzymes, antibiotics, and chemicals. However, the genetic manipulation systems including transformation method has not been maintained in the most *Bacillus subtilis* strains except the well characterized laboratory strain. Natural isolates strains of *Bacillus subtilis* are often show difficulty of transformation due to their lack of natural competency. In this IUMS2014, we will present the in vivo heterogeneous gene transfer system between *E. coli* and *Bacillus subtilis* by using RP4 conjugation system. We used the mobility plasmid pUB307 Tn5, a conjugative plasmid carrying RP1 *tra* genes. pUB307 could be mobilized other plasmids containing transfer origin(*oriT*) sequence. We constructed the series of shuttle vector containing various lengths of *oriT* to assess the effect on heterogeneous DNA transfer. The plasmid containing a Wild Type *oriT* showed well conjugation efficiency between *E.coli* and *Bacillus subtilis*. In contrast, other plasmids containing partially deleted *oriT* showed the smaller frequency. To optimize the inter-genus conjugation, we examined the time required for DNA conjugation. Conjugation for 1 hour gave enough number of the trans conjugants. Finally our optimized conjugation method showed more effective DNA transfer than the natural transformation usually used.

Monday, 28 July 2014 **12:00 - 13:00 Room 516/517 A-B**

Poster Session

BAM-PM1004 - Genome Jigsaw: Implications of DNA fragment position in 16S rRNA gene analysis for bacterial species identification.

Jennifer Mitchell[1], Gabriel Moreno-Hagelsieb[1]
[1]Wilfrid Laurier University, Waterloo, Canada

The small ribosomal subunit (16S rRNA) gene is present within all bacteria, and contains nine variable (V) regions interspersed within conserved regions of the gene. While conserved regions remain relatively constant over time and between bacteria, variable regions can be used for taxonomic purposes. Current methodology for characterizing microbial communities involves sequencing short fragments of this ubiquitous gene, and comparing these fragments to reference sequences in databases to identify the microbes present. Traditionally, 16S rRNA sequences with less than 97% sequence similarity are assigned to different operational taxonomic units (OTUs). This identity threshold was decided because it seemed to be the proper threshold for 16S rRNA gene sequences belonging to the same species. However, a prominent trend to species identification in microbial communities is to sequence small fragments of this gene. This project seeks to evaluate not only the effectiveness of utilizing short fragments, but also implications of the position along the gene these fragments come from in terms of the most appropriate identity threshold so that the OTU clusters would be closer to what a complete gene cluster would provide. In other words, whole gene analysis may be effective for measuring diversity; however, the variable region source of these small fragments may require higher or lower sequence similarity thresholds for accurate measurements. Two algorithms, UCLUST and CD-HIT-EST, have been used to cluster sequences– at the whole gene level and fragments spanning the V13 and V35 regions– from SILVA's Living-Tree-Project (LTP) database into OTUs defined by 97% sequence similarity level. Results so far have shown that clustering fragments from different gene positions impacts OTU generation and composition.

Poster Session

BAM-PM1006 - Crystallization and preliminary crystallographic studies of Lactococcus lactis prolidase

Oarabile Kgosisejo[1], Pawel Grochulski[1], Takuji Tanaka[1]
[1]University of Saskatchewan, Saskatoon, Canada

Lactococcus lactis prolidase (EC 3.4.13.9) is a dipeptidase that hydrolyzes peptides with a proline residue at the C-terminus position of the peptide. In addition to hydrolyzing proline dipeptides, prolidases from different organisms have been shown to hydrolyze and detoxify some organophosphorus compounds, including chemical warfare agents. We have studied L. lactis prolidase and elucidated its catalytic properties, which are different from other prolidases'. This prolidase has 37 % amino acid sequence identity with the bacterial prolidases of which the crystal structures have been solved. This suggests that the low structural similarity with other prolidases is responsible for its catalytic uniqueness. This study focuses on solving the structure of recombinant L. lactis prolidase using X-ray crystallography. Different types of crystals including rod clusters, needles, two-dimensional plates, microcrystals and bipyramids were obtained by the hanging drop vapour diffusion method under different crystallization conditions. Among these, diffraction data of the highest quality was obtained from bipyramidal crystals, which crystallized in 12 – 16 % (w/v) polyethylene glycol 8000, 0.1 M sodium cacodylate (pH 6.5) and 0.1 M manganese chloride. Diffraction data collection was done on beamline 08ID-1 of the Canadian Macromolecular Crystallography Facility at the Canadian Light Source. The crystals belonged to the primitive triclinic space group (P1) with an estimate of six to eight prolidase monomers in an asymmetric unit. The resolution, completeness, R-merge and I/σ (I) of the data were 2.35 Å, 97.80 %, 7.7 % and 10.96, respectively. We are currently working on structure solution using the molecular replacement method with the crystal structure of Pyrococcus furiosus prolidase (PDB accession code 1pv9) as the search model. In addition, we are investigating a wider range of crystallization conditions to determine the conditions yielding crystals that can give higher resolution diffraction data and crystals belonging to a higher symmetry space group.

Poster Session

BAM-PM1008 - Rural residents in China are at increased risk of exposure to tick-borne pathogens Anaplasma phagocytophilum and Ehrlichia chaffeensis

Lijuan Zhang[1], Hong Liu[2], Bianli Xu[3], Zhilun Zhang[4], Yuming Jin[5], Weiming Li[6], Qunqing Lu[7], Liang Li[8], Litao Chang[9], Xiuchun Zhang[10]

[1]National Institute for Communicable Disease Control and Prevention, China CDC, Beijing , China, [2]Centers for Disease Control and Prevention of Anhui Province, Hefei ,China, [3]Centers for Disease Control and Prevention of Henan Province, Zhengzhou ,China, [4]Tianjin Center for Disease Control and Prevention, Beijing , China, [5]Centers for Disease Control and Prevention of Hainan Province, Haikou ,China, [6]Centers for Disease Control and Prevention of Jilin Province, Changchun ,China, [7]Centers for Disease Control and Prevention of Zhejiang Province, Hangzhou , China, [8]Centers for Disease Control and Prevention of Jiangsu Province, Nanjing , China, [9]Centers for Disease Control and Prevention of Yunnan Province, Kunming , China, [10]Beijing Center for Disease Control and Prevention, Beijing , China

Increasing evidence of Anaplasma phagocytophilum and Ehrlichia chaffeensis infection of ticks, rodents and animals has been documented in China. In particular, an unusual transmission of nosocomial cases of human granulocytic anaplasmosis (HGA) occurred in Anhui Province in 2006. To systemically assess the risk of exposure to the two tick-borne acute infectious diseases among rural and urban residents in China, a large cross-sectional serological investigation of A. phagocytophilum and E. chaffeensis was performed in nine provinces and two municipalities from 2007-2009. A total of 7,322 serum samples from rural residents and 819 serum samples from urban residents were collected, and specific IgG antibodies against A. phagocytophilum and E. chaffeensis were detected using an indirect immunofluorescence antibody assay (IFA). The overall seroprevalence of E. chaffeensis and A. phagocytophilum was 9.8% and 15.4%, respectively, in rural residents and 2.4% and 1.5%, respectively, in urban residents. The seroprevalence of both pathogens in rural residents was significantly higher than in urban residents ($P<0.0001$). Variation in the seroprevalence of E. chaffeensis and A. phagocytophilum between different provinces was investigated. Hainan (44.6%), Xinjiang (43.2%) and Beijing (19.4%) shared a higher seroprevalence of E. chaffeensis, whereas Tianjin (41.8%), Hainan (39.2%), Anhui (33.7%) and Beijing (13.6%) had a higher seroprevalence of A. phagocytophilum. The 20- to 29-year-old and 30- to 39-year-old groups had the highest seroprevalence of these two pathogens. Having contact with animals, planting crops, having more employment time and having a fever history were associated with the exposure risk of A. phagocytophilum. Similarly, a fever history was associated with the exposure risk of E. chaffeensis. Here, we concluded that rural residents in China were at a substantially increased risk of exposure to E. chaffeensis and A. phagocytophilum, and even among urban residents, the risk was considerable.

Poster Session

BAM-PM1010 - Multiclonal outbreak of CTX-M-type extended-spectrum β-lactamase-producing Klebsiella pneumoniae in a maternity home, Mongolia

Khosbayar Tulgaa[1], Dongeun Yong[2], Avarzed Amgalanbaatar[3], Ochbadrakh Batjargal[1], Udval Uuganbayar[1], Otgonbayar Bayanjargal[1], Gantulga Chantuu[4], Batbaatar Gunchin[3], Kyungwon Lee[2], Yunsop Chong[2]

[1]Department of Molecular Biology and Genetics, Research Center of Antimicrobial Resistance, Health Sciences University of Mongolia, Ulaanbaatar, Mongolia, [2]Department of Laboratory Medicine and Research Institute of Antimicrobial Resistance, Yonsei University College of Medicine, Seoul, Korea, [3]Department of Microbiology and Immunology, Health Science University of Mongolia, Ulaanbaatar, Mongolia., [4]State Inspection Agency, Ulaanbaatar, Mongolia

Between January 2010 and May 2011, nosocomial outbreaks caused by K. pneumoniae were observed in a neonatal unit of Maternity home, Ulaanbaatar, Mongolia. Surveillance cultures of the healthcare workers' (HCWs) fecal samples, and environmental samples were performed to detect potential reservoirs in addition. A total of 36 K. pneumoniae isolates from the patients (n=31), environment (n=2) and HCWs (n=3) were enrolled in this study. Among 21 cases in the first outbreak in 2010, four and two patients were positive for blood and CSF culture, respectively. Others were positive for either wound or throat culture. During this outbreak, three cases were fatal and attack rates were high in infants delivered with C-section, lower birth score, low birth weight and lower gestational week. The 24 isolates recovered from the neonates and 2 isolates from environmental samples were CTX-3-type ESBL producers with sequence type ST29 and same PFGE pattern. Conjugative plasmid (~50 kb) was replicon type L/M. After closure of the maternity home and moving of all HCWs and patients into other hospital building due to the first outbreak, the second one was observed in neonatal unit. Five isolates in the second outbreak had different resistance gene, blaCTX-M-9 and novel STs. A year later in 2011, three K. pneumoniae isolates recovered from neonates had identical pulsotype with those of the first outbreak strains. Our study shows that the neonatal infections caused by K. pneumoniae observed in 2010 and 2011 were multiclonal outbreaks. Despite intensive efforts to control their spread, the outbreak strains were clonally related each other probably due to overcrowded wards and lack of enough HCWs. Putting more efforts is required to stop the spread of ESBL-producing K. pneumoniae isolates in the Mongolian hospital.

Poster Session

BAM-PM1012 - Larsenia salina gen. nov., sp. nov., a new member of the family Halomonadaceae based on multilocus sequence analysis

Maria-Jose Leon[1], Cristina Sanchez-Porro[1], Rafael R. de la Haba[1], Antonio Ventosa[1]
[1]*University of Sevilla, Sevilla, Spain*

During the course of studies on the microbial diversity of salterns in South Spain we isolated two new halophilic microorganisms, designated as strains M1-18 and L1-16, which according to preliminary identification could represent a new genus and species of the family Halomonadaceae. In this study we determined the taxonomic position of these two strains using a polyphasic approach and a complete multilocus sequence analysis (MLSA). Both strains were motile, strictly aerobic rods, growing in the presence of 3-25 % NaCl (optimal growth at 7.5-10 % NaCl), between pH 4.0 and 9.0 (optimal growth at pH 6-7) and at temperatures between 15 and 40ºC (optimal growth at 37ºC). Phylogenetic analysis based on 16S rRNA gene sequence comparison showed that both strains were related to species of the genera Chromohalobacter and Kushneria, within the family Halomonadaceae, class Gammaproteobacteria. Partial sequences of atpA, rpoD and secA genes were used for a MLSA study and the four genes were concatenated for further analysis. The individual and concatenated trees showed that both strains constituted an independent and monophyletic branch, supporting the new genus status for these strains. The DNA-DNA hybridization between both strains was 82 %, whereas the values between strain M1-18 and the most closely related species of Chromohalobacter and Kushneria were equal or lower than 48 %, showing unequivocally that both strains constitute a new genospecies. The major cellular fatty acids were C18:1ω7c/C18:1ω6c, C16:0, and C16:1ω7c/C16:1ω6c, a profile that clearly differentiate this new taxon from species of the related genera. Overall, the phylogenetic analysis based on the comparison of the 16S rRNA and other three housekeeping gene sequences, and phenotypic, genotypic and chemotaxonomic characteristics, support our proposal of strains M1-18 and L1-16 as a novel genus and species, within the family Halomonadaceae, with the name Larsenia salina gen. nov., sp. nov.

Poster Session

BAM-PM1014 - **Compounds from Parthenium hysterophorus frustrate Helicobacter pylori and adenocarcinoma gastric cell line (AGS) interaction.**

Jazmín Espinosa[1], Erika Rendón[2], Martha Macías[3], Irma Romero[1]
[1]Department of Biochemistry, Faculty of Medicine, UNAM, Mexico City, México, [2]Department of Cellular Biology, Faculty of Medicine, UNAM, Mexico City, México, [3]Department of Natural Products, Institute of Chemistry, UNAM, Mexico City, México

Helicobacter pylori is a Gram-negative bacteria that inhabit the gastric mucosa; it has been recognized as a main factor for the development of chronic gastritis, peptic ulcers and gastric cancer. Fifty percent of the global population is infected with the bacteria. Current therapies are successful but inherit several problems as development of antibiotic resistance and they are aggressive for the patients. Therefore, new bioactive compounds that could decrease infection rates or might prevent the interaction between bacterial adhesins and host cell receptors are in demand. The ethnomedical approach is useful to search bioactive compounds. Parthenium hysterophorus is an herb widely used in Mexico for treatment for digestive illness such as stomachache and gastritis. The aim of this study is to purify bioactive compounds, from P. hysterophorus, that inhibits the interaction of H. pylori with gastric mucosa and to elucidate their possible mechanism of action. To determine adhesion to epithelial cells, FITC-labeled bacteria were co-cultured with adenocarcinoma gastric cell (AGS) monolayers as described in (1), in presence or absence of the herbal extract, fractions or compounds. One hour after infection, the cell monolayers were washed and adherence was quantified by its fluorescence. A dichloromethane-methanol extract obtained from aerial parts of the plant (DMPA) inhibits adhesion in a concentration-dependent manner, reaching 70% of inhibition at 1 mg/ml. DMPA was fractioned by liquid-liquid partition and subsequent chromatography resulting in bioactive fractions that can inhibit nearly 90% of bacterial adhesion so; they are promising in isolation of bioactive compounds. In addition, our results showed that bacteria attachment to AGS cells could be specifically blocked by fucose, sialic acid or neuraminidase pretreatment, so identification of mechanism of action focused in sialic acid or Lewis b antigen-dependent interaction is being currently sought. 1. Beil and Kilian, 2007. Phytomedicine SVI, 5-8. Partially supported by PAPIIT-UNAM IN225711

Poster Session

BAM-PM1016 - Identification of animal-specific virulence factors mediating interactions between the enteric pathogen Pantoea, and the social amoeba, Dictyostelium discoideum

Derek Smith[1], John Stavrinides[1]
[1]*University of Regina, Regina, Canada*

Pantoea are Gram-negative cross-kingdom bacterial pathogens that have been isolated from clinical, plant, and environmental sources. Pantoea are known to cause opportunistic infections that are particularly devastating in hospitals, and which can result in death. Currently, there is limited information on Pantoea host range and the specific disease genes they use to infect their hosts. My research has focused on developing the social amoeba, Dictyostelium discoideum, as a Pantoea pathosystem that can be used to identify virulence factors in Pantoea. A grazing assay was developed and used to distinguish between potentially pathogenic Pantoea isolates by identifying those which could be grazed upon or that were grazing-resistant. Pantoea ananatis BRT175, a grazing-resistant strain, was mutagenized with a mini-Tn5 transposon, and the resulting mutant library was screened for genetic factors essential for grazing resistance. The rhamnolipid surfactant biosynthetic operon, rhlA and rhlB, was shown to be essential for the grazing-resistant phenotype. An analysis of the distribution of the rhlA and rhlB genes showed that a closely related isolate of P. ananatis also possesses both rhlA and rhlB yet does not show the same degree of grazing resistance. This, in addition to the identification of 24 other genes identified in the mutagenesis screen, suggests that there is a complex interplay of numerous factors involved in the interaction with D. discoideum. These data provide the opportunity to study the evolution of an emerging opportunistic pathogen and its suite of animal-specific virulence factors.

Poster Session

BAM-PM1020 - Bioactive molecules in the <10kDa fraction of the Lactobacillus rhamnosus R0011 secretome down-regulate pro-inflammatory biomarkers in intestinal epithelial cells stimulated with pro-inflammatory stimuli

Michael Jeffrey[1], Julia Green-Johnson[1]
[1]*University of Ontario Institute of Technology, Oshawa, Canada*

The human gut microbiome has been associated with maintaining intestinal epithelial cell (IEC) homeostasis by modulating host mucosal immune responses. Members of the Lactobacilli have been shown to suppress the production of pro-inflammatory gene expression in IECs that have been exposed to pro-inflammatory stimuli. However, it is still unclear whether or not these bacteria modulate host immune activity through secretion of bioactive molecules. The aim of this study was to examine the immunomodulatory activity of the L. rhamnosus R0011 secretome on intestinal epithelial cells. The human IEC line HT-29 and rat IEC line IEC-6 were activated with a wide array of innate immune stimulants, to allow delineation of differential effects of the secretome on pro-inflammatory biomarkers induced by varied stimuli. HT-29 IEC were co-incubated with L. rhamnosus R0011 secretome and one of the following pro-inflammatory stimuli: interleukin 1-beta (IL-1β), tumor necrosis factor-alpha (TNF-α); the toll-like receptor (TLR) agonists flagellin (TLR5), poly (I:C) (TLR3), or lipopolysaccharide (LPS) (TLR4); or the nucleotide-binding and oligomerization domain (NOD) agonist Tri-DAP (NOD1) and alterations in production of the pro-inflammatory cytokine interleukin-8 (IL-8) were measured via enzyme-linked immunosorbant assay (ELISA). IEC-6 cells were also co-incubated with L. rhamnosus R0011 secretome and either the TLR agonists flagellin or LPS or the NOD agonist Tri-DAP and alterations in the production of the pro-inflammatory cytokine CINC-1 were measured. The L. rhamnosus R0011 secretome was also subjected to size fractionation to determine the size of the bioactive constituent of the secretome. Down-regulation of IL-8 or CINC-1 was observed in HT-29 and IEC-6 cells co-incubated with the <10kDa fraction of the L. rhamnosus R0011 secretome, for all of the innate immune stimulants tested. These results suggest that L. rhamnosus R0011 secretes a bioactive molecule that modulates host immune responses induced by a broad range of innate immune stimulants.

Poster Session

BAM-PM1022 - Profiling of the multiple adhesion systems of Salmonella enterica serovar Typhi

Yoan Houde[1], Élise David[1], France Daigle[1]
[1]*Université de Montréal, Montreal, Canada*

The Salmonella enterica serovar Typhi genome encodes for 14 putative adhesion systems that are usually not expressed in vitro and only a few of them have been characterized so far. Since specific antibodies against these gene products were found in patients with typhoid, we hypothesized that some systems code for functional adhesins involved in the pathogenicity of serovar Typhi. As no current animal model is available, we investigated the contribution of the adhesion systems during in vitro interaction with human cell lines as well as their expression and regulation. In order to study these different adhesion systems in serovar Typhi, we deleted each one individually and cumulatively by allelic exchange mutagenesis. Hence, we tested every individual mutation and the mutant strain deprived of all 14 systems in adhesion and invasion assays using human INT-407 intestinal epithelial cells. Bacterial uptake and survival was evaluated using human the THP-1 macrophage-like cell line. Several adhesin mutants showed significant differential adhesion and invasion levels to INT-407 cells compared to the wild-type strain. Uptake and survival were also affected in some mutants. Cumulatively, these observations suggested that many putative adhesion systems have indeed different functions depending on the context. We also evaluated expression during growth under laboratory conditions using beta-galactosidase reporter assays. Transcriptional fusion between the promoter of the major subunit gene and lacZ reporter gene revealed that some of the adhesion systems showed considerable activity. These experiments allowed us to discover that many of the S. Typhi adhesion systems are functional and expressed. These observations have the potential to uncover some key elements involved during S. Typhi pathogenesis and suggest new biological targets to either treat multi-drug resistant strains or possibly chronic carriers that do not respond to classical treatments.

Poster Session

BAM-PM1024 - Cytotoxic activity of Serratia marcescens cell-free broths towards the neuroblastoma cell line CHP-212

Mónica L. Pineda Castellanos[1], Jesús Santa-Olalla[2], Eugenia Nuñez-Valdez[1]
[1]Facultad de Ciencias; Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México, [2]Facultad de Medicina; Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México

It has been reported that cell-free broths from clinical isolates of the bacteria S. marcescens are cytotoxic to human cell lines. The aim of this work was to determine whether the entomopathogenic, non-pigmented Sm81 and Sm89 isolates present extracellular cytotoxic activity towards the human cell line CHP-212 (neuroblastoma). The research was focused on the morphology changes observed and the proportion of remaining viable cells after an incubation in the presence of cell-free culture broths from the S. marcescens isolates to evaluate cytotoxic activity by the sulphorhodamine B assay. Results show that both S. marcescens isolates present strong cytotoxic activity and induce cytopathic effects on CHP-212 cells in vitro. Besides, it is shown that the cytotoxic activity is decreased after heat treatment. It is proposed that, compared to clinical S. marcescens isolates, the strains Sm81 and Sm89 might produce protein cytotoxic factors with potential application in cancer therapy.

Poster Session

BAM-PM1026 - Mycobacterial phospholipases C induces alveolar macrophages necrosis through inhibition of PGE2 synthesis

Patricia A. Assis[1], Milena S. Espíndola[1], Francisco W.G. Paula-Silva[1], Sylvia C. Leão[2], Célio Lopes Silva[3], Lúcia H. Faccioli[1]

[1]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Prêto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil, [2]Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, Brazil, [3]Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Prêto, Universidade de São Paulo, Ribeirão Prêto, São Paulo, Brazil

Phospholipases C (PLCs) are virulence factors expressed by several bacteria. In Mycobacterium tuberculosis (Mtb), PLCs are implicated in the pathogenicity of the bacillus, exhibiting cytotoxic effects on macrophages. However, the role of PLCs in macrophage cell death is not fully understood. Our group, using two Mtb clinical isolates carrying genetic variations in PLC genes, previously demonstrated that the isolate 97-1505, which bears plcA and plcB genes, induces higher mortality and significant neutrophil accumulation in the lungs, unlike the isolate 97-1200, which has all PLC genes deleted. Recently, it has been extensive reported that lipid mediators, as PGE2, are modulated by virulent Mtb in order to facilitate necrosis of the host cell and thus, bacterial spread. Here, we used the clinical isolates 97-1200 and 97-1505 Mtb to infect alveolar macrophages in vitro to address the direct effects of PLCs in cell death and eicosanoids biosynthesis. The isolate 97-1505 was more resistant to alveolar macrophage microbicidal activity than the isolate 97-1200 and also induced higher rates of necrosis cell death in alveolar macrophage necrosis. In addition, the PLC-containing isolate 97-1505 inhibited COX-2 expression and PGE2 production. To address the direct effect of mycobacterial PLC on cell necrosis and PGE2 inhibition, both isolates were treated with PLC inhibitors (I-PLC) prior to macrophage infection. Interestingly, inhibition of PLCs affected the ability of the isolate 97-1505 to induce necrosis, leading to cell death rates similar to those induced by the isolate 97-1200. Finally, PGE2 production by Mtb 97-1505-infected macrophages was restored to levels similar to those produced by 97-1200-infected cells. Taken together, our results highlighted mycobacterial PLC as a key virulence factor associated to subversion of PGE2 production and induction of alveolar macrophage necrosis. Financial support: São Paulo Research Fundation (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Poster Session

BAM-PM1028 - Mechanisms by which lactic acid bacteria act on environmental toxins and the potential for fermented foods to reduce host toxin uptake

Jordan E Bisanz[1,2], Joseph Mwanga[3], John Changalucha[3], Megan Enos[1,2], Jeremy P. Burton[1,2,4], Tamara Smokvina[5], Johan van Hylckama Vlieg[5], Gregory B. Gloor[6], Gregor Reid[1,2,4]

[1]CRDC Probiotics, Lawson Health Research, London, Canada, [2]Microbiology and Immunology, Western University, London, Canada, [3]National Institute for Medical Research: Mwanza Centre, Mwanza, Tanzania, [4]Surgery, Western University, London, Canada, [5]Danone Research, Palaiseau, France, [6]Biochemistry, Western University, London, Canada

Toxins in the environment are ubiquitous and often unavoidable but associated with a number of adverse health outcomes. Efforts to reduce exposure often include bioremediation in water and soil, but few have considered use of food grade organisms, such as lactic acid bacteria (LAB), to reduce host uptake from the gut. Eighty strains of Lactobacillus were screened for metal binding and resistance with comparative genomics used to identify putative genes with functions in metal interactions. A cysteine biosynthesis pathway was identified. Also, a mercury resistance element was uncovered in two of these strains. RT-qPCR has verified up-regulation of these genes after exposure to mercury. To validate their importance in resistance and mercury sequestration, we are preparing knockout mutants to examine their phenotype. We also plan an animal study to further show this function in vivo. In two pilot proof-of-concept human studies*, we showed that one month administration of a yogurt supplemented with probiotic Lactobacillus rhamnosus GR-1 to school children in Tanzania showed a trend towards reduction in heavy metals in blood, while this was statistically significant for pregnant women receiving the yogurt for six months. This effect occurred even though the GR-1 strain, while efficient at binding, does not have the mercury resistance pathway, thus we believe there are further effects that can be conferred. Of interest, the intestinal microbiome of the Tanzanian children, assessed by barcoded 16S rRNA Ion Torrent sequencing, showed correlations with higher metal levels and abundance of Succinivibrionaceae. These studies have important implications for people in developing countries, as well as the developed world, where mining and other manufacturing processes appear to be increasing environmental toxin exposure. *This clinical study was not part of the collaboration with Danone Research and TS and JvHV were not involved in the design, execution or interpretation of the results.

Poster Session

BAM-PM1030 - Development of genetically modified bacteria as new bio-indicators to detect silver nanoparticles in aquatic environment

Luc Le Grand[1,2], Jan Roelof van der Meer[2], Karine Lemarchand[1]

[1]Institut des sciences de la mer, UQAR, Rimouski, QC, Canada, [2]dpt. microbiologie fondamentale, Université de Lausanne, Vaud, Suisse

Silver nanoparticles (AgNPs) are commonly used in many consumer products for their antibacterial properties and have the potential to enter aquatic systems. Consequently, since 2009 AgNPs are considered as emerging contaminants in the aquatic environment by the USEPA. Despite the increasing amount of research dealing with AgNPs' behaviour in freshwater and marine environments, the detection of these nanoparticles remains based on the detection of silver without considering its form (nanoparticle or ionic). Although AgNPs are well known for their antibacterial properties, the exact mechanism of their effect on bacteria is still debated. Two main types of effects are proposed: membrane damage and oxidative stress. The aim of our research is to develop a new type of microbiological indicator capable of detecting AgNPs in aquatic environments. We propose to develop genetically modified bacterial strains that could be used as fluorescent microbiological reporters for AgNPs. A GFP-plasmid containing specific promoters induced by nanoparticle action was introduced in Escherichia coli DH5α cells. This modified strain produces a specific fluorescent signal when the target promoter is activated by AgNPs. Two different modified E. coli strains have been developed. The first one reports the activity of the FabA promoter, which is involved in membrane damage repair. The second reports the activity of the SodA promoter, which is involved in the response to oxidative stress. Presently, these two constructions are tested in our laboratory for their ability to detect AgNPs. The use of these microbiological reporters in natural systems may be a fast and cheap method to routinely detect AgNPs in natural systems and could also contribute to our understanding of AgNPs' nanoecotoxicological effects.

Poster Session

BAM-PM1032 - Coordinated rearrangements between domains of the membrane protein complex ExbB–ExbD of *Escherichia coli*

Aleksandr Sverzhinsky[1], Lucien Fabre[2], Andrew Cottreau[1], Damien Biot-Pelletier[1], Sofia Khalil[1], Mihnea Bostina[3], Isabelle Rouiller[2], James Coulton[1]

[1]Dept. of Microbiology and Immunology, McGill University, Montreal, Canada, [2]Dept. of Anatomy and Cell Biology, McGill University, Montreal, Canada, [3]Facility for Electron Microscopy Research, McGill University, Montreal, Canada

Gram-negative bacteria rely on the ExbB–ExbD–TonB system for the import of essential nutrients via the transduction of proton motive force energy from the cytoplasmic membrane to the outer membran This complex is identified as a potential target for antibiotic development due to its central role in energizing the import of many nutrients necessary for the establishment of infection. Despite decades of research, the stoichiometry, subunit organization and mechanism of action of the membrane proteins of the Ton system remain unclear. We focused our studies on ExbB (26.3 kDa), which is considered to be the complex's scaffolding protein, and ExbD (15.5 kDa), which is believed to be responsible for reacting to the proton motive force and charging Ton We co-purified ExbB with ExbD by affinity chromatography in the detergent DDM. While the proteins were free of contaminants, they purified in multiple stoichiometries of hetero-oligomeric complexes. The principal oligomer formed a ~240–260 kDa protein-detergent complex, measured by light scattering and by native gels. Quantitative Coomassie staining revealed a stoichiometry of ExbB4–ExbD2. Negative stain electron microscopy and 2D analysis showed particles of ~10 nm diameter in multiple structural states. Nanogold labeling identified the position of the ExbD periplasmic domain. We used random conical tilt to reconstruct reference volumes in three structural states, then used them to sort the heterogeneous single particles into three data-sets. Each reference volume was refined using its structural state-specific data-set. Our medium-resolution models represent static snapshots of a dynamic system showing the dimerization and flexibility of the ExbD C-termini and rearrangement of the ExbB cytoplasmic domains. Taken together, our data recognize coordinated structural rearrangements between the cytoplasmic domain and the periplasmic domain, concordant with in vivo predictions. We provide the first definitive insights into subunit interactions and structural flexibility of this important membrane protein complex.

Poster Session

BAM-PM1034 - Toxic metal efflux pump in *Bacillus sp.* isolated from heavy crude oil of °API gravity 11.5

Darío Rangel Shaw[1], Jenny Dussán Garzón[1]
[1]*University of Andes, Bogotá, Colombia*

Toxic metals are highly soluble compounds which are present in the environment in low concentrations and principally produced by petroleum and mining industry. Toxic metals are persistent environmental contaminants since they cannot be degraded or destroyed, therefore, bacteria have developed mechanisms to overcome the toxic effects. Due to the impressive physiological and metabolic diversity of Gram-positive bacteria, in this study we examined the mechanisms that indigenous bacteria, member of the genus *Bacillus* use to tolerate and/or resist these pollutants. Tolerance to Pb(II), Cr(VI) and As(V) was determined, as well as the metal removal from the medium. In addition, subcellular distribution of toxic metals, the effect of co-ions on metal-uptake and subcellular distribution of toxic metals, efflux pump kinetics and morphological changes in metal-tolerant bacteria were also determined. The strain *Bacillus sp.* M6 exhibited strong tolerance to metals evaluated, demonstrating the potential of M6 to grow and removal efficiently the toxic metals content in the supernatant accumulating principally in the cell membrane fraction. However, metal removal efficiency and subcellular distribution of metals in M6 is strongly affected by the presence of combinations of ions. Efflux pump kinetics revealed the capacity of *Bacillus* cells to start operating the energy-dependent efflux system to eliminate excessive amounts of ions from the Cytoplasm and Periplasm. This behavior was modeled with a positively underdamped harmonic oscillator equation that significantly fitted to the experimental data. SEM-EDX analysis showed general morphological changes in the case of M6 in presence of metal ions when compared with the control cells without metals. The results in this work indicate the use of several mechanisms in parallel, including efflux pumps, intracellular and extracellular accumulation to resist high metal concentrations of Pb(II), Cr(VI) and As(V) by Gram-positive bacteria.

Poster Session

BAM-PM1036 - Genome manipulation and the identification of toxin-antitoxin and essential genes on the 1.7 Mb pSymB chromid of Sinorhizobium meliloti

George diCenzo[1], Branislava Milunovic[1], Turlough Finan[1]
[1]McMaster University, Hamilton, Ontario

The α-proteobacterium Sinorhizobium meliloti is a N2-fixing endosymbiont of several legumes, which has a tripartite genome consisting of a 3.7 Mb chromosome as well as the 1.4 Mb pSymA and 1.7 Mb pSymB replicons. While traditionally referred to as a megaplasmid, several lines of evidence suggest that it may be more accurate to refer to pSymB as a second chromosome or chromid. To better understand the contribution of pSymB to the biology of this organism, we are using a genome deletion approach to identify loci whose deletion is associated with cell lethality. With this approach, we have identified two essential genes, the sole copy of the tRNAarg-CCG gene and engA, which encodes a protein involved in ribosome biogenesis. Both these genes are related to the translational machinery of the cell and they are situated within a 129 kb region on pSymB. Bioinformatics analyses revealed that a majority of this 129 kb was present as a continuous fragment on the chromosome of recent ancestors of S. meliloti, providing evidence that the essential nature of pSymB evolved through the translocation of core genes from the chromosome. Furthermore, this methodology led to the identification of an active pSymB encoded type II toxin-antitoxin system. This system consists of a toxin and antitoxin belonging to the COG5654 and COG5642 families, respectively. While bioinformatics studies have annotated such gene pairings as toxin-antitoxin systems, our data is the first experimental evidence to support this claim.

Poster Session

BAM-PM1038 - Interrelationship between duf299 operon and chromosome replication in Caulobacter crescentus

Ali Farrokhi[1], James A. Taylor[1], Gregory T. Marczynski[1]
[1]Dept. Microbiology and Immunology, McGill University, Montreal, Canada

In Caulobacter crescentus, the origin of chromosome replication, Cori overlaps a conserved operon beginning with a gene termed “domain of unknown function 299” or “duf299”. We propose that the Cori/Duf299 operon overlap helps coordinate replication and metabolism. This overlap is conserved among the members of α-proteobacteria. Duf299 is a novel Ser/Thr kinase and in E. coli, Duf299 regulates phosphoenolpyruvate synthetase. To address the function of duf299 in C. crescentus, we created an in-frame deletion of duf299. The growth of this mutant strain in glucose media but not xylose media was slower than that of wild-type. Therefore, C. crescentus Duf299 regulates glucose and carbon utilization. Our lab also showed that duf299 transcription is controlled by two promoters from inside Cori; and one promoter is xylose inducible (James Taylor unpublished). The duf299 operon contains four additional genes that may be co-transcribed: maf (multi-copy associated filamentation), skd (shikimate dehydrogenase), dpck (dephospho-CoA kinase) and dnaQ (DNA polymerase ε-subunit). We showed by lacZ experiments that the duf299 operon’s first four genes, duf299, maf, skd, dpck, are transcribed together only by the promoters lying inside Cori and that dnaQ also uses a second promoter. Previous studies showed that Cori replication is the result of competition between replication initiation regulators CtrA and DnaA, each with binding sites in Cori. We determined the duf299 translation start site by lacZ-fusion experiments and site-specific mutagenesis. Interestingly, one CtrA and one essential DnaA binding site overlap the 5’ coding region of duf299. Together, these results imply an unexpected communication between duf299 transcription and translation and the cell cycle regulators CtrA and DnaA. Our working hypothesis is that these regulators time the initiation of replication and determine the optimal expression of the conserved duf299 operon which contains both key metabolic regulators (duf299, maf) and essential metabolic enzymes (skd, dpck, dnaQ).

Poster Session

BAM-PM1040 - Positive selection for gain of RpoS function in Escherichia coli

Steven Botts[1], Sarah Chiang[2], Herb Schellhorn[1]
[1]McMaster University, Hamilton, Canada, [2]Canadian Food Inspection Agency, Markham, Canada

RpoS, while an important stationary phase regulator in the enteric bacteria Escherichia coli, is attenuated by loss of function mutation in a significant fraction of natural populations (nearly 0.3% of environmental E. coli isolates). Null mutations within rpoS are commonly selected by growth on poor carbon sources or during extended incubation (likely due to increases in nutrient transport and/or oxidative metabolism- both of which are traits of rpoS mutants). In contrast, mutations which restore RpoS function may provide a selective advantage for cells exposed to environmental stress. The loss and subsequent restoration of RpoS thus form a molecular switch for adaptation within poor carbon and high stress environments. To investigate selection for RpoS reversion, we exposed rpoS-deficient E. coli to high salt concentrations and assessed the phenotype of presumptive revertants. 3-9% of revertants contained reversion mutations within rpoS (true revertants), while in 91-97% the loss of RpoS was maintained (probable pseudo-revertants). These results show that RpoS function can be restored in deficient E. coli under selective pressure. As osmotic stress is often encountered in the environment, this selection may serve as an adaptive mechanism for competing cells.

Poster Session

BAM-PM1042 - Complete genome sequence of Leptospira interrogans serovar Icterrohaemorrhagiae strain Ictero No. 1 and prediction of genes that contribute to virulence

Kenichiro Iida[1], Takaya Segawa[1], Tian Qin[2], Hideki Hirakawa[3], Ryo Ozuru[1], Xiaokui Guo[4], Shinichi Yoshida[1]
[1]Department of Bacteriology, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan, [2]National Institute for Communicable Disease Control and Prevention, Beijing, China, [3]Kazusa DNA Research Institute, Kisarazu, Chiba, Japan, [4]Shanghai Jiao-tong University School of Medicine, Shanghai, China

The etiologic agent of Weil's disease was first isolated in 1914 and named as Spirochaeta icterrohaemorrhagiae (strain Ictero No.1) by Inada and Ido (1915). Leptospirosis is still an important disease in humans and is widespread throughout the world, especially in developing countries. To further investigate the genomic basis for pathogenicity of this bacterium, we have determined the genome sequence of strain Ictero No.1. This genome was sequenced using the genome sequencer 454 GS (Roche). The strain Ictero No.1 genome was found to be composed of two circular chromosomes of 4,273,003 bp (34.98% G+C content) and 349,926 bp (34.98 % G+C content). It did not contain any plasmids. Gene prediction and annotation were carried out using Meta Gene Annotator software and NCBI database. Sequence analysis showed that the large chromosome (chromosome 1) seems to contain over 3,000 CDS, while the small chromosome (chromosome 2) encodes 883 CDS. The chromosome sequence of strain Ictero No.1 was highly similar to that of previously reported Leptospira interrogans serovar Copenhageni strain Fiocruz L1-130, however, in our laboratory strain Ictero No.1 was found to be avirulent in hamster experiment model. This strain may have lost its virulence through many times of in vitro passages. Comparison of the amino acid sequences of proteins that are coded in open reading frames of strain Fiocruz L1-130 and Ictero No.1 revealed that about 50 genes had amino acid substitutions. The most important of which was the amino acid change in flagella motor protein, which seems to be related to the virulence of Ictero No.1. Moreover, the proteins contributing to potassium metabolism also have amino acid changes seem to be critical. In this meeting, we would like to discuss about the property of strain Ictero No.1 based on the completed annotation results.

Poster Session

BAM-PM1044 - Antagonism effect by a novel bacteriocin of Bifidobacterium bifidum isolated from goat's milk to Helicobacter pylori responsible of the gastro duodenal ulcer disease

Miloud Mahi[1], Ahmed Bensoltane[2]
[1]Laboratory of Food Microbiology, Department of Biologie, Faculty of Natural Science , University of Khemis Miliana, Algeria, [2]Laboratory of Microbiology and Nutrition, Department of Biology, Faculty of Science, University of Oran, Algeria

Helicobacter pylori are pathogenic bacteria, recognized for these implications in the gastroduodenal ulcer diseases. Bifidobacteria play a major role in promoting health and preventing disease. One important mechanism that may explain the beneficials effects of bifidobacteria on gut health is the production of anti-microbial inhibitors other than organic acid, such as bacteriocins. In our studies, three strains of the bifidobacteria have been isolated on MRS-cys medium from goat's milk. The identification has been based mainly in the sequencing DNAr 16s, and we tested the inhibition's activity of our strains towards to Helicobacter pylori. Studies of gastric biopsies taken from 10 hospitalised patients suffering a chronic gastritis and a duodenal ulcer disease in Algeria. We isolated Helicobacter pylori from 7 patients among the10 patients having an infection. The interaction between Bifidobacterium bifidum BSM2015 and Helicobacter pylori BT gave positive results observed by the presence of the zones of inhibitions. Additional tests are necessary to know the exact nature of the inhibiting agent. The results showed that the secretion of the organic acids and the production of a bacteriocin are at the origin of inhibition. The study of the kinetics of Bifidobacterium bifidum BSM2015 shows the decrease of pH in the medium is influenced by the production of the organic acids. The protein nature of this inhibitory compound was confirmed using proteolytic enzymes, proteinase K, alpha-chymotrypsin and trypsin. provoking sharp biochemical studies revealed that this bacteriocin was heat stable 100°C for 10 min. and active over a wide pH range 2-10. The bacteriocin was partially purified by ammonium sulphate precipitation, gel-filtration, and ion-exchange chromatography conserving the antimicrobial activity after every step of purification. The molecular mass of the bacteriocin was analyzed by SDS-PAGE and main band was observed at molecular masses ranging between 3.5 and 6.5 kDa.

Poster Session

BAM-PM1046 - Discovery of a novel antifungal peptide from fermented vegetable: efficacy and mechanism of action

Irfan Ahmad Rather[1], Kong-Ha Choi[1], Yong-Ha Park[1]
[1]*Department of Applied Microbiology and Biotechnology, Yeungnam University, Gyeongsan, Korea.*

Kimchi is a Korean fermented vegetable dish that is known for its richness in lactic acid bacteria (LAB) and diverse bioactive compounds. The identification of a novel antifungal peptide usually requires an exhaustive purification process and laborious characterization. In this study, a proteinacous antifungal compound produced by food-grade LAB, Lactobacillus plantarum YML007, was purified by gel-filtration chromatography and ultra-purified by high-performance liquid chromatography. The mass of the obtained compound was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The compound was found to be a peptide with a low molecular weight of 1256.617 Da. Amino acid sequencing revealed that the peptide contained eleven amino acids and exhibited 30-40% homology with known antimicrobial peptides. The biopreservative efficacy of the bacterium was analyzed using maize and soybean models. The bacterium was found to be effective in preventing fungal growth and aflatoxin production in maize. The morphological changes occurring during the inhibition were assessed by scanning electron microscopy. The antifungal compound restricted mycelial growth of the target fungi and induced marked morphological changes. An animal trail showed that YML007 can be used to preserve the nutritional value of stored grain and ensure the production of better quality feedstuffs that are necessary to improve animal health and performance. The results of this study raise the possibility of preventing the development of specific fungal and bacterial species in food. This may be useful in food industries for the preservation and development of a desirable starter culture for fermented foods, thereby extending the stability and shelf-life of the foodstuff.

Poster Session

BAM-PM1048 - Antimicrobial and anti-hyperglycemic activity of Bryophyllum Pinnatum extracts

Edna Chukwura[1], Ijeoma Nneji[1]
[1]*Nnamdi Azikiwe University, Awka, Nigeria*

Discovering and identifying plants with antimicrobial properties without severe effects for the treatment of diabetes mellitus has become an important goal of research in biomedical science. The qualitative and quantitative phytochemical analyses of Bryophyllum pinnatum were evaluated. Qualitative analysis showed that the leaves of Bryophyllum pinnatum were rich in flavonoids, saponins, tannins, alkaloids and cardiac glycosides. Tannin gave the lowest percentage yield of 0.36% for both the aqueous and methanol extracts while cardiac glycoside gave the highest percentage yield of 65% for the aqueous extract. The methanol and aqueous leaf extracts were screened for their antimicrobial activities against Gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and Gram negative bacteria (Pseudomonas aeruginosa and Escherichia coli) and a fungus (Candida albicans). Agar well diffusion method was used to determine the minimum inhibitory concentration at 100mg/ml, 200mg/ml and 400mg/ml. All organisms except Candida albicans were susceptible to the extracts. The Gram negative organisms were not inhibited by the aqueous extracts. The antidiabetic potential of both extracts of the plant was evaluated in the alloxan monohydrate induced-diabetic albino rats. Graded doses of the aqueous and methanol extracts were administered to experimental diabetic rats for 10 days. Significant reduction in fasting blood sugar values was obtained in the diabetic animals. There were significant differences in the mean values of the parameters evaluated at $P<0.05$ for the methanol-extract treated rats.Hence Bryophyllum pinnatum leaf extracts could be very valuable in the treatment of diabetes mellitus some bacterial associated diseases. Key words Phytochemical, Bryophyllum pinnatum ,diabetes mellitus.

Poster Session

BAM-PM1050 - Silver salts of carboxylic acid terminated generation 1 poly (propyl ether imine) (PETIM) dendron and dendrimers as novel antimicrobial agents against S. aureus and MRSA

Nadia Suleman[1], Chunderika Mocktar[1], Rahul Kalhapure[1], Thirumala Govender[1]
[1]*University of KwaZulu-Natal, Durban, South Africa*

Alternative novel antimicrobial therapeutic strategies are essential to address the current global antimicrobial resistance crisis. Although silver has antimicrobial activity, it displays toxicity to several micro-organisms in its positively charged ionic form. On the other hand, branched monodisperse molecules with multiple peripheral functionalities such as PETIM dendrons and dendrimers have gained interest as antimicrobial agents with low toxicity. Therefore, the aim of this study was to synthesise and evaluate silver salts of generation 1 PETIM dendron and dendrimers. This exploits the multiple peripheral functionalities of biocompatible PETIM dendron and generation 1 dendrimers for the formation of silver salts containing multiple silver ions in a single molecule for enhanced antimicrobial activity at the lowest possible concentration. PETIM dendron, dendrimers and their silver salts were synthesised and characterised by IR, 1H NMR and 13C NMR. The PETIM Dendron/dendrimers, silver nitrate and the synthesised silver salts of dendron (compound A), dendrimer with an aromatic core (compound B) and dendrimer with an aliphatic core (compound C) were evaluated for their antimicrobial activity by the broth dilution method against S. aureus and methicillin-resistant S. aureus (MRSA). The structure and formation of the PETIM dendron/dendrimer and compounds A, B and C were confirmed by IR and/or NMR. Minimum inhibitory concentration (MIC) values of compounds A, B and C against S. aureus were 52.1, 41.7, and 20.8 µg/ml while against MRSA they were 125.0, 26.0 and 62.5 µg/ml respectively. The calculated Fractional Inhibitory Concentration Index further indicated that compound B specifically displayed additive effects against S. aureus and synergism against MRSA. These results confirmed the enhanced antimicrobial activity of the dendron/dendrimer-silver salts. The identification of such novel antimicrobial materials effective against both sensitive and resistant bacterial strains widens the pool of available pharmaceutical materials for optimising the treatment of bacterial infections.

Poster Session

BAM-PM1052 - Action mechanism of riccardin C derivatives for anti-MRSA activity

Daichi Morita[1], Wakano Ogawa[1], Hiromi Sawada[1], Hiroyuki Miyachi[1], Teruo Kuroda[1]
[1]*Grad. Sch. Med. Dent. Pharm. Sci., Okayama Univ., Okayama, Japan*

Methicillin-resistant Staphylococcus aureus (MRSA) is a major problem in clinical place, and the resistance for mostly antimicrobial agents makes difficult to cure MRSA infection. We previously reported riccardin C and its synthetic derivatives exhibited anti-MRSA activity. Riccardin C, a macrocyclic bis (bibenzyl) compound has been discovered in liverworts. It has been reported to have COX-inhibitory activity, HIV-1 reverse transcriptase-inhibitory activity, antifungal activity, LXR-modulating activity, anti-cancer activity, and NOS-inhibiting activity. However, the action mechanism as antibacterial agent is poorly understood. In this study, we clarified the action mechanism of macrocyclic bis(bibenzyl) RC-112 and its partial structure IDPO-9. Survival experiments showed that RC-112 had bactericidal effect for MRSA but IDPO-9 had bacteriostatic effect. IDPO-9 resistant mutants had cross-resistance to triclosan but not to RC-112. Its mutation was identified in fabI enoyl-acyl carrier protein reductase gene, a target of triclosan. On the other hand, addition of RC-112 (but not IDPO-9) caused the accumulation of ethidium and propidium in S. aureus cells. For ethidium-loaded S. aureus cells, RC-112 dependent ethidium outflow was observed. Also, transmission electron microscopy showed that S. aureus cells treated with RC-112 had intracellular lamellar mesosome-like structures. These results indicated that RC-112 would increase membrane permeability for ethidium and propidium with direct membrane damage, and that their inhibitory targets were different between IDPO-9 and others.

Poster Session

BAM-PM1054 - Effect of Simvastatin on the viability and biofilm formation of Staphylococcus aureus

Karina Cogo[1], Talita Graziano[2], Maria Claudia Cuzzulin[2], Humberto Schwartz-Filho[1], Francisco Groppo[2], Gilson Franco[3]

[1]University of Santo Amaro, Sao Paulo, Brazil, [2]Piracicaba Dental School, University of Campinas, Piracicaba, Brazil, [3]State University of Ponta Grossa, Ponta Grossa, Brazil

Nosocomial infections and infections caused by multiresistant microorganisms such as Staphylococcus aureus are a great global concern. Recent studies have shown that statins present antimicrobial against various bacterial and fungal species. The aim of the present study was to evaluate the effect of simvastatin (statin) on the S. aureus viability and biofilm formation, comparing its effects with Vancomycin and Gentamicin. Cultures of 5 strains of S. aureus (ATCC 29213, MRSA 33591, MRSA 43300, 14458 and 6538) were subjected to broth microdilution test for determining the Minimum Inhibitory Concentration (MIC). For evaluating the ability of adhesion and biofilm formation, mono - species biofilms of two strains were subjected to Crystal Violet Binding Assay and Scanning Electron Microscopy (SEM). For statistical analysis we used the Kruskal - Wallis test with a significance level at 5%. The MIC values were: Simvastatin - 15.65 µg/ml (29213) and 31,25 µg/ml for other strains; Gentamicin - 0.78 µg/ml (29213 and 14458), 1.56 µg/ml (6538) and 3.12 µg/ml (MRSA 33591); Vancomycin - 1.56 µg/ml for all strains. Simvastatin could significantly reduce biofilm formation at concentrations up to 8x<MIC for strains MRSA 29213 and 33591 (p<0,05). In addition, at concentrations up to 32x<MIC and 64x <MIC, simvastatin showed a greater ability to reduce S. aureus ATCC 29213 biofilm formation when compared with vancomycin and gentamicin, respectively (p<0,05). Meanwhile, this reduction occurred at concentrations up to 8x<MIC for S. aureus MRSA ATCC 3359 (p<0,05). Images obtained by SEM confirmed the reduction of biofilm formation caused by simvastatin, further indicating a likely reduction in the production of extracellular matrix. In conclusion, simvastatin has inhibitory activity at low concentrations and an excellent ability to inhibit the adhesion and biofilm formation by S. aureus, even at much lower concentrations than the MIC.

Poster Session

BAM-PM1056 - In vitro and in silico evaluation of conventional antimicrobials and new prototypes against Escherichia coli pathotypes isolated from humans and animals in Brazil

Eunice V. Barbosa[1,2], Elenice L. C. Nunes[1,2], Gustavo S. P. Pinto[3], Percilene F. Vegi[3], Mauricio S. Santos[3], Alice Maria R. Bernardino[3], Aloysio M. F. Cerqueira[1], Helena Carla Castro[2]

[1]Departamento de Microbiologia e Parasitologia - UFF, Niterói, Brazil, [2]Departamento de Biologia Celular e Molecular - UFF, Niterói, Brazil, [3]Departamento de Química Orgânica - UFF, Niterói, Brazil

Despite being a commensal of intestinal microbiota, distinct Escherichia coli pathogenic strains are recognized. Several new antimicrobials have been developed but bacterial resistance increasingly occurs. Through the close relationship between humans and animals, and also by the consumption of foods of animal origin, these multiresistant pathogens can be disseminated. This study aims to evaluate the in vitro and in silico activity of conventional antimicrobials and new prototypes, against 15 uropathogenic Escherichia coli (UPEC), 7 Shiga toxin-producing (STEC) and 10 atypical enteropathogenic (aEPEC) strains isolated from humans, cattle and dogs, as well as test their synergistic effects. Disk diffusion agar tests were done with 18 conventional antimicrobials and 28 new synthetic prototypes. The Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations (of conventional antimicrobials and new prototypes that showed better inhibitory growth profile were determined as well an evaluation of synergistic or antagonistic associations.The pharmacokinetic profile (ADMET), toxicology and structure-activity of prototypes was evaluated in silico. UPEC strains were more resistant to conventional antimicrobials, when compared to STEC and aEPEC strains. One ESBL-producing multiresistant (12 drugs) dog UPEC strain carrying the ctx-m gene (A34) was identified. One derivative of the imidazolyl-pyrazole class (MST 03) showed antimicrobial activity against 17 strains with and antimicrobial activity higher than tetracycline and enrofloxacin against the A34 strain. An antagonic association was detected with the MST 03 prototype when combined with sulfametozaxol + trimethoprim, ampicillin and enrofloxacin. The results of in silico studies were satisfactory and show that the MST 03 prototype meets the parameters studied and lead us to believe that it is a derivative with promising antimicrobial profile. The identification of an imidazolyl-pyrazole derivative with significant antibacterial profile may contribute to the development of new antibiotics as new options against multiresistant bacterial infections.

Poster Session

BAM-PM1058 - Characterization of the properties of an antimicrobial mineral clay

Shekooh Behroozian[1], Wanjing Xu[2], Loretta Li[2], Julian Davies[1]
[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada, [2]Department of Civil Engineering, University of British Columbia, Vancouver, Canada

The lack of novel antibiotics and the increase in antibiotic resistance have created a crisis situation in the treatment of infectious diseases worldwide. Natural mineral clays have been employed for therapeutic purposes for centuries and they could provide an effective alternative to antibiotics. However, their mode of action is poorly understood. We describe studies with Kisameet clay (KC) found in British Columbia, Canada and demonstrate that it is an extremely effective broad-spectrum antimicrobial agent. The antimicrobial properties of this clay have been investigated by characterizing chemical, mineralogical, and biological properties of clay which might be involved in cidal activity. We describe our studies devoted to elucidating the mode of action of KC and also the activity of aqueous leachates and organic solvent extracts. KC shows broad spectrum antibacterial activity against clinical-isolated multi-drug resistant bacteria such as Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. Our results demonstrate that the mechanism of antimicrobial action of KC is complex and involves the interaction of different properties of the material. These studies should lead to more defined, homogeneous, and stable preparations of KC for human therapeutic purposes.

Poster Session

BAM-PM1060 - Activity of three Nigerian medicinal plants Lannea welwitschii, Daniellia oliveri and Lophira alata on plasmid-mediated multiple drug resistant enteric bacterial pathogens

Kabiru Akinyemi[1], Christopher Fakorede[1], Bamidele Iwalokun[2], Akin Fasure[1]
[1]Lagos State University, Ojo, Lagos, Nigeria, [2]Nigerian Institute of Medical Research, Yaba Lagos, Nigeria

Enteric pathogens causing enteric infections have developed resistance to some commonly prescribed antibiotics including third generation cephalosporin and fluoroquinolones due to the acquisition of plasmid DNA. Three medicinal plants Lannea welwitschii, Daniellia oliveri and Lophira alata used traditionally for the treatment of gastroenteritis were tested on plasmid-mediated multi-drug resistant enteric bacterial pathogens; Salmonella enterica serovar Typhi, S. Typhimurium, Shigella sp, Klebsiella sp and Escherichia coli. The result of the study indicated that both the alcohol and aqueous plant extracts showed evidence of activity on test strains at varying concentrations with extract of L. Welwitschii exhibiting highest activity on all the strains of the bacteria tested at lower concentrations. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each of the plant extracts on the test strains varied. The MIC of 0.625, 5.0, 5.0, 10 & 10 mg/ml and MBC of 5, 10, 10, 20 & 20 of extract of L. Welwitschii were recorded for S. Typhi, S. Typhimurium, E. coli, Shigella spp and Klebsiella spp respectively. Also, MIC of 10mg/ml of D. oliveri was recorded for S. Typhimurium and E. coli and MBC of 20.0mg/ml and 40mg/ml were obtained for the strains of these two pathogens respectively. While MIC and MBC of the extract of L. alata recorded against each of the strains of S. Typhi and S. Typhimurim were 20 and 40 mg/ml respectively. The results revealed loss of low molecular weight plasmid in enteric pathogens at 5.0 mg/ml of extract of L. welwitschii, also at higher concentration of the extract, loss of high molecular weight plasmid was observed. The synergism of the extract L. welwitschii with conventional antibiotics was established. The phytochemical analysis of the aqueous and methanol extracts of L. welwitschii, D. oliveri and L. alata revealed the presence Alkaloid, Saponins, Tannins, Flavonoids and Anthraquinones.

Poster Session

BAM-PM1062 - Design and production of antibiotic-doped silk-like films

Chong Cheong Lai[1], Zhenwei Gao[1], Daiwen Yang[1]
[1]*National University of Singapore, Singapore*

Besides its outstanding mechanical strength and elasticity, spider silk is biodegradable and biocompatible. Thus it has a great potential in tissue engineering. To reduce infections at the site of tissue implementation, one can attach antimicrobial peptides to silk or silk-like materials. In this study, we fused a human β-defensin mutant to a repetitive domain of a spider silk protein. The defensin mutant is an analog of the human β-defensin-3 peptide that contains three pairs of disulfides and unusual high positive charges, shows a broad spectrum of activity and its activity is relatively insensitive to low salt concentrations. We showed previously that the repetitive silk domain is highly water soluble and forms silk-like fibers in the presence of shear force. We expressed the fusion protein in E. coli and purified it with a Ni-NTA affinity column. The fused defensin mutant was shown to have the correct pairing of the six cysteine residues although the isolated mutant could not be refolded correctly. As expected, the fused mutant still has the antimicrobial activity. Silk-like films were obtained by casting an aqueous solution of the fused protein onto a polystyrene surface and then drying at room temperature. The films were further treated with methanol to enhance the mechanical strength and stability in water. The treated films were demonstrated to have a strong antimicrobial activity.

Poster Session

BAM-PM1064 - WITHDRAWN - Prevalence of Salmonellosis and the susceptibility profiles of Salmonella species in drinking waters in Sokoto State, Northwest Nigeria

Raji Mudasiru[1], Ibrahim Yakubu[2]

[1]Department of Pharmaceutics and Pharmaceutical Microbiology, Usmanu Danfodiyo University, Sokoto, Nigeria,
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria

The safety of drinking water to the people in the Northwest Nigeria has been of major concern as a result of frequently reported incidences of waterborne diseases. Hospital records available in the Basic Health Centers in three towns of Sokoto state (Sokoto, Shuni and Tambuwal) were statistically analyzed. The result revealed that out of 753 cases of waterborne diseases diagnosed, 535 (71.05%) were salmonellosis. Drinking water sources namely borehole, tap, sachet and well waters available to the communities in the three towns were sampled using the membrane filtration technique and analyzed for the presence of some waterborne bacterial pathogens by biochemical tests. Out of 107 waterborne pathogens isolated, 30 (28.04%) were Salmonella species. The susceptibilities of the isolated Salmonella species to some antibiotics commonly used in the communities were determined by the modified Kirby Bauer method. A high proportion of the isolates were sensitive to most of the tested antibiotics (amoxicillin, amoxy-clav, gentamicin, streptomycin, ciprofloxacin, ofloxacin and sparfloxacin). However, 33.33%, 43.33% and 56.67% of the isolates were resistant to chloramphenicol, co-trimoxazole and perfloxacin respectively. The resurgence of multiple antibiotic resistant organisms in the localities has made it difficult to treat salmonellosis with one antibiotic, combination of antibiotics therefore is recommended to produce synergistic effect. Presence of pathogenic waterborne Salmonella species in the water samples and above World Health Organization's recommended limit made the water unhealthy and this has been the cause for rampant cases of salmonellosis in the area. Drinking water in these localities therefore needs to be treated to make it potable.

Poster Session

BAM-PM1066 - Characterization of a defined microbial ecosystem therapeutic for the treatment of Clostridium difficile infection

Christian Carlucci[1], Devon Metcalf[2], Scott Weese[2], Elaine Petrof[3], Michelle Daigneault[1], Joyce Rousseau[2], Emma Allen-Vercoe[1]

[1]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Canada, [2]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada, [3]Department of Medicine, Queen's University, Kingston, Canada

Clostridium difficile infection (CDI) is a severe gastrointestinal disease caused by the Gram-positive, anaerobic, spore-forming and toxin-producing bacterium, C. difficile. Often, antibiotics used to treat CDI further perpetuate gut dysbiosis by suppressing the resident microbiota, leading to recurrent infection characterized by severe diarrhea and inflammation. Fecal bacteriotherapy has emerged as an alternative, highly effective treatment for recurrent CDI, however it is not without risk and is typically reserved for debilitating cases. To circumvent safety issues associated with administration of undefined microbial populations from donor stool, we have developed a defined microbial ecosystem therapeutic, MET-1. This multi-species microbial formulation consists of 33 bacterial strains, and has been used to cure two patients of recurrent CDI in a proof-of-principle trial. The precise mechanisms through which health is restored in recurrent CDI patients after MET-1 treatment are unknown. The present study aims to determine the effects that MET-1 and its component microbes have on C. difficile toxin gene expression, viability, toxin degradation and sporulation. Two clinically relevant C. difficile strains sourced from two individual patient stool samples were thoroughly characterized by ribotyping, toxinotyping, and measurement of growth kinetics. Reverse-transcriptase quantitative (RT-q) PCR assays have been developed to accurately assess C. difficile toxin gene expression in response to MET-1 components. Using a single-stage chemostat model supporting the MET-1 community, we have additionally assessed the behaviour of C. difficile when in the presence of either MET-1 culture or its secreted metabolites. Full characterization of the antagonistic mechanisms of MET-1 against C. difficile, will provide us with valued insight into the therapeutic benefits of our approach, and will indicate research directions through which MET-1 efficacy may be enhanced.

Poster Session

BAM-PM1068 - The role of IL-27 in endotoxin tolerance in Human monocytes

Carlene Petes[1], Christopher Wynick[1], Katrina Gee[1]
[1]*Queen's University, Kingston, Canada*

The cytokine interleukin (IL)-27 has been shown to modulate proinflammatory cytokine and chemokine production in primary human monocytes and has been well described to influence adaptive immunity. The precise mechanisms and cellular processes modulating proinflammatory responses targeted by IL-27 are not well defined in monocytes and macrophages. In these cell types, Toll-like receptor (TLR)-4 mediates cytokine production in response to lipopolysaccharide (LPS). Negative regulatory processes, such as endotoxin (LPS) tolerance and the induction of anti-inflammatory cytokines are important in the control of inflammatory responses, such as sepsis. Endotoxin tolerized cells are characterized by a significant reduction in LPS-induced proinflammatory cytokine production, such as tumour necrosis factor (TNF)-α. Thus these cells lose LPS-responsiveness even though they maintain TLR4 expression. Since our group has demonstrated that IL-27 can enhance TLR4 expression and LPS-mediated cytokine expression, we reasoned that IL-27 may inhibit or break established tolerance. We show that costimulation of cells with LPS plus IL-27 can block endotoxin tolerance. Furthermore, IL-27 works synergistically with LPS in tolerized cells to enhance TNF-α protein and mRNA levels in human monocytic cells treated with LPS compared to untolerized cells. Therefore, our data demonstrates that IL-27 is able to restore the proinflammatory immune response in human monocytic cells inferring that IL-27 could be a possible therapy for post-septic patients to restore their immune function to a pre-septic state.

Poster Session

BAM-PM1070 - Transcriptional profiling of chicken immunity-related genes during infection with Avibacterium paragallinarum

Charlotte Boucher[1], Chrispian Theron[1], Arina Jansen[1], Robert Bragg[1]
[1]University of the Free State, Bloemfontein, South Africa

Avibacterium paragallinarum is the causative agent of Infectious Coryza (IC), which is an upper respiratory tract disease in chickens. The occurrence of outbreaks has emphasized the significance of the disease globally in the chicken industry. Studies have demonstrated that early immune responses are critical in defining the severity and physiological outcome of an infection. This prompted the need to investigate the regulation of immune functions by the number of genes expressed during the chickens' response to A. paragallinarum serovar C3 insult. This study consisted of 15 male leghorn birds that were scored into groups (score 1, 2, 3) according to severity of symptoms after they were challenged. Twelve birds were exposed to pathogen Avibacterium paragallinarum serovar C3. Three birds were unchallenged representing the control group. The birds were scored into groups (score 1, 2, 3) according to clinical symptoms. The quality of extracted RNA was evaluated with a bioanalyzer 2100. RNA with RIN values ≥ 7.5 was selected for hybridizations to a 4 x 44K Agilent Gallus gallus V2.1 microarray slide, followed by data extraction using the Agilent FE software v10.7. Expression patterns of immunity-related genes were followed as symptoms progressed from a disease score of 1 to 3. The data proposed that initial pathogen recognition was either through Toll-like receptors 2 or 4. Unique expression patterns were observed such as the up-regulation of TLR7 which recognizes viral-like particles. This substantiated the presence of prophages reported in the genome of A. paragallinarum. Significant down-regulation of metabolic pathways was observed, which led us to hypothesize that the host may rely on an oxidative stress response as initial immune response. The data sheds light onto the mechanisms that govern the immune system towards infection and / or towards the initial response to infections with highly virulent A. paragallinarum.

Poster Session

BAM-PM1072 - Bistability and antimicrobial peptide resistance in the entomopathogenic bacteria Photorhabdus

Annabelle Mouammine[1], Anne Lanois[1], Alain Givaudan[1]
[1]*UMR 1333 INRA-UM2 DGIMI, Montpellier, France*

Photorhabdus luminescens is a bioluminescent enterobacterium carried as a symbiont in the intestine of infective juvenile stage of Heterorhabditis bacteriophora nematodes. The nematode -bacterium complex can infect and kill a wide range of insect larvae and is used in biological control agent against crop pests. When the bacteria are released into the insect hemolymph, cationic anti-microbial peptides (CAMPs) are produced in response to infection. However the bacteria can resist and kill the insect in about 35 hours. The resistance mechanism against CAMPs in Photorhabdus is known and required the two component system PhoP-PhoQ initially described in Salmonella sp. In Photorhabdus, PhoP regulates the expression of pbgPE operon required for LPS modification. The incorporation of positively charged substituents results in a net loss of negative surface charges, producing bacterial membrane more resistant to CAMPs. Antibiograms and spreading on plates of wild type strain with and without polymixin B revealed that the major part of population was sensible and only about 1% of bacteria can resist to polymixin B. In the course of insect infection, the resistant sub-population is responsible for insect death and this phenomenon is reversible in the cadavers. To have a better understanding of resistance mechanism toward CAMP, transcriptional fusion between the pbgPE promoter and a destabilized GFP associated with flow cytometry allowed us to monitor the expression of resistance gene. We observed that the number of GFP-positive bacteria increased by 65 fold in presence of polymixin B. Also, a 4 fold increase of pbgP transcripts in the population selected with polymixin B was quantified. Altogether these in vitro and in vivo experiments showed that polymixin B selects bacterial cells expressing pbgP genes and the CAMPs-resistant sub-population multiplies in hemolymph and kills the insect. We are now assessing the mechanism involved in the switch between the two sub-populations.

Poster Session

BAM-PM1074 - Determination of enterohemorrhagic Escherichia coli OmpT cleavage sites in human coagulation Factor V

Maranda Baldeo[1], Veena Premjani[1], Samantha Gruenheid[2], Herve Le Moual[2], Jeffrey Weitz[3], John Samis[1]
[1]University of Ontario Institute of Technology, Oshawa, Canada., [2]Department of Microbiology, McGill University, Montreal, Canada., [3]Faculty of Medicine, McMaster University, Hamilton, Canada.

Blood coagulation plays an important role during the host innate immune response for formation of fibrin clots that immobilize and kill pathogenic bacteria. Factor V (FV) in its activated form FVa, is a critical accelerator of coagulation as part of prothrombinase that generates thrombin required for fibrin clot formation. Our previous research indicated that enterohemorrhagic Escherichia coli (EHEC) encodes an outer membrane protease OmpT which specifically cleaved and inactivated FV in human plasma. The purpose of this study was to determine the inactivation cleavage sites of EHEC OmpT within the human FV protein. Purified FV was incubated with an OmpT overexpressing strain of EHEC for 2 and 20 hours at room temperature. Cells were pelleted and fragments of the digested protein were resolved by SDS-PAGE. The FV fragments were electroblotted onto a polyvinylidene fluoride membrane, stained with Coomassie Blue, and subjected to Edman degradation for determination the N-terminal sequences of the protein fragments. The results indicated that OmpT cleaved human FV at five sites: K345, R1034, Q1236, Q1281, and Q1317 resulting in six fragments. The effect of cleavage at R1034, Q1236, Q1281, and Q1317 is unknown as these sites lie within the B domain whose function is presently unclear. Cleavage at K345 within the heavy chain would lead to dissociation of the A1 and N-terminal portion of the A2 domain resulting in loss of a critical region of FV required for FXa and FII binding within prothrombinase. The results indicate EHEC OmpT has evolved to specifically inactivate FV to attenuate fibrin barrier formation and induce an anticoagulant state thereby enhancing bacterial growth and transmission. This inactivation effect of OmpT on coagulation may contribute to the bleeding diathesis often observed during the later stages of severe EHEC infection associated with mucosal damage during hemorrhagic colitis, diarrhea, and disseminated intravascular coagulation.

Poster Session

BAM-PM1076 - Survival, virulence and viable-but-nonculturable (VBNC) status of Legionella pneumophila in response to environmental changes using Fraquil, a defined water medium

Nilmini Mendis[1], Sébastien P. Faucher[1]
[1]*McGill University, Montreal, Canada*

Introduction: Legionella pneumophila is the etiological agent of Legionnaires' disease, caused by inhalation of contaminated aerosols. To date, there is lack of research investigating the relationship of this pathogen with its water environment using well-defined water sources. Results: We tested L. pneumophila survival in a range of temperatures from 4oC to 42oC in Fraquil, a defined, freshwater medium. The bacterial population survived poorly at 37oC and 42oC, the highest temperatures tested, as evidenced by the quick depletion of CFU counts within 2 months of water exposure. In contrast, L. pneumophila survived best at 16oC with unchanged CFU counts for over 6 months. At 4oC, no CFUs were detected after 3 months. Interestingly, a study of the VBNC state at 4oC showed that the bacteria were, in fact, viable for up to 2 weeks after losing culturability on standard laboratory media. This is in comparison to samples incubated at 37oC and 42oC that lost both culturability and viability simultaneously. Then, to test the loss or retention of virulence capacity of bacteria incubated in water for different periods of time, we used L. pneumophila exposed to water for 1 week and 1 month to infect amoeba and human macrophages. No difference in the virulence potential was observed. Conclusions: Using Fraquil, we demonstrate that L. pneumophila is sensitive to high temperatures and that it enters a VBNC state when exposed to 4oC. Our results support growing literature studying VBNC bacteria, in that they may pose significant problems to our current capacity to detect Legionella and control outbreaks of Legionnaires' disease. We also show that L. pneumophila can survive up to 6 months in water at 16oC. Furthermore, we present evidence that this bacterium is able to retain full virulence potential, even after a 1 month exposure time to the nutrient-depleted water environment.

Poster Session

BAM-PM1078 - The glutamate decarboxylase system of *Listeria monocytogenes*: divergence of functionality between related strains

Conor Feehily[1], Kimon Andreas Karatzas[1], Aiden Finnerty[1], Pat Casey[2], Cormac Gahan[2], Conor O'Byrne[1]
[1]Microbiology, NUI Galway, Ireland, [2]Microbiology, University College Cork, Ireland

The food-borne pathogen *Listeria monocytogenes* can survive the transition from food to a mammalian host by deploying an acid protection mechanism known as the glutamate decarboxylase (GAD) system. The conventional view of this system is that extracellular glutamate is transported into the cell in exchange for gamma aminobutyrate (GABA) and the decarboxylation of glutamate to GABA, which consumes a proton, helps to prevent the acidification of the cytoplasm. In this study we examined the role of the GAD system in acid tolerance in 2 well-studied strains of *L. monocytogenes*; 10403s and EGDe, both of which belong to serotype 1/2a. The three glutamate decarboxylase genes (*gadD1*, *gadD2* and *gadD3*) were deleted in both strains and the resulting effects on GABA production and acid survival were measured. To test the role of the GAD system in virulence using the mouse model the 3 deletions were introduced into a murinised strain of EGDe (EGDm), which carries a modified version of the internalin gene allowing productive infections in mice. The analyses showed that only 10403s produces extracellular GABA in response to acidification of the medium. EGDm accumulates intracellular GABA but does not efflux it. In EGDm only the loss of *gadD3* reduced the ability to produce GABA, whereas in 10403s only loss of *gadD2* had this effect. Furthermore only in 10403s did any of the single deletions impact acid survival; loss of *gadD2* produced an acid sensitive phenotype. In EGDm an acid sensitive phenotype was observed only when both *gadD1* and *gadD3* deletions were combined in the same strain. Taken together these results indicate that the GAD systems have evolved to operate quite differently in these two related strains of *L. monocytogenes*. Furthermore in EGDm loss of the decarboxylase genes produced only a modest impact on virulence in mice.

Poster Session

BAM-PM1080 - Importance of reciprocal balance between Th1 and Th17 immunity in Mycobacterium abscessus complex lung disease

Sung Jae Shin[1], Su-Young Kim[2], Hye Yun Park[2], Kyeongman Jeon[2], O Jung Kwon[2], Sang-Nae Cho[1], Won-Jung Koh[2]

[1]Department of Microbiology and Institute of Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul, South Korea, [2]Division of Pulmonary and Critical Care Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

Little information for host immunity against Mycobacterium abscessus complex (MABC) is available regarding changes in serum immunomolecules levels in MABC lung disease during antibiotic therapy. This study was undertaken to investigate whether altered levels of serum immunomolecules during treatment in patients with MABC lung disease can reflect the disease-associated characteristics. A total of 50 cytokines from 24 patients with MABC lung disease were quantitatively assayed using the multiplex bead-based system according to 1) patients with MABC lung disease verse healthy controls and 2) alterations after antibiotic therapy in the MABC lung disease group. In addition, analyses of these cytokines were further performed to determine whether they were specifically associated with the disease phenotype, treatment outcome, and etiological agents. The serum levels of T helper type 1 (Th1)-related cytokines, IFN-γ and IL-12, and Th2-related cytokines, IL-4 and IL-13, were significantly decreased in the patients compared with healthy controls. In contrast, the levels of Th17-related cytokines, IL-17 and IL-23, were significantly increased in the patients compared with healthy controls. With the exception of adiponectin, all of immunomolecules were decreased or not differed at post-treatment compared to pre-treatment. Each level of cytokines between pre- and post-treatment was not different in all cytokines according to patterns of diseases and causative species. In comparing pre- and post-treatment levels in patient subgroups, IP-10 and MIG were significantly lower in patients with successful sputum conversion than in patients with failure of sputum conversion at post-treatment. A low level of Th1-related cytokines and high levels of Th17-related cytokines in patients may be a major cause perpetuating MABC lung disease. Improved comprehension of the role of the investigated cytokines in inducing, maintaining, and regulating immune responses could provide major insights into how a host responds to MABC infection during therapy and could contribute to predicting disease status.

Poster Session

BAM-PM1082 - C. albicans growth, transition and interaction with human gingival fibroblasts were modulated by cigarette smoke

Humaidah Alanazi[1], Abdelhabib Semlali[2], Mohammed Alanazi[2], Mahmoud Rouabhia[1]
[1]Laval University, Dental Faculty, Quebec, Canada, [2]Genome Research Chair, College of Science King Saud University, Riyadh, Saudi Arabia

Microbial biofilms have been described as being present in most bacterial habitats in the human body such as the oral cavity contributing to oral infection1. Furthermore, exposure to cigarette smoke has been reported to promote the formation of biofilm by various oral/respiratory pathogens. Through biofilm, smokers often exhibit periodontal disease that is more severe than in non-smokers2,3. It can also promote oral candidiasis. The aim of the present study was to assess the effect of cigarette smoke condensate (CSC) on C. albicans growth, transition and interaction with human gingival fibroblasts. Using a well-designed experimental protocol that includes C. albicans and CSC, we demonstrated that following contact with CSC, C. albicans showed higher growth and transition. This was basically observed at high concentration of CSC. The effect of CSC on C. albicans was also observed following pre-incubation of C. albicans with CSC and then contact with human gingival fibroblast. CSC-primed C. albicans adhered more to fibroblast. The high adhesion level was observed with 30% CSC. This adhesion was supported by high transition levels of CSC-primed, as compared to non-primed C. albicans. Proliferation of CSC-primed C. albicans was also promoted following contact with fibroblasts. This was at different time points (24, 48 and 72h). It is also important to note that fibroblast growth was reduced following contact with CSC-primed C. albicans. Overall, this study demonstrated that CSC can increase the pathogenesis of C. albicans by promoting the yeast growth, transition and damaging effect on human gingival fibroblast. Tobacco smoke can contribute to Candida oral infection (Funded by the Fonds Émile Beaulieu). [1] Bjarnsholt T. APMIS Suppl.,2013; 121,1–51; [2] Feldman et al., Semin Respir Crit Care Med. 2012; 33, 232–243; [3] Palmer et al., J Clin Periodontol 2005; 32: Suppl, 6180–195.

Poster Session

BAM-PM1084 - Effect of the human intestinal microbiota and Bacteriodes thetaiotaomicron on Escherichia coli O157:H7 transcriptome: multiple aspects of EHEC adaptation

Guillaume Le Bihan[1], Grégory Jubelin[2], Philippe Garneau[1], Annick Bernalier-Donadille[2], Christine Martin[2], Josée Harel[1]

[1]Université de Montréal, Faculté de Médecine Vététinaire, St-Hyacinthe, Canada, [2]INRA, Clermont-Ferrand-Theix, France

Enterohemorragic E. coli (EHEC) are human pathogen frequently responsible for large outbreaks in developed countries. EHEC O157:H7 produce the Shiga toxins and colonize the epithelium of the colon using a type III secretion system (T3SS) which is involved in the formation of attaching and effacing lesions. When EHEC reach the large intestine, they face the digestive environment and compete with the gut microbiota to colonize the epithelial cells. We hypothesise that EHEC adapt their gene expression in response to the intestinal metabolome modulated by human microbiota (HM) and Bacteroides thetaiotaomicron, a predominant bacterial species of the gut. Using microarrays and qRT-PCR we assessed the adaptation of EHEC to the HM and B. thetaiotaomicron. The global gene expression of the EHEC O157:H7 strain EDL933 cultivated in the intestinal content of germ-free rats was compared with that of the strain cultivated in the intestinal contents of rats associated with the HM and also with that of the strain cultivated in the intestinal contents of rats associated with B. thetaiotaomicron. The metabolic profile of EHEC changed in response to the HM by switching from glycolytic to gluconeogenic pattern. Pathways involved in the degradation of sialic acid, ethanolamine, amino acids and microbiota-derived compounds were up-regulated in response to the HM while genes required for the utilization of simple sugars and glycerol were down-regulated. Moreover, the expression of genes encoding for the T3SS and its secreted effectors was decreased in the intestinal content of rats associated with the HM and more importantly with B. thetaiotaomicron. Using transcriptional fusions, we showed that some compounds in the HM, repressed the expression of some virulence genes. The identification of the nutritional niche of EHEC in the intestines as well as compounds regulating virulence genes would allow novel approaches to prevent and fight EHEC infections in human.

Poster Session

BAM-PM1086 - Elucidating the regulatory mechanisms that control phenolic glycolipid (PGL-tb) biosynthesis in *Mycobacterium tuberculosis*

Hanna Ostapska[1], Dominic Nehme[1], Michael Reed[1]
[1]*Department of Medicine & Department of Microbiology and Immunology, McGill University Health Centre, McGill University, Montreal, Canada*

The pathogenicity of *Mycobacterium tuberculosis* (Mtb) is often attributed to components of the unique, lipid-rich mycobacterial envelope. For example, our previous work has shown that a subset of Mtb strains from the East Asian lineage which show hyperlethality in murine models are able to produce the immunomodulatory phenolic glycolipid, PGL-tb. It is interesting, however, that the majority of strains within this lineage do not produce this complex lipid, even though the pks1-15 gene necessary for PGL-tb biosynthesis is intact in both PGL-tb producing and non-producing strains. Indeed, all Mtb strains other than members of the Euro-American lineage carry an intact pks1-15 gene. Therefore, we investigated whether PGL-tb non-producing strains would be capable of synthesizing PGL-tb if induced to do so by introducing a second copy of pks1-15 under control of a constitutive promoter. In this manner, we found that both East Asian and Indo-Oceanic isolates produced large quantities of PGL-tb, which confirmed that these strains were fully competent with respect to PGL-tb biosynthesis. We further addressed this strain variability in PGL-tb production by quantifying pks1-15 gene expression with qRT-PCR and found no correlation between PGL-tb lipid production and the level of pks1-15 transcription in vitro. Our data suggests PGL-tb production is controlled either by post-transcriptional or post-translational mechanisms and that for strains which produce PGL-tb constitutively in vitro, this regulatory mechanism is inactive. Currently, we are raising antibodies against the PKS1-15 protein to ascertain whether the mechanism of regulating PGL-tb synthesis is post-translational. Additionally, we are carrying out macrophage infection studies to determine if these strains that do not produce PGL-tb in vitro, can be induced to do so upon in vivo infection. Together, we anticipate these lines of inquiry will enable us to elucidate the regulatory mechanisms controlling PGL-tb production, and potentially other virulence related lipids, in Mtb.

Poster Session

BAM-PM1088 - Streptococcus agalactiae (GBS) screening among pregnant women in Bukavu City, Democratic Republic of the Congo (DRC)

TK Mitima[1], S Ntamako[2], JK Mutendela[3], A Birindwa[4], N Mukanire[5], K Mubagwa[6]

[1]Laboratoire des Recherches Biomédicales et de Santé Publique (UEA) et ISTM – Bukavu, RD Congo., [2]Université Evangélique en Afrique, Département de Médecine Interne et Pathologies infectieuses et parasitaires, RD Congo, [3]Médecins d'Afrique (MDA), Coordination-Europe, Savigny Sur Orge, France., [4]Département de Pédiatrie, Hôpital Général de Panzi, Université Evangélique en Afrique, [5]Département de Gynéco-Obstétrique, Hôpital Général de Panzi, Université Evangélique en Afrique, [6]International Center for Advanced Research and Training (ICART), Bukavu, DR Congo

Objectives To determine the prevalence of maternal colonization by Streptooccus agalactiae and to identify factors influencing this colonization. Method Vaginal sample were collected during prenatal consultation of women at 3rd quarter of pregnancy. These pregnant women were in total 509, consulting at 12 Health Centers of Bukavu Town. A bacteriological examination was conducted on this vaginal sample. Women with fistulas or those swallowing antibiotics were excluded in this study. Variables included age, socioeconomic level, parity, obstetric- gynecology antecedents, urinary infectious in progress pregnancy and HIV. Results The frequency of colonization was 20%. High level of colonization by GBS is significantly associated by low school education level (OR=2.50, IC 95% 1.57 - 3.97, p=3.10-5), urinary tract infections on progress pregnancy (OR = 3.97, IC 95% 2.46 - 6.39, p=10-7), notion of premature childbirth abortion (OR=8.19, IC 95% 4.49 - 15.01, 10-7) and HIV positive serology (OR=4.22, IC 95% 1.49 - 11.97, p = 10-3). There's a need for further deep study on this issue to enable a follow up of new babies born from mothers infected with GBS. Such study will help in setting strategies for prevention and treatment of babies born from women colonized by Streptococcus agalactiae in South - Kivu Province, Earsten DR Congo.

Poster Session

BAM-PM1090 - Wild and Domestic Green Iguanas (*Iguana iguana*) from Grenada are reservoir for *Salmonella* spp: prevalence, serovars and antimicrobial susceptibility

Victor Amadi[1], Wayne Sylvester[2], Rhonda Pinckney[1], Calum Macpherson[3], John McKibben[4], Rodolfo Bruhl-Day[2], Roger Johnson[5], Harry Hariharan[1]

[1]Pathobiology Academic Program, School of Veterinary Medicine, St. George's University, Grenada, West Indies, [2]Small Animal Medicine and Surgery Academic Program, School of Veterinary Medicine, St. George's University, Grenada, West Indies, [3]Department of Microbiology, School of Medicine and Windward Islands Research and Education Foundation, St. George's University, Grenada, West Indies, [4]Anatomy and Physiology Academic Program, School of Veterinary Medicine, St. George's University, Grenada, West Indies, [5]Office International des Epizooties Reference Laboratory for Salmonellosis, Public Health Agency of Canada, Canada

Salmonellosis is an important public health problem worldwide. In the United States, *Salmonella* spp. cause estimated one million human illnesses annually. The pattern of *Salmonella* infection is associated mainly to animal reservoirs. Many reptiles, including the green iguana, harbor *Salmonella* as part of their normal flora and do not show clinical signs. Rarely, the green iguana may develop a weeping, crusting, vesicular dermatitis due to *Salmonella*. We sampled cloacal swabs from 62 green iguanas, including 47 wild and 15 domestic ones from five parishes of Grenada, West Indies, during a 4-month period of January to April 2013, and examined by enrichment and selective culture for the presence of *Salmonella* spp. Fifty-five per cent of the animals were positive, and eight serovars of *Salmonella* were isolated. The most common serovar was Rubislaw (58.8%), a serovar found recently in many cane toads in Grenada, followed by Oranienburg (14.7%), a serovar that has been causing serious human disease outbreaks in Japan. Serovar IV:48:g,z51:- (formerly, *S*. Marina) highly invasive and known for serious infections in children in the United States, constituted 11.8% of the isolates, all of them being from domestic green iguanas. *Salmonella* Newport, a serovar recently found in a blue land crab in Grenada, comprised 11.8% of the isolates from the green iguanas. The remaining four less frequent serovars included *S*. Javiana and *S*. Glostrup. Antimicrobial susceptibility tests conducted by a disc diffusion method against amoxicillin–clavulanic acid, ampicillin, cefotaxime, ceftazidime, ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, streptomycin, tetracycline and trimethoprim–sulfamethoxazole showed that drug resistance is minimal, with intermediate susceptibility, mainly to streptomycin, tetracycline and cefotaxime. This is the first report of isolation and antimicrobial susceptibilities of various *Salmonella* serovars from wild and domestic green iguanas in Grenada West Indies.

Poster Session

BAM-PM1092 - Roles of intimin, type 3 secretion system and type 1 pilus of atypical enteropathogenic Escherichia coli in the infection of different enterocytes models

Denise Yamamoto[1], Rodrigo T. Hernandes[2,1], Ana M. Liberatore[1], Ivan H.J. Koh[1], Cecilia Abe[3], Rodrigo Barbosa de Souza[1], Tânia Gomes[1]

[1]UNIFESP - Universidade Federal de São Paulo , São Paulo, Brazil, [2]UNESP - Universidade Estadual Paulista Júlio de Mesquita, Botucatu, Brazil, [3]IBu - Instituto Butantan , São Paulo, Brazil

Atypical enteropathogenic Escherichia coli (aEPEC) are agents of diarrhea worldwide. These microorganisms promote attaching-effacing (AE) lesions on enterocytes, whose determinants are located in the locus of enterocyte effacement (LEE) region. Previously, we demonstrated that the aEPEC strain 1551-2 invades differentiated T84 cells, preferentially through the basolateral surface. To reach that surface, Salmonella, Shigella and Yersinia bind to M cells by the Type 1 Pilus (T1P). Here we evaluated the contribution of the adhesin intimin, the translocated intimin receptor, and Type Three Secretion System by single mutagenesis of the LEE genes, eae, tir and escN, respectively, and the fimA gene, encoding the T1P major pilin, in the ability of strain 1551-2 to infect different enterocytes models. The in vitro bacterial translocation (BT) potential was evaluated by infection of M-like cells, obtained by co-culturing Caco-2 and Raji-B cells in Millicell system, and quantifying bacteria recovered from the bottom chamber. In addition, rat ileum fragments (Wistar-EPM rats) were infected ex vivo and analyzed by Transmission Electron Microscopy. For in vivo BT, bacterial suspensions were confined between ligated duodenal and ileum of rats and BT was quantified in Mesenteric lymph nodes (MLN), liver and spleen. Presence of M-like cells increased the aEPEC 1551-2 translocation from 119.6±75.7 (Caco-2 cells only) to 2,869±762.1 CFU ($P<0.05$). Formation of AE lesion and invasion was observed on enterocytes in the ex vivo model infected with 1551-2. In the in vivo BT model, a similar number of CFU of 1551-2 and T1P mutant strain was recovered from MLN (CFU: 6.60±3.14 and 7.82±2.08, $P=0.98$) and liver (0.001±0.0005 and 0.045±0.042, $P=0.32$). Except for the T1P mutant, all the other mutants lost all of these features, suggesting that in aEPEC 1551-2 these virulence factors are essential for M-like cells translocation, AE lesion establishment, enterocyte invasion, and in vivo BT translocation to extraintestinal sites.

Poster Session

BAM-PM1094 - Immunological responses to Malaria and bacterial co-infections

Maria Nelson[1], Patrik Enström[1], Shahab Nahrevanian[1], Ingela Nilsson[1], Anna Fahlgren[1], Johan Normark[1], Sven Bergström[1]
[1]*Department of Molecular Biology, Umeå, Sweden*

In sub-Saharan Africa, concomitant infections occur in high frequencies and have been observed in numerous surveys. Little is known about their impact on the human health despite findings that concomitant infections with malaria aggravates disease leading to increased mortality and morbidity. It is known that a fine tuned balance between pro- and anti-inflammatory responses is required to clear malaria parasites without inducing major host pathology, suggesting that timing and intensity of the different types of responses are crucial for the outcome of infection. In order to study the mechanisms responsible for the pathophysiological consequences seen in concomitant infected patients, we carried out experiments in mouse model using two important pathogenic bacteria found in malaria co-infected patients: Streptococcus pneumonieae and Relapsing fever Borrelia. Serum Analysis from malaria/Borrelia co-infected mice showed diminished bioavailability of NO, which argues for a dysfunctional endothelium. This corresponded to an over expression of ICAM-1 and VCAM by brain endothelial cells, as well as increased sequestration of CD8+ cells in the brain. Cytokine analysis of plasma from these mice showed increased pro-inflammatory response (IL1ß and TNF-α), as well as inability to down regulate the same through IL-10. Our studies show that experimental cerebral malaria (ECM) is induced in co-infected mice due to loss of timing and control over regulatory mechanisms in antigen presenting cells. Preliminary results from the concomitant infection malaria/pneumococci suggest a radically different pathology where co-infection affects both nasopharyngeal carriage and sepsis progression.

Poster Session

BAM-PM1096 - Towards a novel antimicrobial against the opportunistic pathogen Pseudomonas aeruginosa by revealing regulators and inhibitors of the novel virulence factor, AaaA

Esteban Paredes-Osses[1], Stephan Heeb[1], Miguel Camara[1], Charles Laughton[2], Kim R. Hardie[1]
[1]School of Life Sciences, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham, UK,
[2]School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham, UK.

Pseudomonas aeruginosa is a major cause of nosocomial infections, particularly in patients with burns or cystic fibrosis. The P. aeruginosa genome encodes at least four proteins displaying the characteristic three domain structure of autotransporters. Autotransporters are the largest family of secreted proteins in Gram-negative bacteria, and those characterised in pathogens to date are virulence factors. We reported the characterisation of the PA0328 autotransporter as a cell-surface tethered and arginine-specific aminopeptidase that we named AaaA. AaaA offers a fitness advantage in environments where the sole source of nitrogen is peptides with an amino terminal arginine, and is vital for establishing an infection as the lack of AaaA led to attenuation in a murine chronic wound infection which correlated with lower levels of some cytokines. Here, we describe evidence that is shedding light on the regulation of aaaA which includes the identification of regulators that control related metabolic pathways and an alternative sigma factor of RNA polymerase. Moreover, our structural modelling has identified the putative active site of AaaA, and mutants of AaaA with single amino acid changes are enabling us to define the active site of AaaA, thereby facilitating the screening for inhibitors that could be exploited as potential therapeutic agents.

Poster Session

BAM-PM1098 - Vibrio cholerae CpxR is required for activation of resistance-nodulation systems

Nicole Acosta[1], Stefan Pukatzki[2], Tracy Raivio[1]
[1]*Departments of Biological Sciences, University of Alberta, Edmonton, Canada,* [2]*Department of Medical Microbiology & Immunology, University of Alberta, Edmonton, Canada*

The Cpx pathway is a two component system, composed of the sensor histidine kinase CpxA, and the response regulator CpxR. This system regulates crucial envelope stress responses across bacterial species and antibiotic resistance. It regulates protein folding and degrading factors involved in the alleviation of the envelope stress. To identify CpxR-regulated genes, we determined changes of the pandemic V. cholerae El Tor strain C6706 transcriptome after over-expression of the response regulator CpxR. Over-expression of CpxR led to increases and decreases in expression of genes with diverse functions in the cell, mainly genes involved with iron uptake and RND efflux pumps. For example, we found that several genes that encode proteins required for iron acquisition were up-regulated by the Cpx pathway. We determined that some of the novel inducing cues for the Cpx pathway were dependent on the iron status in the inner membrane, as tested using a luminescent reporter.To our knowledge, this is the first demonstration that the Cpx pathway is important for regulation of iron acquisition in V. cholerae. Moreover, activation of the Cpx pathway led to the expression of two resistant nodulation systems (RND) (i.e. vexAB and vexGH), and TolC, the major outer membrane pore in V. cholerae. Additionally, we found that inactivation of this two RND systems activate the Cpx pathway. Our results suggest that iron is an important modulator for the Cpx pathway signaling and the Cpx pathway mediate adaptation to envelope perturbations caused by iron depletion and the presence of toxic compounds.

Poster Session

BAM-PM1100 - IgG antibody in BCG – vaccinated neonates in Asaba, Nigeria

Eyaufe Aidevbo[1], Esemeh Fredrick[1], Adeniran Kayode[2]
[1]Ambrose Alli University, Ekpoma, Nigeria, [2]Federai Medical Center, Asaba, Nigeria

Mycobacterial infection induces cell mediated and humoral responses, although the role of cell mediated immunity is well established. This study examines IgG antibody levels in BCG vaccinated neonates in Asaba Pre and Post BCG vaccination (six weeks after BCG was administered). 182 serum samples from neonates (120 Pre BCG vaccination and 62 Post BCG vaccination) were analysed using the Diagnostic Automation Mycobacterium tuberculosis ELISA kits. TBIgG levels was significantly higher Post BCG vaccination than TBIgG levels Pre BCG vaccination ($P<0.05$). TBIgG levels was similar for both male and female subjects Pre and Post BCG vaccine administered resulted in an increase in TBIgG levels and the response was the same for male and female subjects that enrolled for this study. Therefore the potential role of antibodies in combating mycobacterial infection should be revisited.

Poster Session

BAM-PM1102 - Cigarette smoke rapidly induces genetic and epigenetic changes in Porphyromonas gingivalis.

Iris Zeller[1], Ted Kalbfleisch[1], David Scott[1]
[1]*University of Louisville, Louisville, USA*

The anaerobic, Gram negative bacterium Porphyromonas gingivalis is a causative agent of periodontal diseases that exhibits high phenotypic variation. Smokers are more likely than non-smokers to be infected with P. gingivalis and to exhibit more severe disease despite reduced overt inflammation. Data regarding the impact of tobacco use on the genome of bacteria are sparse, and knowledge about its impact on the bacterial epigenome is absent. The aim of this project was to study the propensity of cigarette smoke extract (CSE) to induce genomic and epigenomic changes in P. gingivalis in the short term. Therefore, the genome of P. gingivalis cultures grown in CSE-conditioned (Kentucky 2R4F reference cigarettes; 1000 ng/ml nicotine equivalents) and control media over 25 passages were compared by single molecule real time (SMRT) sequencing. Even over this short time frame, CSE-exposure induced multiple genomic variations, including deletions and insertions, and altered the methylation status at a cluster of sites in P. gingivalis. The consequences for the emergence of more virulent strains and altered host responses to this important periodontal and systemic pathogen have yet to be ascertained.

Poster Session

BAM-PM1104 - Type III secretion substrate recognition by the Salmonella enterica SPI-2 encoded ATPase SsaN

Sarah Allison[1], Brian Tuinema[1], Ellen Everson[1], Seiji Sugiman-Marangos[1], Kun Zhang[1], Murray Junop[1], Brian Coombes[1]

[1]*McMaster University, Hamilton, Canada*

Salmonella enterica serovars are Gram-negative pathogens that cause a range of human diseases including gastroenteritis and typhoid fever. These pathogens employ two type III secretion systems (T3SSs) for the translocation of bacterial virulence proteins into host cells. An important component of T3SSs is a conserved ATPase that facilitates effector secretion; however, to date, there has been limited work addressing the role of the putative SPI-2 encoded ATPase, SsaN, in effector recognition and secretion. In this study, we undertook a phenotypic and structural characterization of SsaN. We show that a ΔssaN strain is highly attenuated in vivo and is defective for secretion of a subset of SPI-2 effectors. Effectors for which SsaN was dispensable for secretion were shown to be targeted to the T3SS-1 system in the absence of SsaN. To address the mixed dispensability pattern of ssaN for effector secretion, we probed a network of chaperone and effector interactions with SsaN through co-immunoprecipitation experiments. We demonstrate that SsaN interacts with only a subset of chaperones and that SsaN can recognize effector substrates directly, suggesting that effector secretion can occur by both chaperone-dependent and independent mechanisms. In addition, we present the 2.1Å-resolution crystal structure of SsaNΔ1-89 which we have used to begin mapping interaction domains relevant to SsaN function. In summary, our findings address the role of ssaN in the pathogenesis of Salmonella enterica and provide insight into effector substrate recognition and secretion by the SPI-2 encoded T3SS.

Poster Session

BAM-PM1106 - Effect of Stx1-phage on Stx2 production by Escherichia coli O157:H7 strain Sakai

Yongxiang Zhang[1], Wenyu Zhang[1], Chad Laing[1], Roger Johnson[2], Victor J. P. Gannon[1]
[1]Public Health Agency of Canada, Lethbridge, Canada, [2]Public Health Agency of Canada, Guelph, Canada

E. coli O157:H7 is associated with outbreaks and sporadic cases of hemorrhagic colitis and the hemolytic-uremic syndrome in humans. Since its isolation in 1982, this organism has become one of the most important food- and waterborne zoonotic pathogens in the world. Shiga toxin 1 (Stx1) and Stx2 are two of the most important virulence factors produced by this pathogen and are encoded by lambda-like prophages integrated into the bacterial chromosome. It was found that the presence of more than one prophage within a cell can lead to lowered levels of Stx2 production. Our previous study revealed that the specific genetic lineage of O157 strains and the source of their isolation are related to Stx2 production levels. We identified two closely related O157:H7 strains LRH6 and Sakai, which have 99% nucleotide sequence identity between their genomes. LRH6 carries only the Stx2-phage, which is 99% identical to that of Sakai strain, Sakai carries both the Stx1- and Stx2-phage. We found that LRH6 produced 20 times more Stx2 than Sakai. In this study, We created the stx1-phage-deletion mutant ΔΦstx1 from strain Sakai and the stx2 toxoid mutant 299E167Q from strain LRH6 and compared Stx2 production by Sakai, ΔΦstx1 and 299E167Q. The ΔΦstx1 produced 5% more Stx2 than Sakai and 10 folds less than LHR6. We also found that introduction of the CI-repressor from the Stx1-phage in Sakai into 299E167Q resulted in 25% reduction in Stx2 production whereas introduction of CI into strain ΔΦstx1 led to 15% elevation of Stx2 production. Conclusions: While differences in the nucleotide sequence of the Stx2-encoding phage in O157:H7 strains may affect levels of Stx2, regulatory factors located elsewhere in the genome, such as Stx1 phage or its components such as the CI repressor may contribute to the levels of expression of Stx2.

Poster Session

BAM-PM1108 - Plant growth stimulation and root colonisation of soybean (Glycine max L.) by Bradyrhizobium japonicum and Pseudomonas putida as affected by phosphorus and nitrogen source

Dilfuza Jabborova[1], Dilbar Qodirova[2], Dilfuza Egamberdieva[1]
[1]National University of Uzbekistan, Tashkent, Uzbekistan, [2]Termez State University, Termez, Uzbekistan

Soybean (Glycine max L.) is an important legume which contains high amount of protein and considered as an excellent rotation and intercropping crop by improving soil fertility. The available essential nutrient elements such as N, P in soil to the soybean growth are one of the key components to determine their productivity. The effect of N and P availability on the growth and colonisation of Bradyrhizobium japonicum and Pseudomonas putida on the rhizosphere of soybean (Glycine max L.) was studied. Three modified solution HNHP (high N -3000 μmol/L, high P -250 μmol/L), HNLP (high N-3000 μmol/l, low P -50 μmol/l), LNLP (low N-300 μmol/l, low P -250 μmol/l) were maintained in the gnotobiotic sand system. Decreasing of the N and P concentrations in growth medium inhibited the ability of B. japonicum strain NU1 cells to colonize soybean roots, CFU counts decreasing by 20 %, from 107.0x103 (HNHP) to 86.5x103 (LNLP) CFU/cm of root tip respectively. In competitive root tip colonization assay P. putida TSAU1 were better colonizers than B. japonicum NU1. Under Low N and P concentrations the root, shoot length, fresh and dry weight of soybean reduced by B. japonicum. Co-inoculation of soybean with B. japonicum NU1 and P. putida TSAU1 showed the highest stimulatory effect, by increasing significantly dry weight by 39% (HNHP) and 66% (LNLP) in comparison to inoculation with B. japonicum NU1 alone. We observed that plant growth stimulation by co-inoculation of soybean was higher in low N and P condition compared to the single-inoculation.

Poster Session

BAM-PM1110 - *Paenibacillus sp.* - a potential biocontrol agent for black rot of Brassica

Hoda Ghazalibiglar[1], John Hampton[1], Eline van Zijll de Jong[1], Andrew Holyoake[1], Alison Stewart[2]
[1]Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand, [2]Marrone Bio Innovations Incorporation, USA

Black rot caused by a seed-borne bacterial pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) is one of the most devastating diseases of brassicas worldwide and a major problem for New Zealand's seed industry. This research addresses the use of a gram positive bacterium, *Paenibacillus*, for biological control of black rot on cabbage. Twenty-four isolates of *Paenibacillus* were categorized on their interactions with *Xcc* in dual culture assays. Eight *Paenibacillus* isolates with different bioactivity against *Xcc* were selected to be screened for their capacity to reduce black rot symptoms on cabbage in pot trials. From these results one *Paenibacillus* isolate (P16), at the concentration of 5×10^9 CFU/ml was selected. To investigate if the disease control was provided via plant growth promotion, P16 was co-applied with *Xcc* as a seed treatment. In the presence of Xcc, P16-treated seedlings had significantly *($P<0.05$)* greater growth than the control. To determine whether P16 is rhizosphere competent and/or endophytic, a real-time PCR assay using a P16-specific primer set based on the gyrB gene was used to detect and quantify P16 from cabbage seedlings grown from P16-treated seeds (1.5×10^7 CFU/seed) and their rhizosphere and bulk soil over time. The detection limit of the real-time PCR assay for soil and plant samples was determined as 1×10^3 CFU/g. In rhizosphere soil, P16 density had decreased from 9.9×10^5 to 1.1×10^3 CFU/g by 11 days after sowing (DAS), while in the bulk soil it was only detected up to 6 DAS. P16 was not recorded in plant samples, indicating either that P16 is not endophytic or its density in the plant was below the detection limit. Overall, P16 is rhizosphere competent only during early cabbage seedling growth, and is most probably not endophytic. However, it appears that P16, by reducing *Xcc* infection, better enables the seedlings to survive and grow.

Poster Session

BAM-PM1112 - Phylogenetic studies and amplified ribosomal DNA restriction analysis (ARDRA) reveal close association of Pseudomonas oryzihabitans with dieback-affected Dalbergia sissoo trees

Hanny Tantau[1], Annika Stubbe[1], Denise Palm[1], M. Imdadul Hoque[2], Rakha Hari Sarker[2], Sheik Shamimul Alam[2], Mihir Lal Saha[2], Md. Salim Khan[3], Hans-Peter Muehlbach[1]

[1]Biocentre Klein Flottbek, University of Hamburg, Hamburg, Germany, [2]Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh, [3]BCSIR, Tissue Culture Section, Dhaka-1205, Bangladesh

Among plant pathogenic bacteria the ‘genus’ Pseudomonas is one of the most diverse taxonomic groups, which very often underwent revision in the past. In our studies on the bacterial community associated with dieback-affected sissoo trees (Dalbergia sissoo Roxb.) in Bangladesh we found bacteria related to the Pseudomonas group in 75.5% of diseased trees. In contrast, only 13.3% of non-symptomatic trees harboured pseudomonads. The close association with the dieback disease prompted us to study the isolates related to the Pseudomonas group in more detail. DNA based approaches were applied which can reveal genetic diversity in much finer details than classical taxonomic traits. However, by using multilocus sequence typing with several housekeeping genes, allocation of individual isolates to Pseudomonas species was inconsistent, when different genes were applied. Sequencing of almost the complete 16S rRNA gene of 33 selected Pseudomonas isolates finally allowed grouping into only two main clusters. The major one consisted of 19 isolates related to P. oryzihabitans, while in the minor one eight isolates related to P. putida were found together with very few other pseudomonads. The same clustering was obtained with the independent grouping method of amplified ribosomal DNA restriction analysis (ARDRA). These strategies showed that the majority of isolates from dieback affected sissoo samples, which exhibited pathogenic activity on test plants as well as on sissoo seedlings (Valdez et al., Bangladesh J. Bot. 42: 1-16, 2013), are closely related to the species Pseudomonas oryzihabitans.

Poster Session

BAM-PM1114 - Colonization of Bacillus subtilis B26 of Brachypodium distachyon as a model system to study host-endophyte interactions

Francois Gagne-Bourque[1], Boris Mayer[1], Jean-Benoit Charron[1], Suha Jabaji[1]
[1]Plant Science Department, McGill University, Montreal, Canada

A strain of Bacillus subtilis B26, recently reported as an endophytic bacterium of the bioenergy crop switchgrass (Panicum virgatum L.), is nonpathogenic and a growth enhancer of switchgrass. B. subtilis B26 endophytically colonizes swtichgrass seedlings and is vertically transmitted to seeds. Culture filtrate from B. subtilis B26 contains several well-characterized lipopeptide toxins and phytohormones. These qualities suggest that endophytic ability of this strain is a biological requirement for survival in nature and has strong potential as bio-inoculant for biomass enhancement of bioenergy crops. Brachypodium distachyon, is considered a tractable model plant for cereals and bioenergy grass species. Given its rapid cycling time and ease of cultivation, Brachypodium can serve as useful functional model for studying plant-endophyte interactions. Here, we examined the effect of B. subtilis B26 colonization in Brachypodium and the physiological, cellular and molecular responses. B. subtilis B26 successfully colonized all Brachypodium tissues and seeds with the highest DNA copy number found in stem and leaf tissues. Bacterized plants developed faster relative to non-bacterized plants and had a significant ($P < 0.05$) increase of 66%, 64%, 42%, and 376.9% in plant height, stem and root dry biomass, and number of seeds, respectively at 70 days post-inoculation. The ability of B. subtilis B26 to produce indole-3-acetic acid (IAA) and to solubilize inorganic phosphorus could explain this positive response. Under acute and chronic drought stress, bacterized Brachypodium performed significantly better than non-bacterized plants. In response to B. subtilis B26 inoculation, copy number of transcription factors DREB1B- and DREB2B-like that act upstream of genes involved in abiotic stress response (LEA-14-like and COR413), were all substantially up-regulated compared to drought-stressed non-bacterized plants. Our results demonstrate a compatible interaction between B. subtilis and Brachypodium and establish a new model with which to investigate mechanisms underlying resistance to abiotic factors in a tractable monocotyledonous model species.

Poster Session

BAM-PM1116 - Effect of a mixture of branched-chain alkanes on induced systemic resistance and bacterial endophyte populations in Nicotiana benthamiana

Paul Goodwin[1], Weihong Gao[1]
[1]*Environmental Sciences, University of Guelph, Guelph, Canada*

The roots of N. benthamaiana growing in organic soil were treated with water, Civitas which is a commercial mixture of branched-chain alkanes, or an emulsifier, which is used to make a solution of Civitas in water. Civitas induced resistance against Colletotrichum orbiculare and also affected the populations of bacterial endophytes in the plant. Eight endophyte colony types were cultured from roots, stem+petioles and/or leaves corresponding to six species of Bacillus and two species of Pseudomonas. Compared to the water or emulsifier controls, populations of the B. simplex strain LW4 colony type in roots and stem+petioles and populations of the Pseudomonas sp. strain LW3 colony type in roots were significantly increased. Both of those colony types also grew more in minimal media containing Civitas compared to minimal media alone or minimal media with emulsifier, indicating that they have alkane catabolising genotypes. Compared to the controls, populations of the other six colony types generally were not higher in N. benthamiana treated with Civitas or in minimal media containing Civitas. When an isolate of each colony type was inoculated onto N. benthamiana seedlings, B. simplex strain LW4 and Pseudomonas sp. strain LW3 were able to induce systemic resistance. Civitas applied to roots through the soil acts as a selective endophyte growth promoter in plants, increasing the populations of certain bacterial endophytes that have the ability to activate induced systemic resistance.

Poster Session

BAM-PM1118 - Enhancement of photosynthesis, biomass production and nutrient uptake of duckweed by plant growth-promoting bacteria

Tadashi Toyama[1], Yasuhiro Tanaka[1], Kazuhiro Mori[1], Masaaki Morikawa[2]
[1]University of Yamanashi, Kofu, Japan, [2]Hokkaido University, Sapporo, Japan

Duckweed, the common name for main genera of Lemnaceae: Lemna, Spirodela, Wolffia and Wolffiella, is the smallest and fast-growing aquatic plant. Duckweed is commonly used to recover nutrient (nitrogen and phosphorus) from agricultural and municipal wastewaters. On the other hand, duckweed has recently attracted significant attention as a good alternative feedstock for bioethanol production due to its great starch accumulation capability (up to 70% dry weight). Therefore, duckweed has a potential to establish a co-benefit system combining nutrient removal and bioenergy production from wastewater. The objectives of this study were to isolate plant growth-promoting bacteria from duckweed and to examine their effects on photosynthesis, biomass production and nutrient uptake of duckweed. First, we adopted Spirodela polyrhiza (giant duckweed) as model duckweed plant. More than 50 bacterial strains were isolated from S. polyrhiza roots. Sinorhizobium sp. SP4 showed the highest growth-promoting effect on S. polyrhiza in vitro assay. After 3 days co-culture of S. polyrhiza with strain SP4 in Hoagland solution, the rates of biomass (dry weight) growth, photosynthesis activity, nitrate uptake, phosphate uptake and chlorophylls a/b content of SP4-inoculated S. polyrhiza were up to 2.3, 1.9, 1.9, 3.5 and 2.3 times higher, respectively, than those of non-inoculated S. polyrhiza. Strain SP4 also dramatically changed some metabolite levels and gene expressions in S. polyrhiza metabolic pathways, including chlorophylls synthetic pathway, Calvin cycle, carbon fixation, glycolysis pathway and nitrogen uptake pathway. In wastewater treatment experiments, strain SP4-inoculated S. polyrrhiza showed the 2 times higher rates for biomass production, nitrate removal, phosphate removal and starch production compared to the non-inoculated S. polyrhiza. In addition, strain SP4 showed the growth-promoting effect on other 3 duckweed species, Lemna minor, Lemna aoukikusa and Wolffia arrhiza. So, the plant growth-promoting effect of strain SP4 seems to be highly-versatile effect on duckweed species.

Poster Session

BAM-PM1120 - Validation of a novel *mariner* Tn-Seq transposon vector for the study of the soil bacterium *Rhizobium leguminosarum*

Benjamin Perry[1], Chris Yost[1]
[1]*University of Regina, Regina, Canada*

The symbiosis of *Rhizobium leguminosarum* with leguminous plants allows legumes to colonize nitrogen deficient soils, and allows for the production of leguminous food crops without the application of industrially manufactured nitrogen fertilizers. Inoculant strains of *R. leguminosarum* have been commercially developed to enhance the yield of leguminous crops. Inoculant strain effectiveness can be limited by their decreased competitiveness with indigenous *R. leguminosarum* in the soil environment. The identification of cellular traits involved in competitiveness, such as metabolism, stress tolerance, chemotaxis, and signal transduction, has often used forward genetic techniques based on transposon mutagenesis. This technique has limitations due to its often laborious manual screening of thousands of individual transposon mutants. Here we describe the engineering and validation of a transposon vector that allows for the simultaneous screening of every gene in the *R. leguminosarum* genome, by coupling transposon mutagenesis with a next generation DNA sequencing technique known as transposon insertion sequencing (Tn-Seq). The newly developed transposon vector, pSAM_Rl, utilizes a *mariner* class transposon with modified inverse repeats to specifically capture genomic DNA adjacent to the transposon insertion, which is then used for sequencing. pSAM_Rl mutagenized *R. leguminosarum* at a higher frequency than previously used transposons, and functioned within a number of species in the family *Rhizobiaceae*, including *Sinorhizobium* and *Agrobacterium*. *In silica* modelling of pSAM_Rl integration sites in the *Rhizobium* genome found the number of insertion sites per gene was sufficient for robust statistical analysis. A preliminary Tn-Seq screen was performed on complex tryptone-yeast extract media to test the functionality of the transposon in a high-throughput genetic screen and to validate the *in silica* analysis.

Poster Session

BAM-PM1122 - Functional studies of swimming motility-related genes of Pantoea ananatis PA13, causing rice sheath rot

Inyoung Han[1], Yongsang Lee[1], Okhee Choi[1], Jinwoo Kim[1,2]

[1]Division of Applied Life Science (BK21Plus), Gyeongsang National University, Jinju, Republic of Korea, [2]Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea

Bacterial motility is known to play important roles in pathogenicity, biofilm formation, attachment to the host, and survival in ecosystem. Our model bacterium, Pantoea ananatis belonging to the family Enterobacteriaceae in the group Gamma-proteobacteria, is known to cause disease in maize, eucalyptus, onions, and rice. This bacterium is also known as an opportunist human pathogen. Recently, we reported an outbreak of rice sheath rot caused by P. ananatis and its complete genome sequences. Genome information revealed that strain PA13 harbors 46 flagellar biogenesis genes including master regulators FlhDC and specific sigma factor FliA, several chemotaxis genes, and motility-related genes. In this work, we present the results of a large-scale, mariner transposon-based genetic screening. A number of proteins that involved in electro chemical potential, proton motive force, sulfur transfer, nitrogen metabolism, ferrous transporting systems, and protease were found to affect the swimming movement of strain PA13. In addition, we also found that bacterial cell density-dependent expression and nucleotide second messenger, cyclic diguanylate (c-di-GMP) control swimming motility of strain PA13. This work will give novel insights into swimming motility at molecular level, and will contribute to a better understanding of how these proteins interact and function to regulate flagellar motility.

Poster Session

BAM-PM1124 - Quorum signal production in different rhizobial species and their role in the regulation of nod genes and the legume nodulation process

Fabiola E Pagliero[1], Lorda Graciela[1], Jorge Angellini[2], Castaño Carolina[1], Adriana Fabra[2]
[1]Facultad de Ciencias Exactas y Naturales, UNLPam, Argentina, [2]Facultad de Ciencias Exactas, Fisicoquímicas y Naturales, UNRC, Argentina

The rhizobia-legume symbiotic system is one of the most studied because of its importance in agricultural production. The symbiosis is initiated by the exchange of signals between rhizobia and legume roots which enables mutual recognition. Nodulation genes are expressed in response to flavonoids produced by the plant and its expression leads to the synthesis of Nod factors that are inducers of the initial stages of the interaction. The availability of nutrients and other environmental conditions affect this interaction. Regulatory mechanisms based on quorum sensing have been described in bacteria involved in both symbiotic and pathogenic associations with their host plants. The aim of this work was to study the influence of quorum sensing phenomenon on some aspects involved in the rhizobia-legume interaction. Bacteria used in this study were S. meliloti B399, B. japonicum E109, B. elkanii U1302, R. tropici CIAT 899 and Bradyrhizobium sp SEMIA 6144. All rhizobia studied produced quorum signal molecules of the type of AHL (acyl-homoserine lactone). Moreover, it was determined that the regulation of expression of the nod genes by quorum sensing varies with rhizobial species, and in some cases it is dependent on the metabolic state of the microorganism. Given that cell density is associated with the production of AHLs, its effect was studied on the symbiotic behavior of the rhizobia. Its influence on the infectivity was only observed in slow-growing rhizobia. Assuming the ability of microorganisms to sense heterologous AHLs, it was showed that the presence of established populations in the soil affect the infectivity or symbiotic nodulation depending on the system being analyzed. The phenomenon of quorum is involved in the regulation of different aspects of the rhizobia-legume symbiosis and constitutes a contribution to basic and applied knowledge on this type of association.

Poster Session

BAM-PM1126 - Broad-spectrum anti-pathogen activity of new isolates of Bacillus and Pseudomonas bacteria and their suppression of Fusarium crown and root rot disease of tomato

Liang Zhang[1,2], Salah Eddin Khabbaz[1,3], Aiming Wang[1], Pervaiz Abbasi[4]

[1]South Crop Protection and Food Research Center, London, Canad, [2]South China Agriculture university, Guangzhou, China, [3]General Commission for Scientific Agriculture Research, Hama, Syria, [4]Atlantic Food and Horticulture Research Centre, Kentville, Canada

More than 136 bacterial isolates obtained from potato soil and soybean leaves were screened for their growth inhibition of fungal pathogens in plate assays. Eleven of those isolates showed strong antagonistic activity against most fungi. Based on sequence analysis of 16S–23S rDNA gene, five of these isolates were identified as Bacillus amyloliquefaciens, three as B. Polymaxa, two as Pseudomonas chlororaphis, and one as P. fluorescens. These bacterial isolates and their culture filtrates showed variable results in production of HCN, siderophore, phosphate solubilisation, protease, β-1, 3-glucanse, chitinase, IAA, and SA. Antagonistic bacteria showed maximum growth inhibition of pathogen mycelia by the production of volatile compounds, and GC-MS analysis revealed over 15 volatile compounds in these isolates. Liquid culture filtrates of four isolates showed maximum growth inhibition of pathogen mycelia in plate assays. PCR analysis confirmed the presence of antibiotic biosynthetic genes such as phenazine carboxylic acid (PCA), 2, 4 diacetylpholoroglucinol (2, 4 DAPG), pyrrolnitrin, and pyoluteorin in most of these antagonistic bacteria. Bio-control potential of these antagonistic bacteria was evaluated in greenhouse trials. Treatment of tomato roots with irradiated peat formulations of antagonistic bacteria prior to planting in pathogen-infested potting mix provided protection of tomato plants from Fusarium crown and root rot disease caused by Fusarium oxysporum f. sp. radicis-lycopersici and enhanced plant growth.

Poster Session

BAM-PM1128 - The enzymatic shaving of Listeria monocytogenes: an attractive approach to explore its surfaceome

Céline Ribiere[1], Christophe Chambon[2], Mickaël Desvaux[1], Michel Hebraud[1,2]
[1]UR454 Microbiology, INRA Clermont-Ferrand, F-63122, Saint-Genès Champanelle, France, [2]PFEMcp, INRA Clermont-Ferrand, F-63122, Saint-Genès Champanelle, France

Listeria monocytogenes is a Gram-positive bacterium responsible for foodborne diseases. Its persistence in food industry is explained by its important capability of adaptation to hard environmental conditions linked to its ability to adhere and form biofilms on surfaces. The cell envelope and its protein components play a critical role in the phenomena of adaptation, environmental persistence and also in virulence. However, molecular determinants involved in biofilm formation by this pathogen bacterium are not yet well elucidated. The proteomic approach combining the enzymatic shaving of bacterial cell envelope and analysis of resulting peptides by LC-MS/MS is a powerful tool to study the “surfaceome” by tackling the limit of classical in-gel proteomic workflow in extracting and solubilizing cell-surface proteins. In this study, we aimed to optimize and implement a shaving strategy by using trypsin and isotonic buffer for maximizing the surfaceome characterization while reducing contamination by intracellular proteins. This approach was used to compare the surfaceome in exponential and stationary phase of growth but also in planktonic vs biofilm mode of growth in order to explore potential protein determinants involved in biofilm formation. Several experimental conditions were tested to optimize this method (buffer composition, trypsin concentration and incubation time) while cellular lysis was controlled by the presence of nucleic acids in the reaction medium. Extracted peptides were analyzed by nanoLC-tandem mass spectrometry (ESI-IT, LTQ Velos). Despite the optimization of the shaving method, intracellular proteins represent an important part of identified proteins. However, this approach allowed the identification of cell surface proteins whose extraction and separation are never obtained by classical in-gel approaches (for example cell-wall covalently linked proteins), and revealed surface proteins differentially expressed according to growth phase and between planktonic and biofilm mode of growth. This work was supported by the European Framework Program 6 with the ProSafeBeef research consortium (www.prosafebeef.eu)

Poster Session

BAM-PM1130 - Lipidated Dengue-2 envelope protein domain iii independently stimulates long-lasting neutralizing antibodies and reduces the risk of antibody-dependent enhancement

Chen-Yi Chiang[1], Chien-Hsiung Pan[1,2], Chun-Hsiang Hsieh[1], Jy-Ping Tsai[1], Mei-Yu Chen[1], Hsueh-Hung Liu[1], Shih-Jen Liu[1,2], Pele Chong[1,2], Hsin-Wei Chen[1,2], Chih-Hsiang Leng[1,2]
[1]National Health Research Institutes, Miaoli, Taiwan, [2]China Medical University, Taichung, Taiwan

Two important components of a vaccine, the immunogen and immunopotentiator, were combined into a single construct to generate a new generation of vaccines. We selected dengue-2 envelope protein domain III (D2ED III) as the immunogen and expressed this protein in lipidated form in Escherichia coli, yielding an immunogen with intrinsic immunopotentiation activity. The formulation containing lipidated D2ED III (LD2ED III) in the absence of exogenous adjuvant elicited higher D2ED III-specific antibody responses than those obtained from its nonlipidated counterpart, D2ED III, and dengue-2 virus. In addition, the avidity and neutralizing capacity of the antibodies induced by LD2ED III were higher than those elicited by D2ED III and dengue-2 virus. Importantly, we showed that after lipidation, the subunit candidate LD2ED III exhibited increased immunogenicity while reducing the potential risk of antibody-dependent enhancement of infection in mice.

Poster Session

BAM-PM1132 - Implication of the complement system in the adjuvant properties of a plant virus-like nanoparticle

Marie-Eve Lebel[1], Jean-François Daudelin[2], Nathalie Labrecque[2], Denis Leclerc[3], Alain Lamarre[1]
[1]INRS-Institut Armand Frappier, Laval, Canada, [2]Maisonneuve Rosemont Hospital Research Centre, Montreal University, Montreal, Canada, [3]Infectious Disease Research Center, Laval University, Quebec, Canada

Developing new adjuvants and vaccination strategies is of paramount importance to successfully fight against many life-threatening infectious diseases. Very few adjuvants are currently authorized for human use and these mainly stimulate a humoral response. However, specific antibodies are not sufficient to confer protection against persisting infections. We recently showed that Papaya Mosaic Virus-like nanoparticles (PapMV), are highly immunogenic in mice and are a good adjuvant to enhance humoral immune responses against co-administered vaccines. However, the mechanisms that confer these immunomodulatory properties to PapMV and its ability to enhance CD8+ T cell response remain unknown. Using immunization studies in mice, we demonstrate that PapMV induces immune activation through TLR7 ligation and type I interferon production. In addition, administration of PapMV before vaccination with bone marrow-derived dendritic cell (BMDC) loaded with the OVA peptide enhances the number of OVA-specific effector and memory CD8+ T cells and the proportion of CD8+ T cells producing cytokines. In fact, pretreatment with PapMV increases the expression of costimulatory molecules on BMDC, which could help to generate a better CD8+ T cell immune response. The use of PapMV also improves the protection against a Listeria monocytogenes expressing OVA challenge at the memory stage. Moreover, depletion of the complement system in mice increases the adjuvant effect of PapMV. In fact, absence of C3 complement component increases IFN-α production and immune cells activation following PapMV administration and CD8+ T cell response during BMDC immunization in combination with PapMV. In addition, C3 ablation seems to modify the spread and the persistence of PapMV in vivo. Our results demonstrate that PapMV is a suitable adjuvant for BMDC-based vaccines that could be applicable to the development of improved therapeutic DC vaccination strategies against chronic infections. Finally, we highlighted the role of the complement system in the adjuvant effect of PapMV

Poster Session

BAM-PM1134 - Cross-reactivity of anti-pneumococcal surface protein K (PspK) antibodies with pneumococcal strains that express various serotype capsular polysaccharides

In Ho Park[1], Han Wool Kim[1,2], Kyung-Hyo Kim[1,2]
[1]Ewha Center for Vaccine Evaluation and Study, Ewha Medical Research Institute, Soeul, Korea, [2]Department of Pediatrics, School of Medicine, Ewha Womans Univeristy, Seoul, Korea

Streptococcus pneumoniae is a common commensal colonizing the nasopharynx of healthy people and is the leading cause of community-acquired bacterial pneumonia, meningitis, and otitis media. Despite the high effectiveness of vaccine against vaccine-serotype pneumococcal diseases, the introduction of pneumococcal conjugate vaccine (PCV) has led to an increased incidence of pneumococcal disease caused by serotypes not contained in PCV. Our previous studies showed that Nontypeable S. pneumoniae (NTSp), which lacks capsule and is not targeted by the current pneumococcal vaccines, might have new and/or alternative capsule-independent survival mechanisms. Clinical evidence suggest that multiple antibiotic-resistant NTSp can be infectious in humans and cause mucosal infection as well as invasive diseases without capsule and/or with capsule acquired from capsule switching during nasopharyngeal colonization. Among three null capsule clades (NCC) belonging to group II NTSp, NCC1 has our newly-discovered gene, pspK in its cps locus. The pspK gene encodes a protein (PspK) with a long alpha-helical region containing an LPxTG motif and a YPT motif known to bind human polymeric immunoglobulin receptor (pIgR). Previous work showed that PspK binds human secretory immunoglobulin A (sIgA), increases binding of pneumococci to epithelial cells and enhances pneumococcal colonization independently of the genetic background. Based on significance of PspK in NP colonization, we here examined the immune response of PspK against both NTSp and various invasive pneumococci with capsule using flow cytometry, opsonophagocytic killing assay and genetic recombination. Our results showed that rabbit anti-recombinant PspK (rPspK) antibodies bind native PspK on NTSp and kill the bacteria upon complement-activation. It was highly interesting that anti-rPspK antibodies cross-react with acapsular surface antigens expressed on various typeable invasive pneumococcal isolates resulting in oposnophagocytic killing. Conclusively, our experimental data provide the potential possibility of PspK as a protein immunogen which can provide significant protection against both NTSp and typeable S. pneumoniae.

Poster Session

BAM-PM1136 - Structural and immunological features of a recombinant form of the *Streptococcus mutans* phosphate binding protein (PstS)

Ewerton Lucena Ferreira[1], Milene Tavares Batista[1], Rafael Ciro Marques Cavalcante[1], Vanessa Rodrigues Pegos[2], Andrea Balan[2], Luís Carlos de Souza Ferreira[1], Rita de Cássia Café Ferreira[1]
[1]Department of Microbiology, Biomedical Science Institute, University of São Paulo, Brazil, [2]Biosciences National Laboratory (LNBio), Materials and Energy Research Center, Campinas, SP, Brazil

Members of the ABC transporter family (ATP-binding cassete) are widely distributed among bacteria and play crucial role in nutrition. Beside the specific roles as substrate importers, these transport systems also impact the pathogenicity of different bacterial species. In *Streptococcus mutans*, the etiological agent of dental caries, the phosphate uptake system (Pst) system plays an important role in the uptake of inorganic phosphate and affects the adhesion to abiotic surfaces, particularly by the surface-exposed component, the PstS protein. In this study, a recombinant for of the PstS protein, derived from *S. mutans* UA159 strain, was expressed in Escherichia coli and purified by affinity chromatography with a nickel-containing resin. Antibodies raised with the purified PstS recognize both soluble and heat-denatured forms expressed by *S. mutans*. Dot Blot and ELISA analyses showed that antibodies raised with intact *S. mutans* cells recognize the purified protein, suggesting that native epitopes, either linear and conformational ones, are present in the recombinant PstS. Also, spectroscopy characterization of recombinant PstS by Circular Dichroism (CD) and fluorescence emission revealed an organized secondary structure, compatible with similar proteins with solved tertiary structure, and resistance to temperature and pH variation similar to other biding-proteins of ABC transporters. Taken together these results indicate that the recombinant PstS produced in *E. coli* preserves immunological and structural features of the native protein and, thus, could be an useful tool for different applications such as a target antigen in vaccine approaches against dental caries.

Poster Session

BAM-PM1138 - Immune responses of mice immunized with 56 kDa type-specific antigen (TSA56) of Orientia tsutsugamushi causing scrub typhus

Sangho Choi[1], Hang Jin Jeong[1], Byoungchul Gill[1], Young-Ran Ju[1], Kyu-Jam Hwang[1], Jeongmin Lee[1]
[1]*Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongwon-gun, Korea*

Scrub typhus, caused by infection with Orientia tsutsugamushi, is a mite-borne zoonotic disease endemic to the Asian–Pacific area. It is estimated that 1 billion people are at risk for the scrub typhus, and 1 million new cases are diagnosed annually in this region. In 2013, over 10,000 cases of scrub typhus were reported in Korea. The incidence of scrub typhus has increased with climate change. Although the infection is treatable with antibiotics, such as doxycycline and azithromycin, an effective vaccine against O. tsutsugamushi can be more desirable for the control of scrub typhus in endemic areas. In this study, a 56-kDa type-specific antigen (TSA56), which is a major outer membrane protein of O. tsutsugamushi, was investigated for development of a prophylactic vaccine against scrub typhus. We performed both intranasal and intramuscular immunization in mice using recombinant TSA56 (rec56) and TSA56-expressing plasmid DNA (p56), respectively. The heat-labile enterotoxin B subunit of Escherichia coli and plasmid DNA expressing the cytokine regulatory factor were used as a mucosal and DNA vaccine adjuvant, respectively. Intranasal immunization with rec56 induced higher levels of TSA56-specific IgG than did intramuscular immunization with p56. Both intranasal and intramuscular immunization induced a cellular immune response to TSA56, as demonstrated by splenic cell proliferation. Mice immunized with p56 or rec56 showed a protection against homologous challenge with O. tsutsugamushi. Th1 cytokines (interferon-γ, interleukin (IL)-12, and tumor necrosis factor-α) and Th2 cytokines (IL-5, IL-6, and IL-10) were also examined for response to TSA56. These results suggest that TSA56 is a feasible candidate for a vaccine antigen for the prevention of scrub typhus.

Poster Session

BAM-PM1140 - Induction of homologous immune response to Orientia tsutsugamushi Boryong with a 47 kDa outer membrane protein

Sangho Choi[1], Hang Jin Jeong[1], Kyu-Jam Hwang[1], Byoungchul Gill[1], Young Ran Ju[1], Yenong Seon Lee[1], Jeongmin Lee[1]

[1]*Korea National Institute of Health, Cheongwon-gun, Korea*

Orientia tsutsugamushi is the causative agent of scrub typhus, which is a mite-borne zoonotic disease endemic to the Asia-Pacific area. In Korea, the incidence of this disease has increased with climate changes, and over 10,000 cases of infection were reported in 2013. Although the infection is treatable with doxycycline and azithromycin, an effective prophylactic vaccine against *O. tsutsugamushi* would be more desirable for preventing scrub typhus in endemic areas. In this study, we investigated the 47 kDa outer membrane protein (OMP), which is a periplasmic serine protease and a major antigenic outer membrane protein of *O. tsutsugamushi*, as a vaccine candidate. In animal immunization experiments, Intranasal (I.N.) immunization of recombinant 47 kDa OMP (rec47) or rec47 plus heat-labile enterotoxin B subunit from *E. coli* or cholera toxin (CT) induced high level of rec47-specific antibodies compared to intramuscular (I.M.) immunization of the plasmid expressing 47 kDa OMP (p47) or p47 with pBOOST2-samIRF7/3 (pB). Moreover, the combination of rec47 with CT induced strong cellular immune response to 47 kDa OMP, as demonstrated by the splenocyte proliferation assay. I.M. immunization with p47 alone or p47 with pB showed induction of Th1 and Th2 type cytokines, as demonstrated by cytokine ELISA from splenocyte culture. In conclusion, rec47 with CT was the most effective in humoral and cell-mediated immune responses. Furthermore, relatively strong protection against a homologous challenge with O. tsutsugamushi Boryong strain was observed in mice immunized with rec47 plus CT. We expect 47 kDa OMP to be an attractive candidate for a prophylactic vaccine against scrub typhus by *O. tsutsugamushi* infection.

Poster Session

BAM-PM1142 - Classification of Chryseobacterium strains from raw chicken and chicken feather waste in poultry processing plants

George Charimba[1], Piet Jooste[2], Celia Hugo[1]

[1]*University of the Free State, Bloemfontein, South Africa,* [2]*Tshwane University of Technology, Pretoria, South Africa*

Ten yellow-pigmented strains isolated from chicken feather waste, two from chicken feather meal and 17 from a previous study (de Beer, 2005), all isolated from poultry processing plants in South Africa, were screened and identified using 16S rRNA sequencing. Fourteen of the 29 isolates were identified as belonging to the genus Chryseobacterium by 16S rRNA sequencing. Seven isolates (namely; 5_R23647, 8_R23573, 9_R23581 and 10_R23577 from raw chicken and 1_F178, 6_F141B and 7_F195 from chicken feather waste) that belonged to the genus Chryseobacterium and having ≤97% 16S rRNA gene sequence similarity were selected for further 16S rRNA sequencing with two additional primers and subjected to phylogenetic analysis. In a recent study (Charimba et al., 2013); three strains (8_R23573, 9_R23581 and 10_R23577) were described as a new species C. carnipullorum. The remaining four isolates were characterized using conventional phenotypic methods. They were also profiled and identified using the BIOLOG Omnilog Gen III identification system. All four isolates were identified as belonging to the genus Chryseobacterium by the BIOLOG Omnilog Gen III identification system. Four of the nine reference strains (C. gleum, C. indologenes, C. piscium and C. scophthalmum) were correctly identified by the Omnilog system. The others could not be correctly identified because of Omnilog data base limitations. The four unknown strains grouped into three possible new Chryseobacterium species represented by the following strains: 1_F178; 5_R23647; and 6_F141B and 7_F195. All strains require further polyphasic investigations to have full evidence for description as new species.

Poster Session

BAM-PM1144 - The differing biological fates of DNA minor groove-binding (MGB) antibiotics in Gram-negative and Gram-positive bacteria

Leena Nieminen[1], Iain Hunter[1], Colin Suckling[1], Nick P. Tucker[1]
[1]University of Strathclyde, Glasgow, UK

Since the 1940s antibiotics have played a critical role in improving public health. The rise of bacteria resistant to major drugs together with the decline seen in the discovery of potential new antibiotics has caused worldwide concern. A group of compounds called minor groove binding (MGB) compounds have been studied at the University of Strathclyde. MGBs have very high activity against methicillin resistant Staphylococcus aureus (MRSA) yet little is known about their biological mode of action. To find out the mechanism of action of MGBs and to facilitate the rational design of other antibiotic MGBs using synthetic chemistry, a lead compound of the MGB group was studied using a new sequencing technology. It is hypothesized that MGB antibiotics interfere with transcription. In order to identify the specific target genes for MGB and to monitor the bacterial cell stress response to the drug, RNA sequencing technology was applied. The results showed significant changes in the MGB treated bacterial transcriptome where genes were both over- and underexpressed. For example, overexpressed genes included genes belonging to bacteriophages. Small RNAs, known to have regulatory effects to the bacterial gene expression, were also found to play a role. We further identified links between the affected transcripts in order to identify the sequence specificity of the MGB compound. We tested the MGB also against Gram-negative bacteria such as Pseudomonas aeruginosa that is notorious for antibiotic resistance. MGB was active against P. aeruginosa in the presence of cell membrane efflux pump inhibitor. We are using the sequencing technology to further assess the role membrane transport proteins play in resistance and will use this information to design new antibiotics that inhibit their own efflux.

Poster Session

MEM-PM3001 - Endophyte diversity within Mantioban coniferous trees

Lindsay Bowman[1], Paul Holloway[1], Robyn Thorington[1]
[1]*Univeristy of Winnipeg, Winnipeg, Canada*

Foliar endophytes are comprised primarily of ascomycetous fungi. These microorganisms are producers of a variety of novel bioactive natural products, and this diversity is associated with the high variety of endophytic microorganisms. Endophytes are considered to be ubiquitous, yet many remain undescribed. A host plant may harbour many different species, including species-specific endophytes, such as *Rhabdocline parkerii*, which has only been found in *Pseudotsuga macrocarpa*. This study aims to examine the genetic diversity within coniferous tree endophytes in Manitoba, Canada collected from 9 different geographic regions. DNA sequencing of the Internal Transcribed Spacer (ITS) regions has been performed on 33 endophyte isolates, cultured from surface sterilized conifer needles. These sequences were compared to known fungal ITS sequences. 19 sequences showed >97% similarity, 8 showed 90-97% similarity, and 6 were <90% similar to known fungal ITS regions. Of these sequences, there were two genera that had the greatest number of sequences with high similarity: *Alternaria* (13 sequences) and *Coniochaeta* (anamorph *Lecythophora*) (9 sequences). The remaining sequences were most similar to sequences in *Hypoxylon* (2 sequences), *Preussia* (2 sequences), *Cladosporium*, *Sydowia*, *Pyronema*, *Coprinopsis*, *Fimtariella*, or *Paraconiothyrium* genera (1 sequence each). The Shannon-Weaver and Simpsons diversity indices were calculated to be 1.72 and 0.47, respectively. 5 endophyte isolates, from 5 individual conifer trees, collected from 4 different sites, were most similar to *Alternaria alternata*. These were aligned, using AB369425 *Alternaria alternata* as a reference sequence. Pairwise alignment to AB369425 yielded 99.6%, 99.5%, 98.3%, 96.7%, and 95.4% similarity. There was an overall 97.1% similarity when mapped to the reference sequence. These results highlight the molecular diversity of culturable endophytes within Manitoban coniferous trees. It is a step toward greater knowledge of the molecular diversity among different endophytes, which can lead to a greater understanding of secondary metabolite and natural product diversity.

Poster Session

MEM-PM3003 - A research platform for effective identification of secondary metabolism biosynthesis gene clusters from the filamentous fungal genomes

Myco Umemura[1], Itaru Takeda[1,2], Nozomi Nagano[3], Hideaki Koike[4], Jin Kawano[1], Tomoko Ishii[1], Yuki Miyamura[1], Kazuo Shin-ya[3], Kiyoshi Asai[3,5], Masayuki Machida[1,2]

[1]National Institute of Advanced Industrial Science and Technology, Sapporo, Japan, [2]Tokyo University of Agriculture and Technology, Tokyo, Japan, [3]National Institute of Advanced Industrial Science and Technology, Tokyo, Japan, [4]National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan, [5]The University of Tokyo, Tokyo, Japan

Comparison of the genomes three *Aspergillus* species has revealed existence of non-syntenic blocks (NSBs) specifically existing in each species. We have identified the genes responsible to biosynthesis of kojic acid, which is used as a cosmetic whitening agent. The kojic acid biosynthesis gene was successfully identified by the combination of expression, annotation and localization analyses. The genes were located on the NSBs and clustered with the genes encoding a transcription factor and a transporter on the chromosome. In order to effectively identify novel genes for secondary metabolites from fungal genomes, we have developed MIDDAS-M and MIPS-CG based on the observation above, both of which can identify gene clusters for secondary metabolism biosynthesis (SMB) without using any functional knowledge of the genes in the cluster. While MIDDAS-M utilizes cooperative gene regulation of the cluster member genes, MIPS-CG detects the gene clusters by the sequence similarity of the genes in SMB gene clusters from multiple genomes. These methods could effectively detect most of the known SMB gene clusters and additionally detected various novel gene clusters even without so-called core-genes such as PKS/NRPS. It should be noted that MIDDAS-M detected the gene cluster responsible to the production of Ustiloxin B from the *A. flavus* genome. In spite of the chemical structure of Ustiloxin B consisting of five amino acid, one of which is a non-natural amino acid, the corresponding gene cluster did not contain a domain indispensable to NRPS, instead it included *ustA* which encoded the amino acid sequence of Ustiloxin B except norvaline. Detailed analysis of the genes in the cluster revealed a ribosomal peptide synthase (RiPS) pathway. We expect that the new algorithms above in combination with our *de novo* assembling pipeline for SOLiD sequencer can greatly accelerate discovery of novel SMB gene clusters from filamentous fungi.

Monday, 28 July 2014 **11:00 - 12:00 Room 516/517 A-B**

Poster Session

MEM-PM3005 - Molecular epidemiology of dermatophytosis in the Czech Republic: results of a two year survey

Vít Hubka[1,2], Tomáš Větrovský[3], Stanislava Dobiášová[4], Magdalena Skořepová[5], Naďa Mallátová[6], Pavlína Lysková[7], Karel Mencl[8], Helena Janouškovcová[9], Adéla Čmoková[1], Miroslav Kolařík[1,2]

[1]Department of Botany, Faculty of Science, Charles University in Prague, Prague, Czech Republic, [2]Laboratory of Fungal Genetics and Metabolism, Institute of Microbiology ASCR, Prague, Czech Republic, [3]Laboratory of Environmental Microbiology, Institute of Microbiology of the Academy of Sciences of the Czech Republic, [4]Laboratory of Clinical Mycology, Institute of Public Health, Ostrava, Czech Republic, [5]Dermatology and Venerology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic, [6]Laboratory of Mycology and Parasitology, Hospital České Budějovice, České Budějovice, Czech Republic, [7]Laboratory of Clinical Mycology, Institute of Public Health, Prague, Czech Republic, [8]Department of Clinical Microbiology, Pardubice Regional Hospital Inc., Pardubice, Czech Republic, [9]Department of Microbiology, Faculty of Medicine and University Hospital, Charles University, Plzeň, Czech Republic

A large aggregate of clinical isolates of dermatophytes (n=3255) was collected from Czech patients during the first two years of molecular-epidemiological study. The isolates were obtained from clinical specimens provided by the six regional institutions across Czech Republic. Trichophyton rubrum was the causal agent of 79 % of all infections and morphologically typical isolates of T. rubrum were identified to species level only by morphology. The determination of atypical isolates was confirmed by molecular methods (n=189). The molecular part of the study was particularly focused on the non-rubrum dermatophyte species. All of them (21 % of dermatophytoses in the Czech Republic; n=692) were subjected to PCR-fingerprinting method with primer M13-core or comparative sequence analysis by using the ITS rDNA. Altogether, 14 species were identified including T. rubrum. Trichophyton interdigitale together with Arthroderma benhamiae (both 33 %; T. rubrum is no longer included in the percentage) were the most abundantly recovered non-rubrum species followed by Microsporum canis (17 %). All other species comprised 17 % of non-rubrum isolates. Approximately 17 % of isolates identified as zoophilic strains of T. interdigitale by morphology was re-determined as M. persicolor using molecular approach. Microsporum fulvum masquerading as M. gypseum was another discovered cryptic species which comprised 39 % of isolates originally identified as M. gypseum by morphology. Complete list of determined species involved also T. tonsurans, Epidermophyton floccosum, T. terrestre, T. verrucosum and T. erinacei. In addition, two undescribed species of geophilic dermatophytes were isolated and described as T. onychocola sp. nov. and M. aenigmaticum sp. nov. In conclusion, molecular methods are increasingly being used to assist in identification of dermatophyte species and allow us to determine cryptic species easily miss-identified by morphological methods. The identification based on morphology alone may be difficult due to high phenoplasticity and convergent features exhibited by dermatophytes.

Poster Session

MEM-PM3007 - Prostate microbiome analysis using unbiased high-throughput sequencing

Martin Laurence[1], Douglas Brash[2]
[1]Shipshaw Labs, Montreal, Canada, [2]Yale University - School of Medicine, New Haven, USA

Recent advances in prostate cancer research indicate that a fastidious fungus could be infecting the prostate and causing prostate cancer (Sutcliffe, Sfanos, De Marzo et Laurence, 2014). Many medically important fastidious fungus species remain to be discovered (Ghannoum et al, 2010; Paulino et al, 2008; Findley et al, 2013), and substantial evidence indicates that one such species is causing the chronic prostate inflammation which affects over 80% of American men by age 75. Detecting medically important fastidious fungi such as Pneumocystis jiroveci or Encephalitozoon cuniculi in clinical specimens is very challenging. These two species asymptomatically infect a substantial fraction of the population; they do not grow in commonly used culture media, and commonly used consensus PCR primers fail to amplify their ribosomal DNA. The advent of high-throughput sequencing technology has enabled a new microbe detection technique coined “unbiased high-throughput sequencing” (Lipkin 2010). This technique does not use consensus PCR primers, thus can detect all microbes in clinical specimens, including those whose ribosomal DNA region has substantially diverged from related species. We applied this technique to prostate specimens using the Illumina HiSeq 2500 sequencer and the Leif bioinformatics Toolkit, revealing novel sequences which may be originating from a microbe etiologically related to prostate cancer. This systematic microbe detection technique can be applied to other types of clinical specimens, and can detect both known and novel species of bacteria, fungi, protists and viruses. As high-throughput sequencing becomes more affordable, this technique will be widely used in the detection of microbes in clinical specimens. Most importantly, this technique may reveal an infectious etiology for idiopathic chronic inflammatory diseases such as benign prostatic hyperplasia (BPH), chronic prostatitis (CP/CPPS), non-specific urethritis (NSU), reactive arthritis (ReA), ankylosing spondylitis (AS) and prostate cancer.

Poster Session

MEM-PM3009 - Genome sequence of the primary cutaneous mucormycosis pathogen Mucor irregularis and comparison with the broader species

Weida Liu[1], Yongnian Shen[1], Guixia Lu[1], Huan Mei[1], Hongsheng Wang[1], Xuelian Lu[1]
[1]Institute of Dermatology, Chinese Academy of Medical Sciences, Nanjing, China

Mucormycosis diseases have been threatening human health and welfare worldwide. For every 100 million people, over 1.7 million are infected with Mucoromycotina species, including Mu. amphibiorum, Mu. hiemalis, Mu. indicus, Mu. ramosissimus, Rhizomucor miehei and M. irregularis. Thus far, little is known about the molecular mechanisms underlying zygomycosis infection in mammals. Here we report the sequencing of the whole genome of M. irregularis (formerly Rhizomucor variabilis) as a key step toward understanding the virulent factors. The M. irregularis genome, which is 32 megabase in size, consists of 10563 predicted protein-coding genes, 253 tRNA and 11rRNA genes and dozens of DNA transposons. Functional annotation and categorization of the genes highlighted 183 genes to be involved in secondary metabolism, which is considered important for the biochemical features of a fungus and thus provides important clues to deeper exploit the basis of its pathogenicity. Species-specific gene mining enabled by comparing the genomes of M. irregularis and candida and aspergillus, as well as by SSH analysis between M. irregularis and its non-virulent, close relative M. hiemalis revealed 8107 genes that are only present in the M. irregularis genome. This set of M. irregularis-specific genes represents a narrowed-down, highly informative reservoir for exploring virulent factors. To our knowledge, this work represents the first whole genome sequencing effort of a Mucoromycotina subfamily species related human infection, providing valuable resources for the understanding of pathogenicity of M. irregularis as well as the development of new, effective drugs, vaccines and diagnostic tests against zygomycosis.

Poster Session

MEM-PM3011 - Epidemiology of sporotrichosis in China and the study of T-DNA insertional mutagenesis in Sporothrix schenckii

Li Wang[1], Dan He[1], Guangquan Li[1], Yanhua Zhang[1]
[1]Department of Pathogenobiology, College of Basic Medical Sciences, Jilin University, Changchun, China

Sporotrichosis is a world-widely distributed subcutaneous mycosis, mainly caused by dimorphic fungi Sporothrix schenckii complex. In immunocompromised patients, it causes seriously disseminated and systematic infection. In China, this disease has a higher prevalence in the Northeast region. It is usually acquired by traumatic inoculation of this pathogen, often occurs in winter and spring, and more commonly prevalent in women and elderly people. Fixed cutaneous infection is the most common with the lesion usually located on face and upper extremity. Potassium iodide and itraconazole are considered the treatment of choice and have a better therapeutic effect. In an effort to understand the pathogenesis of Sporothrix schenckii better, Agrobacterium tumefaciens -mediated T-DNA insertional mutagenesis (ATMT) technique was used for analysis of the fungus gene function. ATMT is widely used with many advantages, such as high efficiency, random insertion, stable transformants, easy operation and so on. In our study, a T-DNA insertion mutant library of S. schenckii was established using ATMT technique with the optimized factors. Seven mutants with significant phenotypic changes were obtained and the T-DNA insertional flanking sequences were investigated. Bioinformatics analysis suggested one mutant was interrupted with the copper transporter gene, which was considered related to intake of copper ion, growth and reproduction, oxidative stress and pathogenicity of fungi. Compared with wild type, morphological features of the mutant was obviously changed with thinner mycelium, fewer conidia, lower pigment, weakened conversion to yeast, and decreased antioxidative stress ability. In the mouse systemic infection model, the mutant exhibited lower virulence. The results suggested that the function of copper transporter gene was related to spore germination, growth, metabolism, oxidative stress and virulence of S. schenckii. These findings are significant for the diagnosis, prevention and treatment of sporotrichosis.

Poster Session

MEM-PM3013 - Cell cycle regulation and hypoxic adaptation in pathogenic yeast, Cryptococcus neoformans

Susumu Kawamoto[1], Eric Virtudazo[1], Zuzana Moranova[1,3], Misako Ohkusu[1], Kiminori Shimizu[1], Akiko Suganami[2], Yutaka Tamura[2], Vladislav Raclavsky[3]

[1]Department of Molecular Biology, Medical Mycology Research Center, Chiba University, Chiba, Japan, [2]Department of Bioinformatics, Graduate School of Medicine, Chiba University, Chiba, Japan, [3]Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

Cryptococcus neoformans is an opportunistic pathogen of worldwide distribution and responsible for life-threatening infections among immunocompromised persons. We have reported that the cell cycle behavior of this yeast is different from the cell cycle control exhibited by the model yeast Saccharomyces cerevisiae, and also have reported the molecular characterization and physiological roles of the two main eukaryotic cell cycle genes, C. neoformans cyclin dependent kinase 1 (CnCdk1) and cyclin homologues. Only a single Cdk1-related G1 and G1/S cyclin homologue was found in the genome sequence of C. neoformans and designated CnCln1. Surprisingly, CnCln1 was not only able to complement the function of the G1 cyclins of S. cerevisiae, such as ScCln3, but also the G1/S cyclins of S. cerevisiae, such as ScCln1 and ScCln2. Our in silico analysis demonstrated that the CnCln1/ScCdk1 complex was more stable than any of the yeast cyclin and ScCdk1 complexes. These results are consistent with in vitro analysis that has revealed the flexible functional capacity of CnCln1 as a Cdk1-related G1 and G1/S cyclin of S. cerevisiae. In the obligate aerobic yeast Cryptococcus neoformans, limited aeration has been demonstrated to cause slowdown in proliferation and delayed budding, resulting eventually in a unique unbudded G2-arrest. The ability to adapt to decreased oxygen levels during pathogenesis has been identified as a virulence factor in C. neoformans. We identified and characterized the gene that is necessary for the proliferation slowdown and G2-arrest caused by limited aeration. The gene was also identified in parallel studies as homologous both to calcineurin responsive (Crz1) and PKC1-dependent (SP1-like) transcription factors. We have confirmed the role of the cryptococcal homologue of CRZ1/SP1-like transcription factor in cell integrity, and newly demonstrated its role in slowdown of proliferation and survival under reduced aeration, in biofilm formation and in susceptibility to fluconazole.

Poster Session

MEM-PM3017 - Fertility and viability of the hepatic hydatic cysts in Tunisian patients. Results of a prospective study

Akila Fathallah[1], Sondoss Gaïed-Meksi[1], Alia Yaacoub[1], Imen Dhib[1], Fatma Saghrouni[1], Imen Khammari[1], Ridha Ben Hadj Hamida[2], Rached Letaief[3], Moncef Ben Saïd[1]

[1]Laboratory of Parasitology-Mycology, CHU Farhat Hached, Sousse, Tunisia, [2]Service of Surgery, CHU Sahloul, Sousse, Tunisia, [3]Service of Surgery, CHU Farhat Hached, Sousse, Tunisia

Studies on the human hydatic cysts fertility are scarce. The objective of the present study was to assess the fertility and the viability of liver hydatic cysts collected from operated tunisian patients, and to investigate the relationship between both parameters and the following data: age and sex of the patient, echographic type, size, number of cysts, presence of cysto-biliary fistula. For this purpose, we carried out a prospective study on 82 hydatic cysts of the liver taken from 80 patients operated in the surgery services of Sahloul and Farhat Hached teaching hospitals of Sousse, Tunisia, in 2008. The cysts were obtained from 55 women and 25 men aged between 11 and 85 years (average = 43.5 years). The fertility was assessed on the basis of the presence (or lack) of scolex and quantified as the number of scolex in 50µl of pellet of the washed hydatic membranes. The viability was evaluated on the basis of the morphology of scolex upon direct examination and after eosin straining, and then expressed in percentage (live scolex/dead scolex x 100). Out of the 82 examined cysts 73 (89%) were found to be fertile with an average of 790 scolex/50µl. The viability rates of the protoscolex from the fertile cysts varied between 0 and 99% with an average of 31.6%. The analysis of the results showed that: - The type I cysts were significantly more fertile and more viable than the type III and IV cysts. In tunisian patients with liver hydatidosis, both fertility and viability rates are high. Caution needs be taken when operating the patients in order to avoid secondary echinococcosis.

Poster Session

MEM-PM3019 - Amarenographium solium sp. nov. from Yanbu Mangroves in the Kingdom of Saudi Arabia

Ali Bahkali[1], Mohamed Hodhod[1], Mohamed Abdel-Wahab[1], Kevin Hyde[2]
[1]Department of Botany and Microbiology-College of Science-King Saud University, Riyadh, Saudi Arabia, [2]School of Science-Mae Fah Luang University, Chiang Rai, Thailand

During an ongoing study of marine fungi growing on Avicennia marina in the Red Sea coast of Saudi Arabia, a new coelomycete was collected in the genus Amarenographium. The new species is characterized by muriform, brown conidia with one polar gelatinous cap and sheath and holoblastic phialidic conidiogenesis. Amarenographium solium sp. nov. differs from the two known species of Amarenographium by the large size of its pycnidia, a thick (62–75 μm) two-layered peridial wall of the conidiomata that appears as textura epidermoidea in surface view and conidia with one apical appendage. Phylogenetic analysis of SSU and LSU rDNA sequences showed that the new species and thus genus Amarenographium grouped consistently with Medicopsis romeroi with high bootstrap support and form a basal clade to the families: Montagnulaceae and Trematosphaeriaceae, order Pleosporales, Dothideomycetes.

Poster Session

MEM-PM3021 - Anthraquinones promote Botrytis cinerea infected cabbage sucrose sinking

Chuan-Kai Liao[1], Shu-Ying Liu[2], Ming-Yu Hsieh[2], Chaur-Tsuen Lo[3], Kou-Cheng Peng[1]

[1]Institute of Biotechnology, National Dong Hwa University, Hualien, Taiwan, [2]Department of Molecular Biotechnology, Da-Yeh University, Changhua, Taiwan, [3]Department of Biotechnology, National Formosa University, Yunlin, Taiwan

Trichoderma spp. are well known biocontrol agent that can promote growth of host plants. It was assumed that sucrose sinking effect might occur in the symbiosis of Trichoderma and its host plants. Anthraquionones, such as anthraquinone and chrysophanol among many others, are secondary metabolites secreted by Trichoderma to the surrounding environment that possess antimicrobial activities. However, these secretions must serve specific biological purpose to the host plants which is still unclear. Through three-way interaction experiment, in the presence of Trichoderma or anthraquinones around rhizosphere, cabbage leaves infected with Botrytis cinerea demonstrated significant anti-phytopathogenic resistance. Proteomic data showed cabbage proteins related to photosynthesis were highly expressed, namely ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), ribulose-1,5-bisphosphate carboxylase/oxygenase activase (Rubisco activase), and chloroplast ATP synthase. Transcript data supported these findings as well. The analysis of q-PCR showed, in the presence of 10 ppm anthraquinone (A) or chrysophanol (C), the expression of cabbage mRNAs, Rubisco (1.50/A, 1.38/C), Rubisco activase (4.03/A, 22.32/C) and ATP synthase (1.12/A, 1.04/C) along with sucrose 6- phosphate synthase (0.95/A, 1.24/C), sucrose transporter 1 (0.46/A, 1.23/C), 2 (0.82/A, 1.27/C), and 3 (1.38/A, 1.36/C) and sucrose invertase significantly increased. Apparently Trichoderma anthraquinones promoting cabbage photosynthesis in the first place, then glucose was converted to sucrose by sucrose 6- phosphate synthase. Sucrose was further exported and transported by SUT1, 2 and 3 to the root area, where the sucrose was either secreted directly to the rhizosphere or converted to fructose and glucose by sucrose invertase before released to the rhizosphere full of Trichoderma. This study contributes to our understanding of Trichoderma biocontrol mechanism.

Poster Session

MEM-PM3023 - Geographic expansion, genetic evolution, and future risks: a phylogenetic analysis of global subpopulations of the oilseed rape pathogen Leptosphaeria maculans

Sakaria Liban[1], Dilantha Fernando[1]

[1]*Dept. of Plant Science, University of Manitoba, Winnipeg, Canada*

Leptosphaeria maculans is the causal agent of blackleg (aka phoma stem canker), an economically important disease of oilseed brassicas that has led to epidemics in both Australia and France. This pathogen is now a growing concern affecting the Canadian Canola (Brassica napus) industry and has appeared on cultivars rated as resistant. The first reports of this fungus was published in the early 1900's on cabbage in Wisconsin, USA and following the breeding of oilseed rape as a major crop for human consumption and bio-fuel production, it has since spread to major canola growing regions through natural dispersal and trade. It is now found in most countries where Brassica spp. are cultivated and in the last four decades L. maculans has caused significant yield losses in Canada, Europe, and Australia. Moreover there are indications that L. maculans is an expanding species displacing the less aggressive Leptosphaeria biglobosa which has historically colonized oilseed rape crops. The risk of spread to non-host countries (i.e. China) and growing yield losses due to rapid evolution has led to the examination of the genetic diversity between different geographic regions. A set of microsatellites spread among the 76 sequenced supercontigs in the fungal genome were used to screen 96 isolates collected from 7 different countries. Sixty-four unique alleles were used to generate a phylogenetic tree to assess the evolutionary relationship between isolates. The pathogen population showed significant variation both within countries (i.e. Western and Central Canada) and between countries. Overall, genetic diversity was correlated with geographic location, with some exceptions which are posited to be due to trade.

Poster Session

MEM-PM3025 - Efficiency assessment of different concentrations of Saccharomyces cerevisiae and times of action to bind Aflatoxin M1 in milk

Alana Cordeiro[1], Bruna Gonçalves[1], Fernanda Bovo[1], Roice Rosim[1], Carlos Oliveira[1], Carlos Corassin[1]
[1]*School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, Brazil*

The purpose of this study was to evaluate the ability of a Saccharomyces cerevisiae strain to bind aflatoxin M1 (AFM1) in UHT (ultra-high-temperature) milk spiked with 0.05 µg/mL AFM1. All the Saccharomyces cerevisiae (100.000 cells/mL - concentration A and 1.000.000.000 cells/mL- concentration B) cells were heat-killed (100° C, 1 h) and then used for checking the effect of contact time (1 minute, 3 minutes or 5 minutes) on toxin binding in skim milk at 37°C. The mean percentages of Saccharomyces cerevisiae cells concentration B (1.000.000.000 cells/mL) had higher ($P<0.05$) capability to bind AFM1 in milk (69.3±0.3%, 70.2±0.2% and 69.7±0.7% for 1 minute, 3 minutes and 5 minutes, respectively), although there were not significant differences between the contact times evaluated. When using Saccharomyces cerevisiae cells concentration A (100.000 cells/mL) a significant decrease ($P<0.05$) was observed in the percentage of AFM1 bound (61.48±0.1%, 63.00±0.8% and 61.56±0.7%,) during for 1 minute, 3 minutes and 5 minutes, respectively. In this concentration of S. cerevisiae cells (100.000 cells/mL) were significant differences between the contact times evaluated ($P<0.05$). Tree minutes of contact of S. cerevisiae cells at 100.000 cells/mL concentration was the best time of action for concentration A bind aflatoxin M1. Results of this trial indicate that heat-killed Saccharomyces cerevisiae cells, in different combinations of concentration (100.000 cells/mL and 1.000.000.000 cells/mL) and time used (1 minute, 3 minutes and 5 minutes), has a potential application to reduce the concentration of AFM1 in milk.

Poster Session

MEM-PM3027 - Heat resistance of *Leohumicola verrucosa* and *Geminibasidium donsium* isolated from Canadian frozen blueberries

Yutaka Kikoku[1], Koichi Yamane[1], Hiroyuki Nakagawa[1]
[1]*Aohata Corporation, Takehara-shi, Japan*

It is known that adhered heat-resistant molds on fruit materials survive after thermal treatment and cause spoilage in processed fruit products. The prevention of such problems requires measurement of the frequency and the intensity of the heat-resistant molds. Two molds were isolated from Canadian frozen blueberries through heat treatment (80℃). These heat-resistant molds were identified as *Leohumicola verrucosa* and *Geminibasidium donsium* by morphological, ecological and molecular biological character. For sporulation, the molds were incubated at 25℃ on PDA, and then the thermal inactivation rates of the spores were determined in pH3.5 McIlvain buffer. The decimal reduction times (D values) of *L. verrucosa* at 67.0, 74.5, 80.0 and 85.5℃ were 101.0, 54.3, 27.8 and 7.1 min, respectively. The D values of *G. donsium* at 65.0, 67.5, 70.0, 72.5 and 75.0℃ were 2.50, 1.83, 1.13, 0.80 and 0.43 min, respectively. The z values calculated from the thermal death time curves were 16.6℃ and 13.3℃ for *L. verrucosa* and *G. donsium*. The results established by this study may be used by blueberry processors to prevent losses due to spoilage caused by heat resistant microorganisms.

Poster Session

MEM-PM3029 - Optimization of growth conditions for biosynthesis of Laccases enzyme by coriolus versicolor

Zill-e-huma Bilal[1], Sabba Chattah[2], Shakeel Ahmed[1], Arusa Aftab[2]
[1]Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan, [2]Department of Botany, Lahore College for Women University, Lahore, Pakistan

The production of laccase by an indigenous strain of Coriolus versicolor was studied employing submerged fermentation. The physical parameters namely, pH, Temperature, Incubation period and Inoculum size were studied for maximum Laccase enzyme production. The optimum pH for the laccase production was found to be pH 5.0 which showed maximum (12.88 U/ml) laccase activity and pH 10.0 gave the minimum (5.86 U/ml) laccase activity. Of the different temperature (15 oC, 16 oC, 17 oC, 18 oC, 19 oC, 20 oC up to 40 oC) tested for the optimal laccase production 25 oC showed the maximum (11.52 U/ml) laccase activity, while 40 oC shows the minimum laccase activity (4.21 U/ml). Among the different incubation period 7 days supported maximum laccase activity (12.78 U/ml) and 10 days shows minimum laccase activity (5.20 U/ml), while four discs (appx. 12mm) supported the maximum activity (11.80 U/ml) and seven discs shows the minimum laccase activity (5.53 U/ml). The trend of the results was similar for Biomass production. The above results indicate that the Coriolus versicolor can be used as a biotechnological tool.

Poster Session

MEM-PM3000 - Characterization and functional analysis of Amts on pigment production in Penicillium purpurogenum IAM 15392

Kojima Ryo[1], Arai Teppei[1], Motegi Yoshiki[1], Kasumi Takafumi[1], Kato Jun[1], Ogihara Jun[1]

[1]*Department of Chemistry and Life Science,Nihon University College of Bioresource Sciences,Fujisawa,Japan*

The filamentous fungus Penicillium purpurogenum IAM 15392 produces Monascus pigment homologues. The predominant pigments produced are orange pigment (PP-O) and violet pigment (PP-V). PP-V, a polyketide compound, contains an amino group. A most of polyketide compounds involving nitrogen introduce it when those core structures are formed. However, in PP-V biosynthesis, it is believed that the 7-NH is introduced after formation of the pyran ring. Ammonium nitrate is an efficient and stable nitrogen source for PP-V production by Penicillium purpurogenum IAM15392. The structural characteristics of the 7-NH in PP-V suggest the participation of ammonia nitrogen in the production of this pigment. We analyzed the effect of inorganic nitrogen sources on PP-V production and found that ammonium salts were utilized preferentially over nitrate salts. These findings suggest the involvement of ammonium transporter (AMT). Hence, in this study, we identified the Amt/Mep proteins in P. purpurogenum IAM15392. Four AMT genes, AmtA, AmtB, AmtC, and AmtD, were identified from the draft genome sequence database of P. purpurogenum IAM15392. Topology analysis clarified the number of transmembrane helices and the localization of the N- and C-terminus. In addition, phylogenetic analysis revealed that each AMT was present in Mep2 (AmtA) group, MeaA (AmtB) group, and MepA (AmtD) except for AmtC. Furthermore, AMT gene expression was dependent on the pigment production conditions. In PP-V production medium, AmtB and AmtD showed high-level expression. AmtB and/or AmtD were thus down-regulated with RNAi to elucidate the relationship between the two AMTs and pigment production. We found that AmtB and AmtD single silenced transformants could not produce PP-V, whereas AmtB/AmtD double silenced transformants could produce PP-V. The pigment productivity of the constructed silenced transformants indicated that PP-V production depends on the ammonium concentration.

Poster Session

MEM-PM3002 - Evaluation of yeasts isolated from agricultural areas as plant growth promoters

Marcia Rosa-Magri[1], Ana Silvia de Almeida Scarcella[1], Rodolfo Bizarria Junior[1]
[1]UFSCar, Araras, Brasil

Yeasts are employed in several biotechnological processes. This group is naturally present in the rhizosphere, and the plant surface, but their function in these habitats is not known and its potential influence on plant growth promotion is understudied. The objective of this work was the isolation and selection of yeasts isolated from agricultural areas, and the screening for strains with ability to solubilize phosphate and produce indole acetic acid (IAA). Yeasts were isolated from the rhizosphere, leaves and stalks of corn and sugar cane plants; samples were plated in solid culture media. The selection of phosphate solubilizing strains was realized by using solid medium BDYA with phosphate (10% K2HPO4 and 10% CaCl2). The detection of translucent halo around the colony indicated the solubilization of phosphate in the medium. To evaluate the production of indole compounds was used Potato Dextrose liquid sterile medium supplemented with tryptophan. Medium was inoculated with 2 ml of yeast suspension (1 x 107 cells/ml) and kept in shaker at 25°C, 250 rpm for 7 days. After the period, the suspension was centrifuged for 30 minutes at 12000 x g and to the supernatant was added 2 ml of Salkowsky reagent; after 30 minutes the development of pink color indicated the presence of indole compounds. It was isolated a total of 325 yeasts; results of screening indicated a strain able to solubilize phosphate identified as Meyerozyma guilhermondii (3C98); three strains with ability to produce indole compounds were identified as Trichosporon asahii (4C06 and 3S44) and Rhodotorula mucilaginosa (2F32). The conclusions indicate the potential of these strains to promote plant growth, but more detailed studies are being carried out as the competence to root colonization and its effect in vivo on the development of different plants.

Poster Session

MEM-PM3004 - Detection of Trichomonas vaginalis infection among Ghanaian women living with human immunodeficiency virus (HIV) using wet mount microscopy and polymerase chain reaction (PCR) analysis

George Pesewu[1], Marjorie Quarchie[1], Richard Asmah[1]
[1]Department of Medical Laboratory Sciences (MEDLAB), School of Allied Health Sciences, College of Health Sciences, University of Ghana, Korle-Bu, Accra, Ghana

Abstract Background: Trichomoniasis is the most common non-viral sexually transmitted infection (STI) worldwide, with particularly high prevalence in regions of human immunodeficiency virus (HIV) endemicity. Objectives: The aim of the study was to compare and evaluate diagnostic methods such as the wet mount microscopy and polymerase chain reaction (PCR) in the detection and characterization of Trichomonas vaginalis in vaginal discharges of women living with HIV in Accra, Ghana. Methods: A cross-sectional study was performed using women living with HIV (patients) who visit the Fevers Unit of the Korle-Bu Teaching Hospital (KBTH), Accra. In all a total of 120 participants including 100 patients with confirmed HIV status and 20 controls (healthy participants) were tested for the presence of T. vaginalis by the direct wet mount microscopy and PCR analysis using vaginal discharge samples. Two vaginal swabs were taken from each patient, for the detection and characterization of T. vaginalis using Trichomonas specific primers. The DNA sequences for the primer set BTUB 9/2 were designed to target the conserved regions of the three β-tubulin genes of T. vaginalis to improve the analytical sensitivity and specificity. Results: Of the 100 vaginal discharges on swabs from patients and 20 healthy controls, 16 (16%) and 9 (45%) respectively were positive for T. vaginalis by the PCR analysis. The sensitivity and specificity of the wet mount microscopy examination was 0% in both patients and controls ($p < 0.05$). Conclusion: PCR analysis was found to be a good diagnostic tool for the detection of T. vaginalis infection, since it was very sensitive and was able to detect the parasites that were not detected by the wet mount microscopy. However, the major limitation that prevents the usage of the technique in routine laboratory examinations is the availability and cost.

Poster Session

MEM-PM3006 - Identification of a major lipase homolog in cutaneous pathogenic yeast Malassezia restricta

Minji Park[1], Yang Won Lee[2], Dongyeon Kim[1], Won Hee Jung[1]
[1]Chung-Ang University, An-Seong, South Korea, [2]Konkuk University, Seoul, South Korea

Malassezia species are basidiomycetous yeast and opportunistic pathogens often associated with seborrheic dermatitis including dandruff. To date, 14 Malassezia species have been identified, and among them, M. restricta is the most frequently isolated species from the healthy and disease skin. Almost all of the Malassezia species are lipophilic, of which property might be compensated by breaking down the sebum into fatty acid using lipases or phospholipases. Indeed, the recent genome analysis revealed that M. globosa possesses 14 lipases and 9 phospholipases although a role of each enzyme is poorly described. In this study, we aimed to evaluate expression of the genes that encode a lipase or a phospholipase homolog in M. restricta on scalp of the patients with dandruff and to identify the gene that is expressed upon interaction with the human host. Partial sequences of 4 genes of lipase and phospholipase homologs in M. restricta were obtained, and their transcript levels were evaluated in swap samples from 57 patients with severe dandruff. We found that the gene encoding the lipase homolog, MRE_0242, was expressed in almost all the samples while only few samples displayed expression of other homologs. These results suggested that MRE_0242 is the major lipase homolog in M. restricta that is expressed on the skin. Furthermore, we obtained the full length sequences of MRE_0242 by 5' rapid amplification of cDNA ends to characterize the functions of the enzyme. This putative lipase will be subsequently expressed in Pichia pastoris and purified for functional characterization. The results of our study will contribute to understand the interaction between Malassezia and the host.

Poster Session

MEM-PM3008 - In vivo evaluation of Buchenavia tomentosa extract and sodium bicarbonate on Candida albicans infection in an experimental denture model

Guilherme Teodoro[1], Marina Silva[1], Daniel Pereira[1], Cybelle Hiraoka[1], Gabriela Lima[1], Gabriela Melo[1], Fernanda Brighenti[2], Cristiane Koga-Ito[1]

[1]São José dos Campos Institute of Science and Technology- UNESP, São José dos Campos, Brazil, [2]Faculdade de Odontologia de Araraquara- UNESP, Araraquara, Brazil

The study aimed to evaluate the effect of Buchenavia tomentosa aqueous extract and sodium bicarbonate solution on Candida albicans oral infection in an experimental denture model. Experimental acrylic denture devices were intraorally placed in 54 immunosuppressed male rats (Rattus norvegicus Wistar) with mean weight of 350 g. Devices were inoculated with standardized C. albicans suspension (ATCC 18804) and divided into 3 groups, according to the treatment (n=18): B. tomentosa group (6.25 mg/ml); Sodium bicarbonate group (3% in distilled water); and control group (physiologic solution 0.9%). An aliquot of 100 µl of the test substances were applied, twice a day, according to the period of treatment (24, 48 or 72 hours). Animals were sacrificed after the end of the experimental period. Microbiological analysis of the biofilm formed in the denture base and histopathologic study of the palatal mucosa were performed. Data of colony forming units/denture were statistically compared by ANOVA Kruskal Wallis and Dunn's post hoc test ($p<0.05$). One denture per group was analyzed by scanning electronic microscopy. Significantly lower counts of C. albicans were observed among sodium bicarbonate and B. tomentosa groups in relation to the control in the periods of 24 ($p=0.003$), 48 ($p=0.000$) and 72 hours ($p=0.000$). Bicarbonate group showed significantly lower cfu values when compared to B. tomentosa group in the periods of 24 ($p=0.0002$) and 48 hours ($p=0.000$).This difference was not detected after 72 hours ($p=0.050$).Histopathologic analysis evidenced that, after 72 hours, the group treated with B. tomentosa showed the lowest degree of hyphae invasion. It could be concluded that both sodium bicarbonate and B. tomentosa aqueous extract were able to reduce the number of fungal cells. Treatment with B. tomentosa extract was more effective in preventing candidal invasion.

Poster Session

MEM-PM3010 - Cigarette Smoke promotes C. albicans pathogenesis by increasing EAP1, HWP1 and SAP2 Gene Expression

Mahmoud Rouabhia[1], Abdelhabib Semlali[2], Kerstin Killer[1], Humidah Alanazi[1], Witold Chmielewski[1]
[1]Dental Faculty, Laval University, Quebec, Canada, [2]Genome Research Chair, College of Science King Saud University, Saudia Arabia

Background: Smokers are more prone to oral infections than are non-smokers. Cigarette smoke reaches the host cells but also all of the other microorganisms present in the oral cavity. The contact between cigarette smoke and oral bacteria promotes such oral diseases as periodontitis. Cigarette smoke can also modulate C. albicans activities that promote oral candidiasis. The goal of this study was to investigate the effect of cigarette smoke condensate on C. albicans adhesion, growth, and biofilm formation as well as the activation of EAP1, HWP1 and secreted aspartic protease 2. Experimental protocol: Yeast cells were grown in the presence or absence of cigarette smoke condensate (CSC) at various concentrations (10, 20, 30, 40, or 50%). Yeast cultures were then used to assess the adhesion, growth and biofilm formation. Biofilms were examined by Scanning Electron Microscopy and quantitated by crystal violet staining assay. EAP1, HWP1 and SAP2 Gene Expressions were performed by quantitative RT-PCR. Results: Cigarette smoke condensate increased C. albicans (SC5314) adhesion and growth, as well as biofilm formation. This adhesion, growth, and biofilm formation may be supported by the activation of certain important genes. Using quantitative RT-PCR, we demonstrated that CSC-exposed C. albicans expressed high levels of EAP1, HWP1 and SAP2 mRNA and that this gene expression increased with increasing concentrations of CSC. Conclusion: CSC induction of C. albicans adhesion, growth, and biofilm formation may explain the increased persistence of this pathogen in smokers. These findings may also be relevant to other biofilm-induced oral diseases.
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Poster Session

MEM-PM3012 - Sporothrix schenckii biofilms: composition, development and structure

Rocío Sánchez Herrera[1], Lérida Liss Flores Villavicencio[1], Gloria Barbosa Sabanero[2], Myrna Sabanero López[1]
[1]Departamento de Biología, División de Ciencias Naturales y Exactas Campus Guanajuato, Universidad de Guanajuato, Guanajuato, México, [2]Departamento de Ciencias Médicas, División de Ciencias de la Salud Campus León, Universidad de Guanajuato, León, México

A great number of fungal infections are related to biofilm formation on inert or biological surfaces, which are recalcitrant to most treatments and cause human mortality. Sporothrix schenckii complex, a subcutaneous mycosis with a worldwide distribution. Human disease has a broad range of clinical manifestations and can be classified into fixed cutaneous, lymphocutaneous, disseminated cutaneous, and extracutaneous sporotrichosis. However, in S. schenckii has not been described biofilm formation, this issue is addressed in the present research, analyzing the biochemical characteristics of the formation and development of biofilms, using optical microscopy and physicochemical methods. The results indicate that the adhesion, development and formation of the fungal community is time dependent (12, 24 and 48h) and present a metabolic activity (0.05 to 0.62 Abs). The results indicate that the adhesion and development of the fungal community is time dependent (12, 24 and 48h), thereby increasing the biomass (0.19 to 0.5 Abs) and total carbohydrates (1.12 - 3.28µg/µl) is presented. Extracellular polymeric substances, comprising the matrix from biofilm were analyzed by Fourier transform infrared spectroscopy. The SEM shown structurally the development of S. schenckii as networks of hyphae with nucleation centers that consolidate the biofilm. Currently, we address a prospective study of biofilms S. schenckii that would be impact in the pharmacological treatment strategies of fungal infections.

Poster Session

MEM-PM3014 - Translational medical mycology - practice on dermatology clinic in China

Yuping Ran[1]
[1]*West China Hospital, Sichuan University, Chengdu, China*

The fungal infected patients with skin lesions may consult to dermatologists, that is a challenge to diagnose and treatment accurately. Dermatologists take samples from lesion to check the fungal elements under the microscopy by KOH preparation, then to treat the patient. This model has advanced as from bedside to bench, and, from bench to bedside (B to B to B), defined as Translational Medical Mycology. Dermatologists have an advantageous position in finding, isolate, identification the pathogenic fungi and treatment by familiar the usage of antifungal drugs. Samples should be cultured on different media with or without chloramphenicol and cycloheximide and incubated at room temperature and 37°C. No-culture techniques such as PCR based molecular identification, TEM, SEM, bio-chemistry tests, and histopathology are also necessary to confirm identification of the species, especially when the routine culture is negative. We start treatment upon obtaining proof of fungal infection, i.e., KOH positive. Itraconazole, fluconazole, terbinafine, and amphotericin B can be used alone or in combination based on the fungal species and the location. Practice on fungal infection including screen the patient, merge all of the laboratory technique and methods from the microbiologists, pathologists, molecular biologists and other specialists, to find the pathogen and to treatment as earlier as possible, to determine the optimum antifungal drugs and the duration of therapy, based on the drug sensitive test and the time-course culture monitor, from each individual patient.

Poster Session

MEM-PM3016 - Screening and functional analysis of novel pathogenic factor in Aspergillus fumigatus

Kanae Sakai[1], Motoko Oarada[1], Azusa Takahashi[1], Thoru Gonoi[1]
[1]*Chiba University MMRC,Chiba, Japan*

Aspergillus species are the most common molds encountered by humans and can cause variety of diseases. Although they are opportunistic pathogens, they caused severe and usually fatal invasive infection in immunocompromised hosts, such as AIDS and transplantation patients. Among the *Aspergillus* species, *Aspergillus fumigatus* is known as the major causative agent of Aspergillosis. Several antifungal drugs have been used for the therapy, but the recent emerge of drug resistant strains provided a strong incentive for developing new effective medicines in treatments of Aspergillosis. Glycans play variety of biological roles, and their recognition by glycan-binding proteins elaborates complex biological functions and networks. It is demonstrated that the interaction of glycan structure on the surface of pathogen and the receptor (lectin) on the host cell play an important role in the immune system. However, most of these studies focus on the function of receptors on the host cells, little is known about the receptors of pathogen. In this study, we focus on the fungal receptors (lectins) and aim to find the relation between lectins and its pathogenicity. We selected several genes which supposed to be lectin from genome database of *A. fumigatus*, and disrupted them one by one. After that, the mice were infected with wild-type and disruptnat strains to observe the pathogenicity. One of the disruptants, named Δlec3, showed clear increase in its pathogenicity. However, in spite of the difference in pathogenicity no clear morphological difference was observed in culture until now. Our data may suggest there are as yet unknown mechanisms underlying and affecting the pathogenicity. Now, we further study about this disruptant and expect to find new mechanism of infection and development of Aspergillosis.

Poster Session

MEM-PM3018 - Molecular epidemiology of Histoplasma capsulatum in Québec, Canada

Philippe J. Dufresne[1], Soumya Moonjely[1], Thérenne Desmeules[1], Marie-France Sirois[1], Cécile Tremblay[1, 2], Simon-Frédéric Dufresne[1, 3]

[1]Institut national de santé publique du Québec, Ste-Anne-de-Bellevue, Canada, [2]Centre hospitalier de l'Université de Montréal, Montréal, Canada, [3]Hôpital Maisonneuve-Rosemont, Montréal, Canada

Histoplasma capsulatum (Hc) is a pathogenic fungus that naturally thrives in soil rich in organic matter and nitrogen, especially those enriched with decaying bird or bat guano. In North America, it is endemic to Ohio and Mississippi river basin and the St-Lawrence river valley. Histoplasmosis results from the inhalation of aerosolized fungal spores in a contaminated environment. Clinical disease spectrum is diverse and includes progressive disseminated forms, which may be fatal if untreated. Molecular sequence typing of worldwide strain collections has previously revealed 8 distinct phylogeographic species of Hc (North American 1 and 2, Latin American A and B, African, Eurasian, Netherlands and Australian; Kasuga et al. 2003). Little is known of the genetic diversity of Hc isolates found in Canada. We sought to describe the molecular epidemiology of Hc in Québec, Canada. Phylogenic identity of 37 clinical strains from the provincial public health laboratory's (Laboratoire de Santé Publique du Québec) collection was determined using Multi Locus Sequence Typing (MLST). DNA sequences of 4 protein-coding genes (ADP-ribosylation factors – arf; H antigen precursors – H-anti; fatty acid desaturase – ole; alpha tubulin – tub) were compared with the known reference genotypes. Twenty-four new sequence types were identified among the 37 isolates. A high proportion of Latin American genotypes (n=21, 57%) was observed compared to North American genotypes (NAm 2: n=13, 35%; NAm 1: n=0). Eight percent of isolates could not be assigned to a known phylogenetic clade and may represent lone lineages or new cryptic species. The high predominance of Latin American strains suggests that true incidence of "native" histoplasmosis could be lower than anticipated in Québec. These findings bring new insights to the epidemiology of this endemic fungal disease and warrant further research.

Poster Session

MEM-PM3020 - Trichoderma metabolites provoked Brassica oleracea var. capitata resistance against Botrytis cinerea

Chuan-Kai Liao[1], Shu-Ying Liu[2], Chen-Yang Chang[2], Chaur-Tsuen Lo[3], Kou-Cheng Peng[1]

[1]Institute of Biotechnology, National Dong Hwa University, Hualien, Taiwan, [2]Department of Molecular Biotechnology, Da-Yeh University, Changhua, Taiwan, [3]Department of Biotechnology, National Formosa University, Yunlin, Taiwan

Many Trichoderma metabolites showed antimicrobial activities, but little is known about any beneficial effect of these metabolites to the host plants. Chrysophanol and anthraquinone are two major secondary metabolites produced by Trichoderma harzianum. Brassica oleracea var. capitata seeds germination rate can be promoted up to 2 folds while the cultivated soil was pre-treated with 5 ppm of above compound each (seeds germination rate: 20% with water, 48% with anthraquinone, and 44% with chrysophanol). The whole plant development including roots was much better in the presence of both compounds than the control group. Ten ppm of each compound was applied to the rhizosphere of 6-week-old B. oleracea var. capitata for 24 h, leaves then challenged with phytopathogen Botrytis cinerea. The results showed infected area down from 74% to 18% with anthraquinone treatment, and to 28 % with chrysophanol treatment, respectively. Apparently these two compounds provoked acquired resistance of B. oleracea var. capitata against phytopathogens. Further investigation with q-PCR to learn the variation of pathogenesis related proteins including PR-1, chitinase, β-1,3 glucanase, and ascorbate peroxidase. Data exhibited only the expression of ascorbate peroxidase increased significantly but not the others. It seems that prior to phytopathogen infection, minimum amount of reactive oxygen species (ROS) generated by anthraquinones causing B. oleracea var. capitata maintained basal concentration of peroxidase to detoxify the oxidative stress. Once phytopathogens such as B. cinerea invasion occurred, the pre-existing peroxidase apparently contributes immediate response to eliminate the harmful damage of oxidative electrophiles to the host plant thus the spreading of infection was contained. This study enriches our understanding the roles played by Trichoderma metabolites.

Poster Session

MEM-PM3022 - The effect of fungicide and nitrogen on agronomy, diseases, mycotoxins level and quality of 'Princeton' wheat

Ljiljana Tamburic-Ilincic[1], Jonathan Brinkman[2]
[1]University of Guelph, Ridgetown Campus, Ridgetown, Canada

The objective of the study was to investigate the effect of nitrogen, fungicides and their potential interaction on leaf diseases, Fusarium head blight (FHB) symptoms, Fusarium damaged kernels (FDK) level, mycotoxins levels and agronomic characteristics in a hard red winter wheat. Experimental plots of 'Princeton' wheat were planted at Ridgetown, Ontario. The treatments included three spring N rates (75, 100, and 125 kg N ha-1) and three fungicide regimes consisting of an untreated control, QUILT + PROSARO with QUILT applied at flag leaf stage (ZGS 39) followed by PROSARO applied at 50% anthesis (ZGS 60), or PROLINE applied at ZGS 60. Disease severity for powdery mildew and septoria tritici blotch were estimated when present by visually rating the plots on a 0-9 scale. FHB symptoms were recorded as incidence and severity. Deoxynivalenol (DON) concentration was determined by GC-MS method. FDK levels were measured using a SpecStar 2500-X Near Infrared (NIR) Analyzer. There was no significant interaction between N rates and fungicide application for any trait. Both fungicides treatments resulted in increased yield at the 100 kg/ha N component and decreased FHB index at all N levels, when compared to the respective control. Average FDK and DON level across all treatments was 7.5 % and 1.2 ppm, respectively. There was a significant relationship between FDK severity and DON levels; with a tendency for increasing N to increase FDK severity and fungicide application to reduce severity by 30-40% with QUILT+PROSARO. QUILT+PROSARO significantly decreased powdery mildew levels under all nitrogen levels, while PROLINE only reduced the level at the 75 kg/ha N rate. Protein level in grain was significantly reduced by the QUILT+PROSARO treatment at the lowest N level (75 kg/ha). The highest FDK (10.5%) and DON level (2 ppm) was obtained after application of 125 kg/ha of N and without fungicides.

Poster Session

MEM-PM3024 - Zooming on the occurrence of microbodies in cells of some fungal pathogens, and of their possible counterparts in infected host tissues

G.B. Ouellette[1]
[1]*Laurentian Forestry Centre, Quebec, Canada*

Uniform dense bodies are illustrated as occurring in elements of the following fungi (and the hosts they infect): *Ophiostoma novo-ulmi*(elms);*Gremeniella spp.*(coniferous trees);
Sphaaeropsis hypodermic(elms);*Fusarium spp.(carnation and staghorn sumac);*
Verticilliuim dahliae(eggplant);*Coryneum rhoinum*(staghorn sumac). These bodies were observed to occur in various configurations: as pairs of intergrading sizes; in beaded chains; in aggregates; as parts of fine filamentous structures. Abundant occurrence of similar bodies has been noted in vessel elements and adjoining cells in all the systems studied, being highlighted here thus:*O. novo-ulmi*-inoculated elms or injected with the toxin cerato-ulmin; *Verticillium*-inoculated eggplant;*Fusarium*¬-inoculated carnation; linden trees affected with decline. Comparisons are made with like bodies occurring in extracellular matter extending appreciable distance in the surrounding medium regarding, in particular:*O. novo-ulmi* growing on Millipore membranes and*S. Hypodermiain* in cultures. Present observations may also tie in with a seemingly first (and likely unique) report(Wergin 1972), showing the presence of similarly uniform and configured bodies in pathogenic hyphae of *F. oxysporum* f. sp. *lycopersici* and to the present author's unpublished observations (1956) of peculiar growth forms obtained from plated washings of beetles which were artificially contaminated with the DED pathogen and exposed to outdoors desiccating conditions for various periods of time.

Poster Session

MEM-PM3026 - Commensal bacteria are required for the regeneration of damaged skeletal muscle

Kaori Matsuo[1], Motoo Fujita[2], Ryoichi Nagatomi[3]
[1]Tohoku Institute of Technology, Sendai, Japan, [2]Tohoku University Hospital, Sendai, Japan, [3]Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan

BACKGROUNDS: Regeneration of damaged skeletal muscle requires sufficient supply of nutrients. Fully functional intestine and colon assure sufficient supply of nutrients. Since gut commensal bacteria support intestinal function, we hypothesized that elimination of commensal bacteria may affect muscle recovery from damage. OBJECTIVE: To investigate the contribution of commensal bacteria to the recovery of damaged skeletal muscle. METHODS: A cocktail of antibiotics was given orally to eliminate commensal bacteria of 10 weeks old male C57BL/6 mice for 7 days. Control mice received equivalent volume of water for 7 days. On the eighth day, cardiotoxin (CTX) was injected to gatrocnemius muscle to induce muscle damage. On days 3, 5, 7, 10, 14 and 20 after CTX injection, mice were sacrificed. Excised gastrocnemius muscle was subjected to immunohistochemical analyses. In a secondary experiment, LPS was concomitantly given orally with antibiotics before CTX injection to examine if LPS could resume any effect of antibiotics treatment on skeletal muscle regeneration process. RESULTS: Antibiotics treatment effectively reduced gut commensals, both aerobes and anaerobes, to somewhat less than 10-5. Although there was no difference in the body weight of ABx and control mice, recovery of gastrocnemius muscle weight was impaired in antibiotics treated mice even at 20 days after CTX treatment. Regeneration process of gastrocnemius muscle was obvious at day 5 in both groups. Developmental MHC was positive up to day 7 days in the control whereas in the antibiotics, dMHC was continuously positive even at day 20. LPS administration effectively restored the delayed recovery of damaged muscle. CONCLUSION: Our result suggests that components of commensal bacterial such as LPS favor the recovery of skeletal muscle from muscle damage.

Poster Session

MEM-PM3028 - Occurrence of penicilli on Slovak wine grapes from the Small Carpathian region and mycotoxins producing species

Soňa Felšöciová[1], Ľubomír Rybárik[1], Dana Tančinová[1], Zuzana Mašková[1]
[1]*Slovak University of Agriculture, Nitra, Slovakia*

The Slovak wine-growing region is divided into six viticulture areas. The largest in size and the most important over the centuries has been the Small Carpathian area. From the twelve vineyards were collected 14 grape varieties during harvesting 2011-2013. The objectives of this study were to gain more knowledge about mycobiota on grapes originating from Slovakia, with a focus on genus Penicillium and potentially toxigenic species tested by thin layer chromatography for the ability to produce selected mycotoxins. Fifty wine grapes per bunch that showed no symptoms were plated onto Dichloran Rose Bengal Chloramphenicol agar medium. The plates were incubated at 25±1°C for 7 days in the dark. From the 2795 fungal strains detected, 18 genera were identified. The genus Penicillium colonised 93% of samples. Thirteen species of Penicillium (251 colonies) were isolated and identified from exogenous mycobiota, namely P. aurantiogriseum, P. citrinum, P. coprophilum, P. crustosum, P. expansum, P. funiculosum, P. glabrum, P. griseofulvum, P. chrysogenum, P. oxalicum, P. purpurogenum, P. roqueforti, P. thomii. The main occurring penicillium species were P. chrysogenum (36%) which had the highest level of the isolated strains (63%), followed by P. crustosum (29% of the samples and 13% of the isolated strains), P. griseofulvum (21% of the samples and 9% of the isolated strains) and P. expansum (13% of the samples and 6% of the isolated strains). Seven potentially toxigenic species were tested for their toxigenic ability. We confirmed the production of citrinin, griseofulvin, patulin, penitrem A, roquefortin C. Out of 104 strains, 69% produced at least one mycotoxin. Ochratoxigenic microfungi P. verrucosum and P. nordicum were not found. The study was supported by the project: Development of International Cooperation for the Purpose of the Transfer and Implementation of Research and Development in Educational Programs conducted by the Operational Program: Education, ITMS code: 26110230085

Poster Session

VIR-PM2001 - Soraphen A mediated inhibition of acetyl CoA carboxylase activity represses HCV replication

Geneviève Desrochers[1], Ragunath Singaravelu[2], Shifawn O'Hara[3], Prashanth Srinivasan[2], Rodney Lyn[1], John P. Pezacki[1,2,3]

[1]Department of Chemistry, University of Ottawa, Ottawa, Canada, [2]Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Canada, [3]National Research Council of Canada, Ottawa, Canada

Hepatitis C virus (HCV) is a major cause of liver transplantation and hepatocellular carcinoma. Over 50% of HCV infected patients suffer from hepatic steatosis, a by-product of the high cellular lipid levels required for the replication and proliferation of the hepatitis C virus. HCV induces increased intracellular lipid levels through upregulation of de novo lipogenesis and repression of lipid secretion and catabolism. The rate-limiting step of fatty acid synthesis is catalysed by the enzyme acetyl-CoA carboxylase (ACC). In this study, we used a nanomolar inhibitor of ACC, Soraphen A, to investigate its role in HCV pathogenesis. Using coherent anti-Stokes Raman spectroscopy (CARS), we have shown that Soraphen A lowers the total cellular lipid volume in Huh7 hepatoma cells. More specifically, we have shown that triglyceride levels in Huh7 cells decrease as a result of a Soraphen A treatment. These effects on total lipid volume and triglyceride levels were also observed in Huh7 models stably expressing, respectively, a sub-genomic and a full-genomic HCV replicon of genotype 1b. Soraphen A treatment resulted in a potent nanomolar inhibition of HCV replication in Huh7 cells stably expressing either the subgenomic or full-length replicon. (IC50=10 nM) Repression of HCV replication in response to Soraphen A treatment was also observed in the genotype 2a full-length infectious model JFH-1, demonstrating that the inhibitory effect is conserved across genotypes. Native PAGE analysis revealed that Soraphen A treatment decreases polymerisation of acetyl-CoA carboxylase in hepatoma cells stably expressing HCV. This is consistent with previous findings, which suggests that Soraphen A acts by preventing the polymerisation of long active ACC polymers from inactive dimers. Collectively, our results demonstrate that Soraphen A mediated inhibition of ACC polymerisation inhibits HCV replication, and that Soraphen A is a valuable probe to study ACC's role in HCV pathogenesis.

Poster Session

VIR-PM2003 - Characterization of PPAR-α regulated miRNAs and their influence on Hepatitis C virus pathogenesis

Prashanth Srinivasan[1], Ragunath Singaravelu[1], Daniel Jones[2], Curtis Quan[1], Rodney Russell[2], John Paul Pezacki[1]
[1]National Research Council Canada, Ottawa, Canada, [2]Immunology and Infectious Diseases, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Canada

MicroRNAs (miRNAs) are small RNAs that post-transcriptionally regulate gene expression. Their aberrant expression is commonly linked with diseased states, including hepatitis C virus (HCV) infection. HCV hijacks lipid metabolism to facilitate its viral lifecycle. Clinically, this manifests as steatosis in 50% of HCV-infected patients. One pathway implicated in the development of HCV steatosis is PPAR-α signalling, which regulates fatty acid catabolism. Previously we have shown that HCV modulates PPAR-α expression through activation of miR-27, contributing to the accumulation of lipid droplets in hepatocytes. In this study we have performed functional miRNA profiling using a PPAR antagonist to identify miRNAs that are regulated by the PPAR-α pathway in human hepatoma cells. Our profiling resulted in the identification of 20 miRNAs implicated in the signalling pathway. In order to validate these miRNAs association with fatty acid catabolism, Coherent anti-Stokes Raman scattering (CARS) microscopy, for label-free imaging of cellular lipid content, was performed. It was found that a subset of these miRNAs regulate hepatic lipid homeostasis. We are currently investigating the relevant biological targets of these miRNAs as well as their potential anti-HCV activity. Our work suggests that HCV-induced downregulation of PPAR-α signalling through miR-27 induces dysregulation of other miRNAs, which together contribute to altered hepatic lipid homeostasis.

Poster Session

VIR-PM2005 - Ecology and evolution of influenza A viruses circulating in the populations of wild birds in the Kazakhstan part of Caspian Sea region (2002-2012)

Aidyn Kydyrmanov[1], Kobey Karamendin[1], Marat Sayatov[1], Kainar Zhumatov[1], Saule Assanova[1], Klara Daulbaeva[1], Yermuhammet Kassymbekov[1], Yelizaveta Khan[1]
[1]Institute of Microbiology and Virology, Almaty, Kazakhstan

The nourish species diversity of aquatic and near water system birds and natural habitats determine the North Caspian region as one of the key points for the studying of ecology and evolution of avian influenza viruses. A total of 76 influenza A virus isolates were allocated from four duck, five gull, two grebe species, and by one from Pelecanidae, Rallidae and Corvidae birds as the result of virological investigation of 2716 samples from birds of 44 species in the Kazakhstan part of Caspian sea during 2002-2012. Influenza viruses with six hemagglutinin and four neuraminidase subtypes in six different combinations were identified. The HA and M genes of influenza A virus isolates were characterized in this study. The HA genes of the Caspian H1N2 influenza viruses are not phylogenetically related to the pandemic strains cluster H1.3. The H4N6 viruses are probably reassortants of H4N8- and H4N6-like variants isolated in South Africa and China in 2004-2005. The HA gene of isolate A/mute swan/Aktau/1460/2006 (H5N1) fell into the separate group of low pathogenic H5 viruses branching from the sub-lineage of European strains circulated in 2005-2008. HA gene of H11 subtype virus A/herring gull/Atyrau/2180/2007 isolated in Northern Caspian belongs to American lineage. Influenza A/H13 virus isolates form a group of viruses phylogenetically ascending to prototype strain A/gull/Maryland/704/1977 (H13N6). The viruses of H16N3 subtype were close to that of Scandinavian strains forming sub-lineage, not related with the Caspian isolates of 1970-80th. M gene of the isolate A/black-headed gull/Atyrau/4379/2010 (H1N2) belongs to the gull lineage of Eurasian viruses and shows a close relatedness to Mongolian H13N6 isolates of 2006-2008, which keep them away from the viruses genetically and geographically typical for the North Caspian region. The analysis indicates that the Northern Caspian region is actively involved in the process of influenza virus global circulation and evolution.

Poster Session

VIR-PM2007 - Quantitative evaluation of the impact of host species on the genetic variation and evolution of avian influenza virus

Weifeng Shi[1,2], Xianbin Li[3,4], Zhong Zhang[1,2], Michael Carr[5], Di Liu[6], Weimin Zheng[7], Yanzhou Zhang[8], Chaodong Zhu[8], Fumin Lei[8]

[1]School of Basic Medical Sciences, Taishan Medical College, Taian, Shandong, China, [2]Collaborative Innovation Center for the Origin and Control of Emerging Infectious Diseases, Taishan Medical College, Taian, Shandong, China, [3]Shenzhen Institute of Advanced Technology, Chinese Academy of Science, Shenzhen, China, [4]University of Chinese Academy of Sciences, Beijing, China, [5]National Virus Reference Laboratory, University College Dublin, Dublin 4, Ireland, [6]Network Information Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, [7]Institute of Information Engineering, Chinese Academy of Sciences, Beijing, China, [8]CAS Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Avian influenza viruses (AIVs) annually cause high morbidity and mortality in human zoonotic transmission events with considerable pandemic potential and are also responsible for substantial economic losses in domestic poultry in East and Southeast Asia. AIVs infect a wide range of hosts, including migratory and domestic birds, fowls, and mammals. However, it remains unclear the contribution of each host to the genetic variability and evolution of AIVs. In this study, we perform a bioinformatics analysis of the HA gene sequences derived from H5N1, H6, H7 and H9N2 subtypes from different hosts. We apply BEAST to estimate evolutionary parameters, and Migrate to calculate the gene flow of AIVs between different hosts. PACT is used to reconstruct the trunk of the genealogy and study the annual change of the contribution of each host to the virus. Our results show that for H5N1, viruses from chickens and domestic ducks account for 36% and 24% of the virus variation, while those from wild ducks contribute approximately 10%. For the H6 subtypes, the percentages are 66% for domestic ducks and 20% for wild ducks. The top three hosts for the H7 subtypes are wild ducks (47%), domestic ducks (21%), and chickens (18%). In contrast, chickens (91%) and land fowl (7%) almost entirely contribute to the observed variations of H9N2 AIV. Furthermore, apart from H6, the contribution of the hosts to virus variation varies annually for H5N1, H7 and H9N2. To our knowledge, this is the first study providing quantitative evidence for the host contribution to viral genetic variation and evolution. Our results provide new insight into the AIV evolution among hosts, and highlight that surveillance and control strategies should be formulated for AIVs from different hosts and subtypes.

Poster Session

VIR-PM2009 - A point mutation prevents the accumulation of influenza virus M1protein to ND10

Toshikatsu Shibata[1], Mitsuhiko Moriyama[1], Tatsuo Yamamoto[1], Satoshi Hayakawa[1], Kazumichi Kuroda[1]
[1]*Nihon University School of Medicine, Tokyo, Japan*

We have previously shown that a part of M1 protein molecules accumulated to ND10 (PML bodies) in influenza virus infected cells (Shibata T et al. J Virol Methods. 2009) The accumulation was observed by the expression of N-terminal region of M1 protein, but not by that of C-terminal region. This result clearly showed the important role of N-terminal region. The N-terminal region of M1 protein consists of 9 alpha helixes and 8 loops (loop1-loop8). To reveal which part of N-terminal region was essential for the accumulation to ND10, the alanine substitution of the 8 loops was conducted. Alanine substituted N-terminal region of M1 protein was expressed in 293 T cells. Detection of the mutated N-terminal regions was done using tetra-cysteine tag introduced at the C-terminus of N-terminal region. In 8 loop-mutants of N-terminal region, only loop5 mutant was defective in accumulation to ND10. Loop5 mutant of authentic M1 protein was also lacked accumulation to ND10. Interestingly, M1 protein was detected in the supernatant of the loop5 mutant transfected cells. The loop5 corresponds to the amino acids at positions 86-90 (GNGDP). To examine which amino acid(s) was essential for the accumulation, each of 5 single amino acids was substituted by alanine. The D89A mutant lost the ability of accumulation to ND10, while the other mutants showed the accumulation. The generation of influenza virus with D89A mutation using plasmid-based reverse genetics was not successful. These results suggested that aspartic acid at position 89 should be crucial for accumulation of M1 protein to ND10. We assume that the lack of the accumulation might cause the lethal defect in influenza virus replication.

Poster Session

VIR-PM2011 - Different pathogenicity of H5N1 highly pathogenic avian influenza viruses determined by a single amino acid in the M1 protein

Naganori Nao[1], Masahiro Kajihara[1], Junki Maruyama[1], Rashid Manzoor[1], Mieko Muramatsu[1], Reiko Yoshida[1], Masatoshi Okamatsu[2], Hiroshi Kida[1,2], Ayato Takada[1]

[1]*Division of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan,*
[2]*Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan*

In 2010-2011, multiple outbreaks of highly pathogenic avian influenza (HPAI) occurred in Japan. Prior to these outbreaks, two HPAI virus strains, A/duck/Hokkaido/WZ83/2010 (H5N1) (WZ83) and A/duck/Hokkaido/WZ101/2010 (H5N1) (WZ101) were isolated from wild migratory ducks in Hokkaido, Japan. Although both WZ83 and WZ101 caused 100% mortality in 4-week-old chickens, WZ101 killed chickens more rapidly than WZ83. Moreover, WZ101 was highly lethal to 2-week-old ducks, whereas WZ83 caused no or only mild clinical symptoms in ducks. WZ83 and WZ101 are genetically similar (overall nucleotide sequence homology is 99.99%), and there are only two amino acid differences in the PB1 and M1 proteins (amino acid positions 317 and 43 in the PB1 and M1 proteins, respectively). To determine the critical factor(s) for the different pathogenicity between WZ83 and WZ101, we generated reassortant viruses between these viruses and compared their pathogenicity for chickens and ducks. We found that WZ83 whose M gene was replaced with that of WZ101 killed chickens more rapidly than wild-type WZ83 and caused severe clinical symptoms (e.g., neurological symptoms and collapse) in ducks with 60% mortality. In contrast, all ducks infected with WZ101 whose M gene was replaced with that of WZ83 survived without or with only mild clinical symptoms. Exchange of the PB1 gene did not affect the pathogenicity of the viruses for chickens and ducks. These results suggest that the difference between WZ83 and WZ101 in the pathogenicity for chickens and ducks is determined by the single amino acid in the M1 protein.

Poster Session

VIR-PM2013 - Application of the ion torrent high throughput sequencer to genetic analysis of avian influenza A viruses

Jessica Benkaroun[1], Yunyun Fu[1], Yanyan Huang[1], Gregory Robertson[2], Davor Ojkic[3], Hugh Whitney[4], Andrew Lang[1]
[1]Memorial University of Newfoundland, St. John's, Canada, [2]Environment Canada, Mount Pearl, Canada, [3]University of Guelph, Guelph, Canada, [4]Newfoundland and Labrador Department of Natural Resources, St. John's, Canada

Wild birds are the natural reservoir of influenza A viruses (IAVs), although many other species can act as hosts. IAVs have segmented negative-sense RNA genomes and evolve quickly due to an error-prone replicase enzyme and through the process of segment reassortment. Migratory birds move IAVs around the globe, contributing another important facet to IAV dynamics. The island of Newfoundland, Canada, represents an interesting location for the study of avian influenza A viruses (AIVs) because it has migratory bird connections with the mainland of North America and also with Europe. To better characterize the prevalence and the dynamic diversity of AIVs in wild birds in Newfoundland, Canada, we have applied the Ion Torrent high throughput sequencer to AIV sequencing. AIV viruses were cultured in embryonated specific pathogen-free chicken eggs from wild bird swab samples identified to contain AIV by real-time reverse transcriptase PCR. RNA was extracted from allantoic fluids and subjected to reverse transcriptase PCR to amplify the eight genome segments. The resulting DNA was used to create 200-bp read libraries, with 10 viruses barcoded and pooled together for sequencing on a single chip. The coverage varied for different viruses, with complete sequences obtained for all 8 segments for some and only partial sequences for some of the 8 segments for others. Therefore, some optimization of the procedure is still required. In comparison to the traditional Sanger sequencing method we were previously employing, this application of the Ion Torrent high-throughput sequencing technology has demonstrated great promise and will allow us to fully sequence the genome of multiple viruses more quickly and at lower cost.

Poster Session

VIR-PM2015 - Peripheral blood leukocyte responses to influenza virus infection in the ferret

Nedzad Music[1], Adrian J. Reber[1], Ruben O. Donis[1], Jacqueline M. Katz[1], Ian A. York[1]
[1]*Influenza Division, Centers for Disease Control and Prevention, Atlanta, USA*

Similarities in the pathogenesis observed between humans and ferrets have made ferrets a useful animal model for the study of influenza virus infection. However, the lack of reagents for this species has made assessment of viral pathogenesis and immunity difficult. We identified or confirmed a panel of antibodies that react with ferret leukocyte subsets. Daily monitoring of influenza virus-infected ferrets revealed rapid, marked changes in peripheral blood leukocyte (PBL) subsets. T cells, especially CD8+ cells, rapidly dropped to 5-20% of baseline levels on the first or second day post-infection, then partially recovered, but remained significantly lower than baseline levels for about 7 days post-infection. B cells showed a transient reduction in the first few days after infection, and a transient moderate granulocytosis also occurred. Although immunization with inactivated influenza vaccine made little difference in clinical signs and viral shedding upon H3N2 challenge, vaccinated animals restored pre-infection levels of leukocytes more rapidly than naïve ferrets. We also compared the effects of infection with the same dose of two different influenza A viruses, A/Perth/16/2009 (H3N2) and A/New York/21/2009 (A(H1N1)pdm09). Animals challenged with A(H1N1)pdm09 virus had more rapid weight loss, earlier onset of fever and 3- to 7-fold higher virus titers in nasal washes than those infected with H3N2 virus. The increased severity of A(H1N1)pdm09 infection was paralleled by PBL subsets, which showed evidence of more rapid and severe inflammation in A(H1N1)pdm09 virus infection. While H3N2 virus-infected animals showed little change until d2 post-infection, A(H1N1)pdm09-infected animals showed significant changes in all subsets examined on d1 after challenge. A(H1N1)pdm09-infected ferrets also showed a more gradual recovery to baseline levels of T cells. In addition to helping understand influenza virus pathogenesis, monitoring PBL subsets may be useful in evaluating disease severity and response to different influenza viruses.

Poster Session

VIR-PM2017 - The genomic RNA signatures of Influenza A virus that activate host innate immune response

GuanQun (Leonard) Liu[1,3], Hong-su Park[2,3], Qiang Liu[1,2,3], Yan Zhou[1,2,3]
[1]School of Public Health, University of Saskatchewan, Saskatoon, Canada, [2]Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada, [3]Vaccine and Infectious Disease Organization-International Vaccine Center (VIDO-InterVac), University of Saskatchewan, Saskatoon, Canada

Influenza A virus (IAV) infection of mammalian cells triggers innate immune response through recognition of viral RNA by pathogen recognition receptors (PRRs). Although the authentic IAV ligands that stimulate the host interferon system remain elusive, accumulating evidence has revealed the association of RIG-I with IAV genomic RNA in this process. The “panhandle model” of IAV genome structure illustrates the 5’ triphosphate and double strandedness formed by the 5’ and 3’ extremities which meet the criteria for RIG-I recognition. In this study, we defined IAV genomic RNA signatures located within the panhandle structure that contribute to the host interferon response. Using refined site-directed mutagenesis and ribonucleoprotein (RNP) reconstitution system, we tested the immunostimulatory activity of various subgenomic and genome-length RNA variants with mutant panhandle structures in porcine alveolar macrophages. These cells robustly produce type I interferon upon RNA transfection through RIG-I pathway. We found that the base-pairing of the segment specific region (5’ 14-16:3’13-15) within the proximal stem of the panhandle structure was dispensable for viral RNA promoter activity and immunostimulatory activity of reconstituted RNAs. Strikingly, substitution of the partially double-stranded terminal stem by full complementarity retained normal promoter activity but augmented the immunostimulatory ability. Similar enhancement was observed with 5’-end deletion of the unpaired adenosine or 3’-end insertion of an uridine residue at position 10. Taken together, we identified a minimal IAV genomic RNA signature located within the panhandle structure that efficiently stimulated host interferon response. This physiological relevant RNA structure offers insights into antiviral development by targeting RIG-I signaling.

Poster Session

VIR-PM2019 - Analysis of the role of chitinase (chi2) from the entomopathogenic fungus Metarhizium anisopliae as an insecticide against Spodoptera frugiperda larvae infected with recombinant baculoviruses

Anabele Lima[1], Bergmann Ribeiro[1], Renato Resende[1], Bergmann Ribeiro
[1]*Department of Cell Biology, University of Brazilia, Brazilia, Brazil*

Brazil stands out for being one of the largest exporters of agricultural products in the world, therefore the use of chemical pesticides impacts on both the environment and public health. An alternative to this practice is the use of biological agents to control pests. Spodoptera frugiperda is a species of polyphagous larva that attacks several economically important cultures in various countries, and is a major pest of maize in Brazil. Some pest control policies use viruses such as baculoviruses as a biological control tool. Baculoviruses are arthropod-specific double-stranded DNA viruses. In order to increase the pests speed of kill, thus improving its efficiency as a biopesticide, recombinant baculoviruses have been constructed using genes from the M. anisopliae fungus, which belongs to a group of entomopathogenic microorganisms that produce chitinases to penetrate the host´s body. The analysis of chitinase overexpression in insects infected by a recombinant baculovirus containing both the viral chitinase gene (chiA), as the fungus M. anisopliae chitinase gene (chi2), shows that it might increase its virulence, enhancing insect cuticle degradation and also intensifying peritrophic membrane degradation, hence allowing virus entry in the intestine of more susceptible insects. This study aimed to assess whether the expression of fungal chitinase enzymes during viral infection was able to increase viral pathogenicity in S. frugiperda larvae. Constructs were made inserting a chitinase gene (chi2) from M. anisopliae into the genome of AcMNPV virus, in order to evaluate the effect of heterologous expression in recombinant virus pathogenicity. The LC50 presented by the recombinant virus suggests an increase in virulence when compared to the wild type virus, furthermore, recombinant virus-infected cells showed higher levels of enzymes when compared to wild type virus-infected cells.

Poster Session

VIR-PM2021 - The complete genome sequence of alfalfa dwarf virus, a new putative cytorhabdovirus

Nicolas Bejerman[1,2], Fabian Giolitti[1], Soledad de Breuil[1], Veronica Trucco[1], Sergio Lenardon[1], Ralf Dietzgen[2]
[1]Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA) Córdoba, Argentina., [2]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Australia

Alfalfa dwarf virus (ADV) was detected in lucerne (alfalfa) crops distributed throughout Argentina. Diseased plants had shortened internodes, a bushy appearance, deformations, puckering, epinasty of leaflet blades, vein enations, and varying sized papillae on the adaxial leaflet surfaces. The full-length nucleotide sequence of the genomic RNA of ADV was obtained using a combination of deep sequencing of small RNAs and Sanger sequencing of PCR-amplified fragments. ADV genome comprised 14,491 nucleotides and the analysis of the antigenomic sequence of the negative-sense viral RNA genome revealed seven open reading frames (ORFs). Each of the ORFs found, except that encoding the polymerase gene, was amplified and sequenced to confirm the results of the in silico analysis of the sequence. The genome organization of ADV was similar to that of strawberry crinkle virus (SCV), a member of the genus Cytorhabdovirus: 3'-N-P-P3-M-G-P6-L-5', where N is the nucleocapsid protein gene, P the putative phosphoprotein gene, 3 and 6 are genes coding for proteins of unknown function, M the putative matrix protein gene, G the glycoprotein gene, and L the putative polymerase gene. ADV formed a distinct cluster with viruses in the genus Cytorhabdovirus: persimmon virus A (PeVA), lettuce necrotic yellows virus and lettuce yellow mottle virus in a phylogenetic tree based on the N protein sequence, which confirmed previous results based on alignments of a partial fragment of the L gene. Furthermore, amino acid sequence comparisons with the corresponding sequences of other rhabdoviruses revealed the closest relationship to PeVA (genome sequence of SCV is not publically available), with identities ranging from 17.4% for the matrix proteins and 43.1% for the L proteins. These results indicate that ADV may be classified as a new species in the genus Cytorhabdovirus.

Poster Session

VIR-PM2023 - Molecular epidemiology of Measles isolates from Nigeria between 2010 and 2011

Adedayo Faneye[1], Johnson Adeniji[1]
[1]University of Ibadan, Ibadan, Nigeria

BACKGROUND AND AIMS An estimate of 139 300 in 2010 deaths in children has been attributed to measles and the WHO African region accounted for 36%. Nigeria still records epidemics of measles which impacts on its goal of reducing preventable child mortality. Case-based surveillance with molecular characterization of wild- type measles isolates is one of the strategies employed by WHO in control of measles. This study employs case-based surveillance and molecular characterization of wild type measles isolates to provide an update on molecular epidemiology of measles virus (MV) isolates from Nigeria. METHODS Blood, throat swab and Urine samples were collected from 1200 children presenting with fever and maculopapular rash in South-Western Nigeria. Blood samples were screened for MV IgM antibodies using Enzyme Linked Immunosorbent Assay (Enzygnost, Germany) and MV N gene was detected and amplified from throat swabs and Urine samples of the children who tested positive to measles IgM by PCR amplification. The amplified product was then directly sequenced. The sequence data was edited and analysed using Mega5 software. RESULTS Measles IgM antibodies were found in 128 (10.6%) children. Measles RNA was detected from 23 throat swabs and urine samples. Sequences were obtained from 12 of the 23 PCR products. All the isolated strains were identified to be genotype B3 cluster 1 except one isolate that is of Clade A CONCLUSIONS It is interesting to report the first MV of clade A from Nigeria. This clade was previously known to circulate in Europe and Americas. Also, a high rate of divergence among the predominant circulating genotype of B3 cluster 1 was observed.

Poster Session

VIR-PM2025 - Genome organization, comparative sequence analysis and phylogeny of Datura yellow vein nucleorhabdovirus

Ralf G. Dietzgen[1], David J. Innes[2], Nicolas Bejerman[1]
[1]QAAFI, The University of Queensland, St. Lucia Qld, Australia, [2]QDAFF, Agriscience Queensland, Brisbane Qld, Australia

Datura yellow vein virus (DYVV) is a distinct plant-infecting nucleorhabdovirus with enveloped bacilliform particles that accumulate in the perinuclear space of infected cells. There are two known strains of DYVV in Australia, which infect Datura stramonium and the ornamental Black-eyed Susan (Thunbergia alata), respectively. We have used a combination of Roche 454 second-generation sequencing of oligo dT-enriched RNA from virus-infected plants and Sanger sequencing of RT-PCR amplified overlapping fragments to determine the complete 13,188 nucleotides genome of the T. alata strain. The 3' and 5' ends of the genome were confirmed by RACE. The negative-strand RNA genome of DYVV encodes six genes: nucleoprotein (N), phosphoprotein (P), putative movement protein (3), matrix protein (M), glycoprotein (G) and polymerase (L) in the order 3'-N-P-3-M-G-L-5'. The genome also features complementary 3' leader and 5' trailer sequences and conserved intergenic regions that are typical for all rhabdoviruses. The highest sequence identity matches of the viral open reading frames in Blast X searches were with cognate sequences of Sonchus yellow net virus (SYNV) N, G and L proteins with 51%, 45% and 45%, respectively. Phylogenetic analysis with other plant rhabdoviruses identified SYNV as the closest relative, clustered together in a separate branch among other viruses in the genus Nucleorhabdovirus.

Poster Session

VIR-PM2027 - Nucleocapsid assembly of Measles virus requires a phosphorylation modification on the core domain of nucleoprotein

Akihiro Sugai[1], Hiroki Sato[1], Kyoji Hagiwara[1], Hiroko Kozuka-Hata[1], Masaaki Oyama[1], Misako Yoneda[1], Chieko Kai[1]
[1]*The Institute of Medical Science, University of Tokyo, Tokyo, Japan*

The most defined role of measles virus nucleoprotein (N protein) is encapsidation of viral genomic and antigenomic RNA, where N proteins tightly associate with the viral genome to form an N-RNA complex that is template for viral transcription and replication. We had previously demonstrated that the major phosphorylation sites of N protein (S479 and S510) are involved in various stages of the viral replication. Additionally, we also found that a double-mutant of both major phosphorylation sites remained weakly phosphorylated, suggesting that unidentified phosphorylation site exists within the N protein. This minor phosphorylation residue has yet to be identified, and their functions are poorly understood. In this study, we identified nine putative phosphorylation sites by MALDI-TOF/TOF mass spectrometry. We prepared alanine substitution mutants for each putative phosphorylation site of N protein and examined the phosphorylation levels by ^{32}P labeling. Among these nine putative phosphorylation sites, the T279 site was remarkably phosphorylated. Minigenome expression assays revealed that the eight putative phosphorylation sites other than T279 were not required for N-protein function, but the T279 site was found to be functionally indispensable. Limited proteolysis and electron microscopy suggested that a T279A mutant lacked the ability for RNA encapsidation but was not denatured. Moreover, dephosphorylation of the T279 site by alkaline phosphatase treatment caused deficiencies in nucleocapsid formation. Taken together, these results indicate that phosphorylation at T279 is a prerequisite for functional nucleocapsid formation.

Poster Session

VIR-PM2029 - Detection and transcriptional mapping of the messenger and leader RNAs of orchid fleck virus, a bisegmented negative-sense RNA virus

Hideki Kondo[1], Maruyama Kazuyuki[1], Sotaro Chiba[1], Nobuhiro Suzuki[1]
[1]*IPSR, Okayama University, Kurashiki, Japan*

Orchid fleck virus (OFV) has two-segmented negative-sense RNA genome that resembles monopartite genome of plant nucleorhabdoviruses (family Rhabdoviridae). OFV RNA1 encodes N, P, ORF3, M and G proteins, whereas OFV RNA2 encodes L polymerase. These ORFs except for G have been shown to be expressed in OFV-infected plants possibly from the as-yet-undetected corresponding monocistronic mRNAs. It was unexplored whether transcriptional regulation of OFV genome is similar to that of rhabdoviruses. Hence, in this study, the transcripts of six genes encoded by OFV were molecularly characterized. All of the OFV mRNAs were started with a 5'-AA-3' sequence including one to three non-viral adenine nucleotides which were added at the 5' end of each mRNA, while their 3' termini were ended with a 5'-AUUUAAAUAAAA(A)n-3' sequence. These data revealed that the conserved 18 nt stretch [3'-UAAAUUUAUUUUGU(U/A)(G/A)UU-5'] in the gene junction sequences of RNA1 consists of a 12 nt gene end (3'-UAAAUUUAUUUU-5'), a tetra-nucleotide nontranscribed intergenic [3'-GU(U/A)(G/A)-5'] and a di-nucleotide gene start (3'-UU-5') sequences. Similar semi-conserved sequences are also present in RNA2. In the 3'- and 5'-terminal regions of the OFV genomic RNAs, there are putative extragenic regions called the 3' leader and 5' trailer, respectively. Therefore, we conducted similar analyses and showed that the polyadenylated plus- and minus-strand short RNA transcripts were generated from the 3'-terminal regions of both the OFV genomic and antigenomic RNAs, providing the first example of plus- and minus-strand leader RNAs in a segmented minus-strand RNA virus. This study strongly suggests that the transcription strategies of OFV and plant nucleorhabdoviruses are very similar, in which both having the well-conserved gene-junction sequences and generating monocistronic mRNAs and polyadenylated plus-strand leader RNAs. Our data provide further evidence that OFV and nucleorhabdoviruses might have evolved from a common ancestor, even though OFV has a bipartite genome.

Poster Session

VIR-PM2031 - Mechanisms of differential sensitivity of RSV and HPIV3 replication to curcumin

Genevieve Robinson[1], Beau Rabishaw[1], Kelsey Young[1], Kathryn Wright[1]
[1]*Department of Biochemistry, Microbiology, and Immunology, Ottawa, Canada*

Respiratory syncytial virus (RSV) and Human parainfluenza virus type 3 (HPIV3), members of the Paramyxoviridae, are the two most common viruses associated with severe respiratory infections requiring hospitalization in children under 2 years of age. Curcumin is a natural product that has been reported to restrict the replication of influenza virus, and more recently RSV. We have also tested the ability of curcumin to reduce replication of RSV in human monocyte (THP-1) and lung epithelial cell lines (A549, Beas2b), but found limited effect at doses that were not directly toxic to the cells. However, curcumin significantly reduced replication of HPIV3 in THP-1 and Beas2b cells, while having only a minor effect in A549 cells. We have carried out a more detailed investigation of the mechanisms of inhibition of HPIV3 replication. We have shown that addition of curcumin to cells as late as 24 hr post infection was as effective in reducing virus production as adding curcumin before or at the time of infection. These results suggest that curcumin affects viral replication after the binding and entry stages. As well, we are assessing whether inhibitors of signalling pathways known to be affected by curcumin also reduce HPIV3 replication. Inhibition of the MAPkinases p38 and MEK/ERK reduce HPIV3 replication, while inhibition of JNK had no effect. Inhibition of p38 also reduced RSV replication to a small extent. Other cellular pathways/molecules affected by curcumin are mTOR1 and hsp90; inhibition of mTOR1 with rapamycin had no effect on the replication of either virus, while HPIV3 displayed increased sensitivity to a hsp90 inhibitor compared to RSV, consistent with our findings with curcumin. Ongoing experiments are confirming phosphorylation of MAPkinases after infection, and examining viral transcription and replication in the presence of inhibitory compounds.

Poster Session

VIR-PM2033 - A potential role for the nucleolar protein Fibrillarin in henipaviruses replication

Celine Deffrasnes[1], Cameron Stewart[1], Paul Monaghan[1], Christina Rootes[1], Glenn Marsh[1], Andrew Bean[1]
[1]*CSIRO-AAHL, Geelong, Australia*

Zoonotic viruses from wildlife and domesticated animals pose a serious threat to human health. Henipaviruses, Hendra (HeV) and Nipah virus (NiV), are zoonotic paramyxoviruses which cause severe neurological and respiratory disease in human with an associated ~60% mortality rate. HeV is transmitted from bats to horses and infected horses then spread the virus to humans. Similarly, NiV is transmitted from bats to humans via diseased pigs or in some cases directly from bats. There is currently no approved treatment or vaccine against these for humans and therefore new therapeutic strategies are required to deal with these infections. A RNA interference whole genome screen performed by our group identified several host genes required for HeV and NiV replication. The top candidate host genes included the nucleolar protein fibrillarin. Gene silencing of fibrillarin greatly reduced HeV and NiV replication as measured by TCID50 and antigen staining. Nucleolar proteins such as fibrillarin are involved in nucleolar assembly, ribosome biogenesis, cell cycle regulation, cell proliferation and can act as a chaperone for the import of proteins into the nucleolus. Fibrillarin has previously been shown to interact with viral proteins, we therefore investigated the relationship of HeV viral proteins with fibrillarin. We found that HeV matrix protein travels to the nucleolus where fibrillarin resides. Furthermore, HeV infected cells with reduced fibrillarin expression appear more susceptible to apoptosis, which may be related to the observed antiviral activity. To investigate the specificity of the observed impact on viral replication by fibrillarin gene knockdown we evaluated the impact of knocking down fibrillarin expression on the replication of Dengue virus (flavivirus) and H5N1 avian influenza virus. Intriguingly, the impact appeared to be restricted to the Henipahviruses. These data support the approach that modulation of host gene expression impacts viral replication and may provide a novel approach to antiviral strategies.

Poster Session

VIR-PM2035 - Wild-type measles viruses with non-standard genome lengths

Bettina Bankamp[1], Chunyu Liu[1], Yayati Bera[2], Pierre Rivailler[1], Susmita Shrivastava[2], Ewen F. Kirkness[2], William J. Bellini[1], Paul A. Rota[1]

[1]Measles, Mumps, Rubella and Herpes Virus Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, USA, [2]J. Craig Venter Institute, Rockville, USA

The length of the genome of measles virus (MeV) is highly conserved at 15,894 nucleotides (nt). Here, we report the results from complete genomic sequencing of wild-type isolates of MeV with genome lengths of 15,900 nt. Two genotype D4 viral isolates had a 7 nt insertion in the 3' untranslated region (UTR) of the matrix (M) gene and a 1 nt deletion in the 5' UTR of the fusion (F) gene. The net gain of 6 nt complies with the rule-of-six required for replication competency of the genomes of morbilliviruses. The insertions and deletion (indels) were confirmed in one patient sample. MeVs can be grouped into eight clades, subdivided into 24 genotypes, based on the highly variable 450 nucleotides coding for the carboxyl-terminus of the N protein (N-450). The positions of the indels were identical in both viral isolates, even though epidemiological data and the three nt differences between the N-450 of the two genomes suggested that the isolates represented separate chains of transmission. Identical indels were found in the M-F intergenic regions of 14 additional genotype D4 isolates that were imported into the US during 2007-2010. Two viral isolates with indels produced titers in A549/hSLAM and Vero/hSLAM cells that were less than one log TCID50 different from titers produced by two MeVs with standard genome lengths. There were no differences in the size of plaques produced by viruses with and without the indels. This is the first report of wild-type MeVs with genome lengths other than 15,894 nt and demonstrates that the length of the M-F UTR of wild-type MeVs is flexible.

Poster Session

VIR-PM2037 - Stress granule-like structures are not involved in recognition of Sendai virus infection

Asuka Yoshida[1], Kawabata Ryoko[1], Takemasa Sakaguchi[1], Takashi Irie[1]
[1]*Hiroshima University, Hiroshima, Japan*

Type I interferon (IFN), one of the most important cytokines in the host innate immune system, is induced by cellular recognition of pathogen-associated molecular patterns (PAMPs), followed by expression of hundreds of IFN-stimulated antiviral genes. As for RNA viruses, viral RNA species, such as double-stranded (ds) RNA, produced as a by-product of viral replication have been reported to serve as PAMPs and to be sensed by Toll-like receptors or cytoplasmic RIG-like receptors (RLRs). However, it remains unclear what RNA species serve as PAMPs in real viral infection and how RLRs encounter the viral RNAs in the infected cells. Recently, an antiviral stress granule (avSG) was reported to function possibly as a general platform for many viruses to initiate antiviral signaling (Onomoto et al., PLoS One 7:e43031, 2012). In this study, we found that a representative IFN-inducible virus, Sendai virus (SeV; strain Cantell), a prototype of paramyxoviruses, did not induce avSG formation, as in the case of strain Z, which has been well known for its strong antagonism against the IFN system. However, a series of SeV recombinants with a lack of substitutions of amino acids within the IFN-antagonistic accessory protein C were able to induce avSG formation in an IFN-inducibility-dependent manner. SeV antigens were not detected in the avSGs even in this case. These results suggest that, unlike other viruses previously reported, such as influenza A virus, avSG formation is not required for detection of SeV infection.

Poster Session

VIR-PM2039 - Human parvovirus B19 infection and rheumatoid arthritis clinical course

Svetlana Kozireva[1], Anda Kadisa[1,2], Zaiga Nora-Krukle[1], Natalja Kakurina[1,2], Milda Naciute[3], Rita Rugiene[3], Diana Mieliauskaite[3], Irute Girkontaite[3], Modra Murovska[1]

[1]August Kirchenstein Inctitute of Microbiology and Virology, Riga Stradins University, Riga, Latvia, [2]Riga East Clinical University Hospital, Riga, Latvia, [3]Department of immunology, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania

Human parvovirus B19 (B19) could be the cause of the initial immune process that promotes the development of rheumatoid arthritis (RA) in predisposed humans. It also may influence the clinical course of RA – disease activity and aggressivity. This study aims to assess whether B19 infection makes course of RA more active and more aggressive. 88 RA patients (51 - from Latvia and 37 from Lithuania) and 25 healthy individuals were examined for the presence of anti-B19 antibodies and B19 virus specific DNA by ELISA and recomLine B19 IgG and IgM test, and nested PCR, respectively. The proliferative activity of T-lymphocytes was estimated on the 3rd and 6th day of cultivation in the presence of virus or B19 VP1/VP2 peptide, using 3H-thymidine incorporation technique. Indices of RA activity and aggressivity were analysed depending on the presence of B19 infection markers. In the patients group with high disease activity B19 active infection markers (virus specific IgM antibodies and/or viremia) were detected in 20/45 patients, in the group with middle disease activity - in 6/30 patients (p=0.025). In the groups with low disease activity and remission the patients and positive cases number were not sufficient for comparative analysis. Also detection frequency of B19 latent or past infection markers in the group with high disease activity was higher than in the patients group with middle disease activity (25/45 and 24/30, respectively, p=0.025). No patients without B19 infection markers were identified. In patients with B19 active infection markers the higher mean indices of disease aggressivity were determined. Lymphocytes of RA patients responded to B19 antigens quicker and more frequently than those of healthy individuals (50.0% versus 8.0% on the 3rd day, and 73.1% versus 38.0% on the 6th day). Active B19 infection mostly increases RA activity but also have an effect on disease aggressivity.

Poster Session

VIR-PM2041 - VP4 plays a pivotal role in both virion assembly and viral RNA synthesis during enterovirus infection

Yong Wah Tan[1], Wan Jin Hong[3], Justin Jang Hann Chu[1,2]

[1]Institute of Molecular and Cell Biology, Collaborative Translation Unit for HFMD, Singapore, Singapore, [2]National University of Singapore, Department of Microbiology, Singapore, Singapore, [3]Institute of Molecular and Cell Biology, Protein Trafficking and Cancer Cell Biology, Singapore, Singapore

As an emerging public health threat, outbreaks of hand, foot and mouth disease (HFMD) has had an apparent increase in both frequency and severity in the recent years, particularly in Asia. The disease can result from infections by a plethora of enteroviruses, with type A enteroviruses, human enterovirus 71 (HEV71) and coxsackievirus A16 (CVA16) being the most common aetiologic agents isolated during HFMD outbreaks. The enterovirus capsid is composed of four structural proteins, VP1, VP2, VP3 and VP4. Previous studies on poliovirus, a type D enterovirus, reported the importance of N-myristoylation of capsid protein VP4 in virion assembly. The N-terminal myristoylation signal (MGXXXS) of viral capsid protein VP4 is an extremely well conserved feature of enteroviruses, highlighting the integral role this co-translational modification plays in enterovirus replication. Inhibition of myristoylation using small interfering RNA (siRNA) to human N-myristoyltransferases (hNMTs) confirmed the importance of myristoylation in the replication of HEV71. To gain a deeper understanding of the role myristoylation plays in viral replication, we used myristic analogues, 2-hydroxymyristic acid (2OHM) and 4-oxatetradecanoic acid (4O) to inhibit myristoylation of VP4. The two analogues exhibited differential effects on the replication of HEV71 in rhabdomyosarcoma (RD) cells, with 2OHM preferentially affecting VP0 cleavage into mature proteins VP4 and VP2 while 4O affected viral replication on general. This implies the involvement of the myristoylated VP4 in viral RNA synthesis in addition to virion assembly.

Poster Session

VIR-PM2043 - Development of alternative vaccines against Foot-and-mouth disease virus by empty capsids expression in insect cells

Claudine Porta[1,3], Silvia Loureiro[2], Eva Perez[1], Liz Fry[3], David Stuart[3], Ian Jones[2], Bryan Charleston[1]
[1]The Pirbright Institute, Pirbright, UK, [2]University of Reading, Reading, UK, [3]The Wellcome Trust Centre for Human Genetics, Oxford, UK

Whole viruses inactivated by a chemical process constitute the current vaccines against Foot-and-mouth-disease (FMD). Grown in mammalian cells, their main disadvantages are: (1) the instability of the killed virions at ambient temperature requiring maintenance of a cold chain between manufacture and administration (2) the hazards associated with dissemination of this airborne virus, which leads to the need for strict bio-containment laboratories for vaccine production. FMDV is comprised of a capsid made up of 60 copies each of 4 proteins and a single positive strand of RNA. As an alternative approach to FMDV vaccination we are developing a strategy for the generation of virus-like particles (VLPs). Their main advantages are: (1) preservation of the three-dimensional structure of the virus capsid and hence they will, unlike subunit vaccines, provide the full range of viral antigenic sites (2) absence of the viral genome and thus infectivity, which allows their production in standard facilities. FMDV comprises 7 serotypes; these vary in the level of stability of the virus particles and in their concomitant ability to produce empty capsids, as a by-product during virus multiplication. No natural empty capsids are generated by serotype O1M, our chosen target for VLPs synthesis via baculovirus-mediated expression in insect cells. A genetic cassette encoding the precursor of the structural proteins P1 and the viral protease 3C, that cleaves it into mature capsid proteins, was designed. To overcome the well documented cellular toxicity of 3C, variants of this cassette were tested in order to try and improve expression of P1 whilst producing 3C at reduced levels of synthesis and/or activity. In addition in an effort to generate capsids with improved morphology, the need for myristoylation for FMDV particle morphogenesis was re-assessed through N-terminal mutagenesis of P1.

Poster Session

VIR-PM2045 - Detection and genetic characterization of human cosavirus in a pediatric patient with diarrhea, Japan

Shoko Okitsu[1,2], Pattara Khamrin[3], Aksara Thongprachum[2], Shuichi Nishimura[4], Sayaka Takanashi[2], Hiroyuki Shimizu[5], Satoshi Hayakawa[1], Hiroshi Ushijima[1,2]

[1]Nihon University School of Medicine, Tokyo, Japan, [2]The University of Tokyo, Tokyo, Japan, [3]Chiang Mai University, Chiang Mai, Thailand, [4]Nishimura Clinic, Kyoto, Japan, [5]National Institute of Infectious Diseases, Tokyo, Japan

[Introduction] In Japan, the mortality of acute gastroenteritis (AGE) in children under 5 years old is low, however, prevalence of the disease is still high. The detections of diarrheal viruses including norovirus GI and GII, group A rotavirus, sapovirus, human astrovirus and adenovirus are routinely conducting, however, almost half of samples remained to be negative for those viruses. Human cosavirus (HCoSV) is a newly identified virus in the family Picornaviridae. This virus has been detected in stool samples of children with diarrhea or non-polio acute flaccid paralysis worldwide. In this study, HCoSV was detected in a fecal sample of pediatric patient with AGE in Japan. [Material and Methods] A total 630 stool samples were collected from children with AGE in 6 pediatric clinics, in Japan, between November 2011 and April 2012, and screened by (RT-) PCR method for detection of 13 diarrheal viruses including HCoSV, noroviruses GI and GII, group A and C rotaviruses, human astrovirus, adenovirus, sapovirus, Saffold virus, Aichivirus, human parechovirus, enterovirus, and human bocavirus. [Results] HCoSV was found in a single sample (10928/JPN) from a boy aged six month. This sample was negative for other 12 diarrheal viruses. The clinical feature of patient was severe diarrhea, and no other findings including vomiting, fever and upper respiratory symptoms. Analysis of complete coding-sequence revealed that the HCoSV strain seemed to be classified as a new genotype in HCoSV-A,. [Discussion] To our knowledge, HCoSV has been found in the samples with diarrhea as well as healthy condition, and the pathogenicity of HCoSV remains to be uncertain. In this study, HCoSV strain 10928/JPN was isolated from a pediatric patient with AGE, and other common diarrhea-caused viruses were not found in the sample. Therefore, HCoSV is seemed to be the viral agents that cause AGE in this patient.

Poster Session

VIR-PM2047 - A potexvirus that produces attenuation over a potyvirus in plants

Gabriela Chavez-Calvillo[1], Carlos A. Contreras-Paredes[1], Tzvetanka D. Dinkova[2], Javier Mora-Macias[3], Laura Silva-Rosales[1]

[1]Departamento de Ingeniería Genética. CINVESTAV Irapuato, México, [2]Departamento de Bioquímica Facultad de Química, UNAM. México, D. F., [3]Langebio. CINVESTAV Irapuato, México

Synergism in plants is the classic example of potex-potyvirus interaction and has been reported in different hosts. Antagonism has been described only between phylogenetic related viruses. Our studies reveal that two non-related RNA viruses: Papaya ringspot virus (PRSV), a potyvirus; and Papaya mosaic virus (PapMV), a potexvirus produce a contrasting phenotype in mixed infections on papaya plants. The outcome of the disease depends on the order of arrival and infection time to their host: a synergistic (detrimental) or an antagonistic (beneficial) interaction. We observed a remarkable synergistic phenotype when the host was simultaneously infected with both viruses or when PRSV was firstly inoculated. In both synergistic cases, PapMV was able to increase its transcripts (as has previously reported), but its translation decreases. Our polysome profiling analysis suggests that PRSV interferes with the cellular translation machinery preventing the association of PapMV RNA with the polyribosomes and inducing its massive genomic RNA accumulation. Unexpectedly, when PapMV was primary infected, PRSV symptoms decrease concomitant with a genomic RNA reduction. However, its Coat Protein accumulation decreases only 30% as compared to its single infection. Our polysome analysis shows, that he small amount of PRSV-RNA locates in the highly active translational fractions, allowing its CP accumulation. Also the endogenous EIF1α messenger seems to be affected when PRSV is present. We estimated that PapMV moves faster in the plant and produces more viral RNA than PRSV, but PRSV-RNA is more efficiently translated producing more protein per RNA molecule. PRSV symptom attenuation by PapMV is correlated with a prolonged production of Systemic Acquired Resistance, detected by the induction of the Pathogenesis-related protein 1 gene and a significant increase in the amount of Reactive Oxygen Species shown to be involved as a plant response.

Poster Session

VIR-PM2049 - Polyprotein processing of Cocksfoot mottle sobemovirus

Gabriela Uffert[1], Merike Sõmera[1], Kärt Sõber[1], Kätlin Kivisaar[1], Grete Nummert[1], Karin Valmsen[1], Erkki Truve[1]
[1]Tallinn University of Technology, Tallinn, Estonia

Cocksfoot mottle virus (CfMV) belongs to the genus Sobemovirus. It has a positive-sense polycistronic RNA genome. An ORF2a/2a2b encodes polyprotein(s) exhibiting several different conserved motifs, including the motif characteristic of a serine protease (Pro) which proposed consensus is H(X32-35)[D/E](X61-62)TXXGXDG. However, the protease function has been proven only in case of Sesbania mosaic virus (SeMV). Also, the 3D-structure of SeMV Pro is available. A modeling based on the alignment of the sequences of CfMV and SeMV Pro-s and the resolved structure of SeMV demonstrates the similarities between them. For SeMV, it has been demonstrated that four cleavage sites are utilized during a polyprotein cleavage to subsequent proteins. For CfMV, two polyprotein cleavage sites have been identified during mass-spectrometric analysis of the N- and C-terminal sequences of VPg that is encoded also in composition of polyprotein. Here we demonstrated that a point mutation in either cleavage sites abolished infection. Secondly, we have introduced a point mutation into catalytic triad of CfMV putative Pro. Experiments with different host plants showed that inactivation of viral Pro completely abolished infection. Thus, the correct cleavage of VPg carried out by viral Pro is essential for CfMV infection. In addition to these two cleavage sites, we have earlier predicted that there is a non-conventional but well conserved cleavage site E/M between an N-terminal transmembrane anchor and serine protease domain of CfMV. A point mutation in that putative cleavage site abolished infection. Hence this site indeed might be important for polyprotein processing. A search for the fourth possible cleavage site in CfMV polyprotein C-terminus is under the process. The preliminary results of a study about C-terminal stop mutants suggest that the last 20 amino acids are not needed for viral infection.

Poster Session

VIR-PM2051 - Dissecting functions of the Polerovirus P0 protein silencing suppressor in evasion and elicitation of plant defenses

Melanie Sacco[1], Ken-Der Wang[1]
[1]*California State University, Fullerton, USA*

The Polerovirus protein P0 is a viral silencing suppressor (VSR) that inhibits host RNA silencing, an anti-viral defense mechanism, by targeting Argonaute (AGO) proteins for degradation. We previously found that the P0 from three Polerovirus species, Beet Western Yellows Virus (BWYV; P0BW), Cucurbit aphid-born Yellows Virus (CABYV; P0CA) and Potato Leafroll Virus (PLRV; P0PL), are recognized as avirulence determinants in Nicotiana glutinosa and induce either resistance with cell death (hypersensitive response; HR) or extreme resistance in the absence of cell death, depending on the plant accession challenged. To identify amino acids that are critical for P0 activities, the P0BW gene was analyzed by serial deletion and by directed mutagenesis at several sites conserved in all three P0 proteins. We also performed systematic mutation analysis of the entire length of the P0 protein by sequential substitution of 6 amino acid blocks with the sequence NAAIRS. We predicted that the modifications could potentially cause loss of VSR activity and/or loss of HR elicitation in N. glutinosa accession TW59, or gain of HR in N. glutinosa accession TW61 where no cell death response against P0BW previously existed. Mutant clones were co-infiltrated with GFP into the leaves of N. glutinosa and N. benthamiana to observe for suppression of RNA silencing and induction of cell death, or with AGO1 to monitor P0-induced degradation. Lysine substitution of two conserved central arginines caused P0BW to elicit more rapid and robust HRs on TW59. Modifications within or deletion of the last 22 carboxy-terminal amino acid residues (227-249) resulted in P0BW mutants that maintained VSR activity, but displayed impaired ability to elicit HR on TW59, suggesting that P0 activities in suppression of silencing and in elicitation of HR may be distinct.

Poster Session

VIR-PM2053 - Subcellular localization of viral protein that induces developmental abnormalities in stem and leaf tissues

Go Atsumi[1], Reiko Tomita[1], Ken-Taro Sekine[1]
[1]*Iwate Biotechnology Research Center, Iwate, Japan*

We identified a novel virus, Gentian Kobu-sho-associated virus (GKaV) as a candidate of Kobu-sho pathogen in gentian. Kobu-sho is a syndrome that causes tumorous or hyperplastic disorders on stems, nodes and roots of gentians. GKaV sequence has a single large ORF that encodes a potential polyprotein of about 7,400 amino acids. The amino acid sequence did not show significant similarity to any plant viral proteins. We found that expression of GK32 fragment, a partial 810 nt fragment of the large ORF, induced a typical symptom of Kobu-sho using a transient assay based on Nicotiana benthamiana-Potato virus X vector system. GK32 expression induced ectopic development of lignified cells in stem cortex, resulting in tumorous symptom on stems. Furthermore, GK32 expression induced an ectopic development of leaf-like tissue on the abaxial side of veins in normal leaves. In this study, we examined the localization of GK32 protein fused with YFP by confocal microscopy in N. benthamiana using Agrobacterium-mediated transient assay. We found that GK32-YFP formed many speckles or plate-like structures in the nucleus. GK32-YFP colocalized with Arabidopsis thaliana SmB and SmD3 fused with mCherry that are marker proteins of Cajal bodies in the nucleus. In contrast, GK32-YFP did not colocalize with coilin-mCherry, another established marker of Cajal bodies. Because SmB and SmD3 are components of mRNA splicing, localization of GK32-YFP was compared with that of other mRNA splicing factors, U1-70K, U2B″ (found in Cajal bodies) and SR proteins (SR34, SC35 and RS2Z33: found in nuclear speckles but not in Cajal bodies). The results revealed that GK32-YFP colocalized with U1-70K- and U2B″-mCherry but not with SR proteins-mCherry.

Poster Session

VIR-PM2055 - Evidence for independent selection of BNYVV resistance breaking strains by Rz1 in sugar beet and analysis of associated fitness loss by means of deep sequencing

Kathrin Bornemann[1], Mark Varrelmann[1]
[1]*Institute of Sugar Beet Research, Göttingen, Germany*

Beet necrotic yellow vein virus (BNYVV), vectored by Polymyxa betae, causes rhizomania in sugar beet. For disease control cultivation of hybrids carrying Rz1 resistance is crucial but compromised by resistance-breaking (RB) strains with specific mutations in the P25 pathogenicity factor at amino acid 67-70 (tetrad). All RB-strains described so far in US and ES possess an A67V amino acid exchange within the RNA3-encoded P25 pathogenicity factor (tetrads VLHG and VCHG). BNYVV recently isolated from Rz1 plants collected in UK, NL and D, displaying patches of strong rhizomania symptoms, however, displayed the yet unknown P25 tetrad composition AYPR. Loading of virus –free P. betae population and subsequent resistance test supplied evidence for Rz1 resistance breaking abilities and demonstrated geographically independent selection of BNYVV RB-strains with different composition of the pathogenicity factor. To demonstrate selection by Rz1 and effect of RB mutations on relative fitness, competition experiments between strains were performed. Following mixture of strains with four RNAs, a shift in tetrad variants was observed, suggesting that strains did not mix or transreplicate. The plant genotype exerted a clear influence on the frequency of RB tetrads. In Rz1 plants, the RB variants out-competed the WT variants, and mostly vice versa in susceptible plants, demonstrating a relative fitness penalty of RB mutations. The strong genotype effect supports the hypothesized Rz1 RB strain selection with four RNAs suggesting a certain tetrad needs to become dominant in a population to influence its properties. Tetrad selection was not observed when a RB strain, with an additional P26 protein encoded by a fifth RNA, competed with a WT strain supporting its role as second BNYVV pathogenicity factor and suggesting reassortment of both types.

Poster Session

VIR-PM2057 - A multi-target, non-infectious and clonable artificial positive control for PCR-based assays

Donna Caasi[1], Mohammad Arif[1], Mark Payton[2], Ulrich Melcher[3], Louise Winder[4], Francisco Ochoa-Corona[1]
[1]Oklahoma State University, NIMFFAB, Stillwater, USA, [2]Oklahoma State University, Dept Statistics, Stillwater, USA, [3]Oklahoma State University, Dept Biochem & Cell Biol , Stillwater, USA, [4]Lincoln University, Lincoln, Canterbury, New Zealand

Positive controls are essential for PCR reliability and are challenging to obtain for rare, exotic and/or emerging pathogens and pose biosafety risks if manufactured using infectious pathogens. Custom synthetic DNA inserts can be designed *de novo* in tandems of forward and reverse complement priming sequences to be inserted in circular plasmid vectors. To test this concept, artificial positive controls (APCs) for use in PCR were synthesized to contain primer sequences targeting four viruses (Barley yellow dwarf virus, Soilborne wheat mosaic virus, Triticum mosaic virus and Wheat streak mosaic virus) pathogenic to wheat and, as internal control, the plant mitochondrial nad5 gene. Thermodynamics and folding parameters of twenty-four APC inserts were assessed in silico. Two thermodynamically different APCs, designated optimal and sub-optimal, were cloned and tested using end point PCR. The optimal APC had a 100% amplification rate, while only 92% of virus-infected plant tissues, commonly used as reference positive controls, amplified. An array of APC priming sequences from different organisms and/or previously tested primers can be accommodated in a large and flexible number of positive control targets. APCs will streamline and standardize routine PCR, improve reliability and biosafety, and create opportunities for development and commercialization of new synthetic positive control sequences.

Poster Session

VIR-PM2059 - Bipartite genomic RNAs of Red clover necrotic mosaic virus use different eukaryotic translation initiation factors for their cap-independent translation

Yuri Tajima[1], Masanori Kaido[1], Kazuyuki Mise[1], Tetsuro Okuno[1]
[1]*Graduate School of Agriculture, Kyoto University, Kyoto, Japan*

Plant RNA viruses often lack both a 5' cap and a 3' poly(A) tail in their genomic RNAs. Instead, cap-independent translation enhancer elements (CITEs) located in the 3' untranslated region (UTR) mediate their translation. Although eukaryotic translation initiation factors (eIFs) or ribosomes have been shown to bind to the 3' CITEs, our knowledge is still limited for the mechanism, especially for cellular factors. A positive-strand plant RNA virus Red clover necrotic mosaic virus (RCNMV) does not possess a 5' cap and a 3' poly(A) tail in its genomic RNAs, RNA1 and RNA2, and they initiate translation in a cap-independent manner. RNA1 has a 3' CITE named 3'TE-DR1 that is essential for cap-independent translation. In addition, RNA1 has an adenine-rich sequence (ARS) upstream of 3'TE-DR1 in the 3' UTR. Poly(A)-binding protein directly binds to ARS and the binding is required for recruiting 40S ribosomes. On the other hand, RNA2 does not possess RNA elements like 3'TE-DR1 and ARS, and translation from RNA2 is coupled to RNA2 replication. Precise mechanisms of cap-independent translation of RCNMV remain elucidated. Here, we screened Arabidopsis thaliana mutants to identify which eIF4F/eIFiso4F components promote the cap-independent translation of RCNMV genomic RNAs. We found that RCNMV requires all eIF4F/eIFiso4F component genes for infection in planta. Using Arabidopsis protoplasts, we show that eIF4E, eIF4G, and eIFiso4G2 are required for RCNMV replication. We confirmed the requirement of eIF4E and eIF4G for 3'TE-DR1-mediated translation of RNA1 using luciferase assays in Arabidopsis protoplasts and an in vitro translation system. Furthermore, we found that eIFiso4E and eIFiso4G1 are required for translation of RNA2. These results show that the requirements of eIF4F/eIFiso4F for cap-independent translation differ between RNA1 and RNA2. Such differential preferences of eIFs between viral genomic RNAs might contribute to regulating viral gene expression during RCNMV infection in host plants.

Poster Session

VIR-PM2061 - Physiological and biochemical modifications induced by viral infection in garlic (allium Sativum L.)

Michelle de Souza Fayad-André[1], André Nepomuceno Dusi[2], Augusto César Franco[3], Renato de Oliveira Resende[4]
[1]Department of Plant Pathology, Institute of Biology, University of Brasilia, Brazilian, Brazil, [2]Brazilian Enterprise for Agricultural Research, Brasilia, Brazil, [3]Department of Botanic, Institute of Biology, University of Brasilia, Brazilian, Brazil, [4]Department of Cell Biology, Institute of Biology, University of Brasilia, Brazilian, Brazil

Garlic is one of the major horticultures of the world. Its use is related mainly to cooking and phytotherapy, and Brazil ranks 29th worldwide and 2nd in South America in its production. However, due to phytosanitary issues associated to some species of Potyvirus, Carlavirus and Allexivirus, total yielding can be reduced up to 53%. When infection process begins, plants carry on with physiological and biochemical changes related to activation/blocking of specific metabolic pathways. Since little is known regarding the accurate physiological and biochemical description of such modifications associated to garlic viral complex infection, we chose it to be one of the objectives of our research group. In order to proceed with accurate description comparing virus-free (HP) and infected plants (IP), we used 'Cateto Roxo' cultivars, in a 2 x 10 bioassay in green-house. Seeded garlic cloves were evaluated at 30, 60 and 90 days post-seeding (d.p.s.) and at the end of culture cycle for physiological (plant height, dry and fresh weight, total leaf area, bulb weight) and biochemical (photosynthetic and rubisco activity and total starch, soluble sugars and proteins) traits. Data was analysed using a T-test (5%). HP developed better than IP for all physiological traits, except bulb weight at 30 and 60 d.p.s. Also, CO_2 assimilation was 20% higher for HP than IP, and contents of chlorophyll and carotenoids was significantly lower for IP, at all measuring points. Such results may be related to plant symptomathology, which reduces photosynthetic activity by destroying chlorophyll molecules in affected tissues. There was no significant difference in total soluble sugars and total proteins between HP and IP, but more scrutinized investigations using HPLC will be performed to elucidate our results. Starch analysis showed that garlic plants do not accumulate starch, or if they do so, it stacks at very low levels.

Poster Session

VIR-PM2063 - Genetic variability of Watermelon mosaic virus infecting cucurbits in Southern United States

Akhtar Ali[1], Osama Abdalla[2]
[1]*The University of Tulsa, Tulsa,USA*

Cucurbits (cantaloupe, cucumber, pumpkin, squash and watermelon) are economically important cash crops that are mostly grown in southern United States. Many viruses are known to infect cucurbits and cause significant losses to cucurbit production annually. During 2008-2012 growing seasons more than 700 cucurbit samples showing virus-like symptoms were collected from nine southern States. All samples were screened against the antisera of Watermelon mosaic virus (WMV) by dot-immunobinding assay (DIBA). The results showed that 30% of the samples were positive to WMV. Total RNA was extracted from randomly selected 57 DIBA positive WMV samples. Coat protein genes of WMV were amplified using reverse transcription-polymerase chain reaction (RT-PCR) followed by cloning and sequencing. Phylogenetic analysis of both nucleotide and amino acid sequences of these 57 isolates and previously WMV isolate reported from Florida reported in 1990 showed high degree of variation in nt sequences of CP genes. Nucleotide sequences of all WMV isolates from USA were also compared with the available sequences of WMV isolates in the NCBI database and are discussed.

Poster Session

VIR-PM2065 - Mapping and characterisation of broad spectrum resistance to Potato Virus Y in Solanum phureja

Lesley Torrance[1], Stuart MacFarlane[1], Graham Cowan[1], Karen McLean[1], Glenn Bryan[1]
[1]The James Hutton Institute, Dundee, Scotland UK

Potato virus Y (PVY), the type species of the Genus Potyvirus, is the most important viral pathogen of potato worldwide and is economically damaging in related crops such as pepper, tomato and tobacco. PVY is spread rapidly by aphids often being transmitted before insecticides repel or kill the vector making host resistance the most effective control method. A few sources of resistance have been reported in wild species of potato; these are Rysto from Solanum stoloniferum, Ryadg from S. tuberosum spp. andigena and Rychc from S. chacoense, and they have been mapped to potato chromosomes XII, XI and IX, respectively. To date, none of these resistance genes have been precisely mapped or cloned. We have previously reported the identification S. phureja accessions resistant to PVY (strains PVYO, PVYC, PVYN and PVYNTN) and PVA. PCR markers published for Rysto and Ryadg did not associate with resistant plants suggesting that the phureja resistance is novel (1). Further genetic analysis of segregating populations has mapped the resistance to the lower half of chromosome IX, a location not previously associated with any known virus resistances. However, more detailed analysis suggests that resistance is influenced by a second locus in chromosome IV. We found that resistance in some clones was non-functional when the plants were maintained at 28°C whereas it remained functional in others. The mechanism of resistance was studied by conducting comparative transcriptomic analysis of infected plants at 22°C and 28°C. In addition, a GFP-tagged PVY clone was used to study the virus replication and movement in phureja resistant plants compared with plants containing Rysto. The results of these studies will be presented. (1) Torrance, L., Liu, H., Cowan, G., Bradshaw, J., MacFarlane, S (2009). Extreme resistance to potyviruses in Solanum tuberosum group Phureja. Aspects of Applied Biology 94, 1-4

Poster Session

VIR-PM2067 - Salicylic acid restricts the Ti-plasmid-based CMV vector lacking the 2b gene into veins when inoculated by agroinfection

Noriho Fukuzawa[1], Katsunori Murota[1], Noriko Itchoda[1,2], Takeshi Matsumura[1], Chikara Masuta[3]
[1]National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan, [2]Hokusan Co., Ltd, Kitahiroshima, Japan, [3]Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

Cucumber mosaic virus (CMV) contains three genomic RNAs (RNAs 1-3). We have previously developed the CMV vector, which is an engineered RNA2 to express a heterogous gene. When N. benthamiana was inoculated with in vitro transcripts of the CMV vector expressing green fluorescence protein (GFP), we found GFP fluorescence all over the inoculated and upper leaves. We here constructed the CMV vector in a Ti plasmid. Those constructs of RNA1 and RNA2 were designated pBI-CR1 and pBI-CR3, respectively. RNA2 was modified as a viral vector to create pBI-CR2Δ2b. We inserted the GFP gene in pBI-CR2Δ2b (pBI-CR2Δ2bGFP) and inoculated it onto N. benthamiana together with pBI-CR1 and pBI-CR3 using agrobacterium. The result showed that GFP fluorescence was localized only in the veins of the inoculated and upper leaves. Therefore, CMV spread was different, depending on the inoculation method. Because 2b is an RNA silencing suppressor (RSS), we initially thought that RNA silencing might be involved in the CMV vein localization. We then inoculated the CMV constructs in the presence of either 2b or HC-Pro, another RSS, onto N. benthamiana. When HC-Pro was co-expressed, GFP was again localized in the veins while the 2b expression allowed CMV to spread. We therefore suspected salicylic acid (SA)-mediated resistance of restriction of CMV in veins, and co-expressed the SA glucosyltransferase (SGT) gene with the CMV constructs; SGT can reduce the amount of SA. The results showed that CMV could spread all over the inoculated and upper leaves, suggesting that SA should be erased to improve the spread of the CMV vector lacking 2b. This work was supported in part by grants from the Ministry of Economy Trade and Industry in Japan.

Poster Session

VIR-PM2069 - Involvement of VPg and eIF4E in the cellular tropism of Barley yellow mosaic and Wheat yellow mosaic bymoviruses

Huangai Li[1], Yukio Shirako[2]
[1]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, [2]ANESC, The University of Tokyo, Tokyo, Japan

Barely yellow mosaic virus (BaYMV) and wheat yellow mosaic virus (WYMV) are bipartite, plus-sense RNA viruses belonging to the genus Bymovirus within the family Potyviridae. The only known host of BaYMV is barley, and that of WYMV is wheat in fields. WYMV replicates successfully in mesophyll protoplasts from wheat (cv. Shiranekomugi) whereas BaYMV shows only limited replication. By contrast, mesophyll protoplasts from barley (cv. Ryofu) support the replication of BaYMV but not WYMV. The present study examined the role of the eukaryotic translation initiation factor 4E (eIF4E) in determining the host range of BaYMV and WYMV. Two mutant RNA2 cDNA constructs were generated by replacing a viral gene dispensable for replication with genes encoding Hv-eIF4E (from barley) or Ta-eIF4E (from wheat). Co-expression of Ta-eIF4E enabled WYMV to replicate in barely mesophyll protoplasts, and co-expression of Hv-eIF4E increased BaYMV replication in wheat mesophyll protoplasts. These results suggest that eIF4E is an important cellular factor that determines the host range of BaYMV and WYMV. The role of the viral genome-linked protein (VPg) was also examined. A BaYMV RNA1-derived WYMV VPg chimera (BY1.WY-VPg) was constructed by replacing the RNA1 BaYMV VPg gene with the WYMV VPg gene. The BY1.WY-VPg chimera successfully replicated in wheat mesophyll protoplasts but lost the ability to replicate in barley mesophyll protoplasts. Further analysis showed that co-expression of RNA2 Ta-eIF4E restored the ability of BY1.WY-VPg to replicate in barley mesophyll protoplasts. These results suggest that both VPg and eIF4E are involved in defining host cell tropism.

Poster Session

VIR-PM2071 - Highly stable systemic expression of GFP derived from plant virus-based vector through serial passages in the plant

Da-Young Sim[1], Eun-Gyeong Song[1], Byoung-Eun Min[1], Mi-Yeon Lee[1], Ki-Hyun Ryu[1]
[1]Seoul Women's University, Seoul, South Korea

Pepper mottle virus (PepMoV)-based viral vector containing the nucleotide sequence corresponded to green fluorescent protein (pSP6PepMoV-Vb1/GFP) showed highly infectious expression on the entire plant of Nicotiana benthamiana and specific pepper species (Lee et al., 2010). In this study, the stable expression of foreign gene and the population diversity of the progeny virus from the PepMoV viral vector were examined. Transcript generated from pSP6PepMoV-Vb1/GFP was initially inoculated on the N. benthamiana (Passage 0). Six serial passages were performed in the same host. The symptoms and GFP signals under UV light in the plant during passages were investigated and the nucleotide and amino acid sequence variation (e.g. transition, insertion and deletion) of the coat protein region was compared with wild type virus sequence. The phenotype and virus concentration of plants during all passages were similar to wild type virus-infected plant. The RNA sequence encoding the virus coat protein varied between individual plants. The majority of mutations consisted of silent mutations that it indicated that progenies of PepMoV viral vector are under the stabilized selection during the host passage and in the systemic host. This result could support the guideline for biosafety risk assessment of genetically engineered vector for developing living modified organism.

Poster Session

VIR-PM2073 - The necessity of VPg phosphorylation of Cocksfoot mottle virus is host-dependent

Kristjan Kamsol[1], Allan Olspert[2], Erkki Truve[1]
[1]Department of Gene Technology, Tallinn University of Technology, Tallinn, Estonia, [2]Department of Plant Sciences, University of Cambridge, Cambridge, UK

Cocksfoot mottle virus (CfMV) is a member of the genus Sobemovirus – a small group of plant viruses with small icosahedral virions and a positive-sense ssRNA genome. The genome is attached to a viral protein genome-linked (VPg) at the 5' end and lacks a 3' poly(A) tail. Usually VPgs are natively unfolded proteins which are covalently linked through a phosphodiester bond to 5' end of viral RNAs and subgenomic RNAs. Two amino acid residues of CfMV VPg have been shown to be phosphorylated. It has been proposed for other viruses that different phosphorylation patterns of VPg may regulate interactions between VPg and certain host or viral factors which in turn may be involved in viral replication and/or movement. Therefore it was decided to study the necessity of phosphorylation of the two amino acid residues in VPg for CfMV viability. Five single mutants and one double mutant virus were constructed. Phosphorylated amino acid residues T340 and S391 were mutated into alanine one by one and together, the amino acid residues were replaced with each other or S391 was replaced with glutamic acid to mimic the charge state of phosphorylated serine. Different host plants were infected – oat cv „Jaak", wheat cv „Zebra" and barley cv „Golden Promise". As a result, it was seen that the necessity of VPg phosphorylation for CfMV is host dependent. Mutant S391A and double mutant T340A&S391A were unable to infect oats whereas infection was established in other hosts. No differences from wild type virus were detected with any of the other mutants regardless of the host specificity. In conclusion we demonstrate that the necessity of VPg phosphorylation of CfMV is host-dependent.

Poster Session

VIR-PM2075 - Rapid sampling of microorganisms and nucleic acids for PCR assays using a novel elution-independent collection device

Donna Caasi[1], Mohammad Arif[1], Denise Altenbach[2], Francisco Ochoa Corona[1]
[1]Oklahoma State University, NIMFFAB , Stillwater, OK, USA, [2]BIOREBA AG, Christoph Merian-Ring 7, Reinach, Switzerland.

Collecting and archiving nucleic acids (NA) are key steps in detection and diagnosis when using PCR for medical, biosecurity, or microbial forensics applications. Paper-based technologies for collection offer advantages, such as storage of NA at room temperature. Recovering NA from paper requires only a few steps, but direct PCR is hampered by the residual paper matrix. Soluble biomaterials, of different thicknesses were tested for suitability as matrices in PCR amplification protocols. Scanning electron microscopy showed that pore spaces and crevices of the biomaterials, either dry or wet, and with or without bacteria (*Pseudomonas syringae* pv. tomato), were retained after wetting. When the absorbance of residual materials was measured at 260 and 280 nm matrix residues were highest in the thickest biomaterial, but were not inhibitory to PCR amplification. A novel elution-independent collection device (EICD), Oklahoma State University patent pending (reference-2010.26), designed for rapid collection of microorganisms and recovery of nucleic acids, uses the soluble matrix to collect fluids by contact and lateral flow. Minute pieces (1.2 mm diameter) of the soluble element dissolve directly in commercial PCR mixtures without an intermediate elution step, thereby streamlining PCR based assays. Eleven different viruses, fifteen bacteria, one fungus, one insect and one plant gene (used as internal control) were assessed in one-step RT-PCR assays without intermediate RNA extraction. The resulting EICD prototype was effective for use with sap from infected tobacco plants and insects. The EICD will be commercialized for agricultural diagnostics by license-holder Bioreba Ag, Switzerland, whot found the device useful when targeting the plant pathogenic bacteria
Clavibacter michiganensis, *Ralstonia solanacaerum*. EICD is a simple, rapid sampling choice for in medical, veterinary, plant health biosecurity, forensics, and food quality applications.

Poster Session

VIR-PM2077 - High-throughput sequencing of a Hepatitis Delta Virus population indicates a conserved structure for an RNA promoter for RNAP II

Yasnee Beeharry[1], Lynda Rocheleau[1], Martin Pelchat[1]
[1]*University of Ottawa, Ottawa, Canada*

Hepatitis Delta Virus (HDV) is one of the smallest RNA animal pathogens known and, despite its simplicity, causes fulminant hepatitis. HDV is a satellite virus that requires the Hepatitis B Virus proteins for its encapsidation, and hijacks its host RNA polymerase II (RNAP II) to transcribe and replicate its genome. However, it remains unresolved how this virus is able to use the human RNAP II with an RNA template instead of a DNA template. We previously identified the right terminal domain of genomic HDV RNA as a promoter for RNAP II. Mutagenesis suggested that the secondary structure might be important for RNAP II acting on this RNA promoter. In the present study, we aimed to identify RNA promoter features important for HDV replication. We serially passaged an HDV population replicating in 293 cells over a year in order to amplify the viral sequences able to replicate. Then we performed deep-sequencing of the promoter region of this HDV population. We generated 473,139 sequences representing 2,351 new HDV variants for this region. Analysis of positions of nucleotide conservation and covariation indicated that the sequence of this region is heterogeneous and that the rod-like conformation is conserved for both polarities of the HDV RNA genome. In addition, we identified conserved nucleotides at the tip of the rod-like structure, near the proposed initiation site of transcription. We corroborated our findings with sequences from HDV variants isolated from various hosts. Altogether, we identified a conserved RNA structure and localized nucleotides and base-pairs likely important for an RNAP II RNA promoter. We also developed a method to analyze both sequence heterogeneity of a viral population and RNA structural features from high-throughput sequencing data. This approach will be useful to study viral RNA structures involved in the life cycle of other RNA viruses.

Poster Session

VIR-PM2079 - Functional analysis of phospholipase D and phosphatidic acid in a plant RNA virus replication

Kiwamu Hyodo[1], Masanori Kaido[1], Kazuyuki Mise[1], Tetsuro Okuno[1]
[1]*Graduate School of Agriculture, Kyoto University, Kyoto, Japan*

Eukaryotic positive-strand (+)RNA viruses replicate using the membrane-bound replicase complexes, which contain multiple viral and host components. Cellular membranes are thought to facilitate the building of viral factories, to promote a high concentration of membrane-bound viral proteins, and to provide protection against cellular nucleases and proteases. The membrane lipids and proteins may serve as scaffolds for targeting the viral replication proteins or for the assembly of the viral replicase complex. However, our understanding of the roles of various lipids and lipid biosynthesis enzymes in (+)RNA virus replication is limited. In this study, by using two-step affinity purification and liquid chromatography-tandem mass spectrometry analysis, we identified two Nicotiana benthamiana phospholipase D (PLD), PLDα and PLDβ, as interaction partners of Red clover necrotic mosaic virus (RCNMV) replication proteins. PLD hydrolyses structural phospholipids, such as phosphatidylcholine and phosphatidylethanoamine, to form phosphatidic acid (PA) and remaining headgroup. PLD and PLD-derived PA are known to be involved in several abiotic and biotic stress responses in plants. Gene-silencing and pharmacological inhibition approaches showed that PLDs-derived PA played a positive role in viral RNA replication. In consistent with this, direct application of PA to virus-infected plant cells or plant-derived cell-flee systems enhanced the viral RNA replication. Using a lipid overlay assay, we showed that p27 RCNMV replication protein is a PA-binding protein. RCNMV-infected plant leaves showed high accumulation of PA, leading to the possibility that RCNMV hijacks host PA signaling pathways for successful RNA replication.

Poster Session

VIR-PM2081 - Combining Hepatitis B surface antigen (HBsAg) with Anthrax (rPA) for a single oral vaccine

Mani Bhargava[1], Saurabh Bhargava[2]
[1]ICFAI University, Dehradun, India, [2]Manav Bharti University, India

Vaccination has not only become vital but a lot of revolutionary changes are being observable in the field of vaccine delivery. Vaccine antigens administered by the oral route are often degraded during gastrointestinal transit. Bile salt stabilized vesicles i.e. bilosomes are found to be effective in preventing antigen degradation and enhance mucosal penetration. The aim of the present work was to prepare a combination vaccine system against hepatitis-B (HBsAg) and anthrax(rPA). Oral immunization induces both mucosal and systemic immune responses, whereas mucosal responses are not generally observed following systemic immunization. Bilosomes provide needle free, painless approach for immunization, thereby increasing patient compliance and consequently increasing vaccination coverage. Bilosomes containing HBsAg and rPA were prepared by a lipid cast film method. Antigen loaded bilosomes were characterized in-vitro for their shape, size, percent antigen entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake of bilosomes. The in-vivo study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA. Bilosomes formed were multilamellar and were stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken up by the gut associated lymphoid tissues. In-vivo data demonstrates that bilosomes produced both systemic as well as mucosal antibody responses upon oral administration at higher dose levels as compared to intramuscular immunization but fail to produce any synergistic effect. Thus, HBsAg potentiates the production anti-rPA antibody. Also measurable sIgA in mucosal secretions were observed. Thus, the bilosomes are a promising carrier for oral combination vaccines. This approach could be adapted for human use because the mucosal surfaces are the initial sites of infection and it therefore seems logical to attempt to develop vaccination strategies that evoke appropriate localized responses to counteract the early events of pathogenesis.

Poster Session

VIR-PM2083 - Papaya mosaic virus virus-like particles adjuvant improve the mucosal immune responses of the trivalent inactivated flu vaccine

Gervais Rioux[1], Claudia Mathieu[1], Alexis Russell[1], Marilène Bolduc[1], Marie-Eve Laliberté-Gagné[1], Pierre Savard[2], Denis Leclerc[1]

[1]Department of Microbiology, Infectiology and Immunology, Infectious disease research center, Laval University, Quebec City, Canada, [2]Department of Neurosciences, Laval University, Quebec City, Canada

Trivalent inactivated flu vaccines are currently the best means to prevent influenza infections, even though their immunogenicity against the large variety of circulating strains is weak. Since the respiratory tract is the favoured site of influenza infections, mucosal vaccines and adjuvants could greatly boost the protection against this disease. The papaya mosaic virus (PapMV) virus-like particles (VLPs) have already been shown to enhance the immune response against the trivalent inactivated flu vaccine and broaden the protection when administered subcutaneously and therefore acts as an adjuvant. We therefore wanted to determine if PapMV VLPs could improve the immune response against influenza in the lungs by mucosal vaccination. In the present study, we show that PapMV VLPs adjuvanted influenza vaccine immunized by the intranasal route significantly increase the amount of IgG, IgG2a and IgA in the lungs of vaccinated mice as compared to mice that received the vaccine alone. The addition of PapMV VLPs to the vaccine in both routes elicits a broadening of the lungs immune response to the conserved influenza nucleoprotein. Vaccination with the adjuvanted formulation injected intranasally robustly protects mice against an influenza infection by an heterosubtypic influenza strain, as observed by the lack of symptoms and weight loss during the challenge. The intranasal route is also more efficient than the subcutaneous route to protect mice from an influenza infection. We demonstrate for the first time that PapMV VLPs are effective and potent as a mucosal vaccine adjuvant, in addition to its previous beneficial effects in subcutaneous immunizations.

Poster Session

VIR-PM2085 - Recombinants based on Poxvirus as vaccines against Mycobacterium tuberculosis

Hsi-Hwa Chi[1], Ru-Wen Chou[2], Chia-Tsui Yeh[1], Wen Chang[3], Chi-Feng Lee[1]
[1]National Defense Medical Center, Taipei, Taiwan, ROC, [2]Centers For Disease Control, Taipei, Taiwan, ROC, [3]Academia Sinica, Taipei, Taiwan, ROC

Recombinants based on poxviruses have been used widely as foreign gene delivery systems to study many biological functions of target genes and as vaccines against many pathogens, particularly in the scientific and clinical field. Based on safety issue, effective expression and ability to activate specific antibacterial immune responses, one of the most promising poxvirus vectors for human use are the attenuated modified vaccinia virus Ankara (MVA) strain. Because of the scientific and clinical interest in the platform for the next generation of recombinant poxvirus vectors, here we developed a recombinant multivalent vaccine multi-rTB encoding the highly immunogenic Mycobacterium tuberculosis culture filtrate proteins for inducing protective immunity against tuberculosis. Our results shown that multi-rTB containing novel TB genes encoding 30-kDa antigen complex, 25-kDa membrane protein, 40-kDa phosphate transporters homologous, and 6-kDa early secreted antigen target protein were effective molecules for inducing protective immunity against tuberculosis in vaccinated animal models. The multistage strategy not only protected against initial illness, but controlled reactivation of latent infection and reduced bacterium levels in the lung of vaccinated animal more effectively than tice BCG alone. Several unique features make MVA recombinants excellent candidates as vaccine vectors: Firstly, the safety and stability of attenuated vaccine ease for manufacture and administration. Secondly, the cytoplasmic site of foreign antigen expression with the packing flexibility of the genome, which allows large amounts of the genome to be deleted and foreign genes to be integrated with infectivity and thirdly, the ability to induce both antibody and cytotoxic T cell immune responses against many pathogens with long lasting immunity after a single vaccination. Conceivably, these studies should provide evidence of the potential utility of multi-rTB for the development of a genetically modified MVA vaccine against tuberculosis.

Poster Session

VIR-PM2087 - Protective efficacy of baculovirus displayed hemagglutinin against highly pathogenic influenza H7 subtypes

Mookkan Prabakaran[1], Subaschandrabose Rajesh Kumar[1], JiYong Zhou[2], Jimmy Kwang[1]

[1]Animal Health Biotechnology, Temasek Life Sciences Laboratory, National University of Singapore, Singapore, [2]Key Laboratory of Animal virology of Animal Virology of Ministry of Agriculture, Zhejiang University, Hangzhou, PR China

The outbreak of human infections with avian-origin H7N9 influenza has raised global concerns about a potential human pandemic. However, most of the previous H7 vaccine studies showed less immunogenic in humans and animals. Therefore, the generation of simple and reliable newer vaccines is high priority for pandemic preparedness. In this study, we aimed to develop a recombinant vaccine by expressing HA of H7N9 (A/Shanghai/2/2013) on the surface of baculovirus (BacHA). Further, live or inactive form of BacHA (H7N9) vaccine was immunized twice either intranasally or subcutaneously into mice. The immunogenicity and cross-protective efficacy of the BacHA (H7N9) vaccine was assessed against homologous H7N9 or heterologous H7N7 subtype challenge. The results showed that mice immunized subcutaneously with adjuvanted inactive BacHA (H7N9) induced robust cross-neutralizing antibody responses against H7 subtypes (H7N9, H7N7 and H7N3) compared to subcutaneous or intranasal immunization of live BacHA. In contrast, mice immunized intranasally with live BacHA stimulated higher HA-specific mucosal IgA levels in the upper airways, the port of virus entry. Also, intranasal immunization of BacHA of either H7N9 or H7N7 completely protected against 5 MLD50 of heterologous H7 subtype infection. An overall study revealed that intranasal immunization with live baculovirus displayed HA of either H7N9 or H7N7 vaccine induces antigen specific humoral and mucosal immunity, and provides cross-protection against heterologous H7 subtype.

Poster Session

VIR-PM2089 - Mumps virus circulation in Argentina. Three closed grown-up groups, outbreak analysis

Romina Bonaventura[1], Daniel Cisterna[1], Leila Martinez[1], Sandra Sagradini[2], Mariel Caparelli[2], Carla Vizzotti[2], Maria Cecilia Freire[1]

[1]Servicio de Neurovirosis, INEI, ANLIS "C.G. Malbrán", [2]Programa Nacional de Control de Enfermedades Inmunoprevenibles

Parotitis, an illness characterized by swollen parotids, varies from a subclinical disease to meningoencephalitis. EBV, parainfluenza virus; enterovirus and adenovirus can cause the illness but mumps virus (Paramixoviridae family) is the most frequent. Mumps is a vaccine preventable disease. Argentina has incorporated the vaccine since 1998.Thirteen genotypes from A to N have been described so far. All are defined on the basis of the gen of the SH protein. In Argentina we are unaware of which genotypes are circulating nowadays. The aim of this study was to describe a mumps outbreak which affected students from military schools and which took place in 2012 located in San Juan and Buenos Aires provinces.We received saliva, serum and urine samples from 71 adult patients affected with severe disease in some of them. They were analyzed by serology and RT-nested-PCR amplifying a fragment (205bp) of the SH gene that was subsequently sequenced. Positive results: Buenos Aires province (n=48), IgM33 ; IgG 37, PCR saliva 36,PCR urine 15,sequenced 35. San Juan province (n=23) IgG 14, IgM 10, PCR saliva 18, PCR urine 7, sequenced18. The sequencing showed 48/53 (90,5%) samples as genotype K. The higher homology found was with previous sequences detected in Brazil 2007.This homology represented 97,5-98 % from all samples analyzed. Five cases (9,5 %) were vaccine associated adverse events in patients recently vaccinated (Urabe strain). Results show the circulation in Argentina of one wild genotype of mumps virus. As this circulation occurred in an adult military non- vaccinated closed community we suggest the vaccination of all persons who enter there. Despite the MMR vaccine administration in the young population, it was impossible to eliminate wild Mumps virus circulation . It would be important to integrate the laboratorial virus surveillance to define how many of parotitis cases are caused by Mumps virus.

Poster Session

VIR-PM2091 - Immune response and protective efficacy of a live attenuated H7N9 influenza vaccine against homologous and heterologous H7 wild-type viruses in ferrets

Yumiko Matsuoka[1], Mariana Baz[1], Zhongying Chen[2], Janine Lu[2], Myeisha Paskel[1], Celia Santos[1], Hong Jin[2], Kanta Subbarao[1]

[1]NIH, Bethesda, USA, [2]MedImmune LLC, Mountain View, USA

Live attenuated H7N9 influenza vaccine viruses that possess the hemagglutinin (HA) and neuraminidase (NA) gene segments from the newly emerged wild-type (wt) A/Anhui/1/2013 (H7N9) and six internal protein gene segments from the cold-adapted influenza virus A/Ann Arbor/6/60 (AA ca) were generated by reverse genetics. Immunogenicity and protective efficacy of H7N9 ca vaccine virus was evaluated in ferrets and compared with a previously generated H7N7 ca vaccine (based on A/Netherlands/219/2003: Min et al, 2010, J Virol 84:11950-60). One dose of H7N9 ca or H7N7 ca vaccine induced robust serum neutralizing antibodies against homologous virus that cross-reacted with heterologous wt parent virus. Two doses of the H7N9 ca vaccine, or sequential doses of heterologous vaccines (H7N7 ca followed by H7N9 ca) greatly boosted serum antibody titers. Ferrets that were vaccinated with one or two doses of H7N9 ca or sequential H7N7/H7N9 ca vaccines were fully protected from replication of the H7N9 wt challenge virus in the nasal turbinates and lungs. Ferrets challenged with H7N7 wt virus were also well protected from viral replication in both tissues. Based on the promising immunogenicity and protection data in ferrets, the H7N9 ca vaccine is being evaluated in a phase I clinical trial.

Poster Session

VIR-PM2093 - DNA-launched vaccines against flavi- and alphaviruses

Igor Lukashevich[1], Peter Bredenbeek[2], Peter Pushko[3]
[1]University of Louisville, Louisville, USA, [2]Leiden University Medical Center, Leiden, The Netherlands, [3]Medigen Inc., Frederick, USA

Live attenuated vaccines (LAVs) are the most effective platform to control infectious diseases. LAVs have a highly favorable benefit-risk profile and excellent cost-effectiveness. Among FDA-approved vaccines, 60% are LAVs. Nevertheless, here is a remote possibility that a LAV may revert to a virulent form. The maintenance of “cold chain” accounts for up to 80% of vaccination cost in tropical endemic areas. Each LAV is a heterogeneous population of genetically distinct virus sub-types, some of which may be responsible for adverse reactions. Testing of LAV stocks on contaminations, heterogeneity, potency, safety, and efficacy is one of the regulatory challenges for manufactures of LAVs. To improve LAVs and methods of their application we invented the “infectious” DNA (i-DNA), a novel vaccination technology combining efficacy of LAVs and advantages of DNA immunization (Pushko & Lukashevich, 2008). A unique feature of this technology is that the full-length copy of LAV is placed in the recombinant DNA plasmid in the context of optimized eukaryotic promoter and regulatory sequences. Thus, a LAV can be launched in vivo directly from an optimized i-DNA plasmid. Since LAV i-DNA represents a molecular clone, it will generate a uniform population of LAV potentially improving safety profile. i-DNA vaccines can be easily genetically modified to secure attenuated genotype/phenotype and reduce viral fitness. Recombinant i-DNA manufacturing is much easier for standardization and for control (purity, heterogeneity, and potency) then manufacturing of LAVs. In addition, (i) application of i-DNA is not dependent on cold-chain; (ii) bacterially-produced i-DNA will induce TLR9-mediated innate immune responses which will provide “priming” effects before recovery of LAV from i-DNA; and (iii) will promote development of virus-specific adaptive immune responses. We used this technology to design and launch by DNA immunization of experimental animals Yellow Fever 17D vaccine, Venezuelan Equine Encephalitis TC-83 vaccine, and Chikungunya 181/25 vaccine.

Poster Session

VIR-PM2095 - Type 2 dengue virus NS5 protein expressed in Escherichia coli preserves immunological properties of the native protein

Rúbens Alves[1], Jaime Amorin[1], Raíza Bizerra[1], Lennon Pereira[1], Sara Pereira[1], Rafael Cavalcante[1], Luís Ferreira[1]
[1]Department of Microbiology, Biomedical Science Institute, University of São Paulo, São Paulo, Brazil

Dengue is a disease caused by one of the four dengue virus serotypes (DENV 1-4), affecting millions of people worldwide and causing a significant number of deaths annually. There are no effective treatments or vaccine approaches capable of preventing such infection. Anti-DENV vaccine strategies based on nonstructural proteins as antigens have been shown to be safer than those based on structural proteins. In addition, anti-DENV cellular immune response against nonstructural proteins has shown to play a major role in controlling viral replication. The DENV nonstructural protein 5 (NS5), the most conserved protein among all serotypes, plays a crucial role in viral replication and contains most of the DENV major CD8+ T lymphocyte-specific epitopes. In this study, we generated a recombinant form of DENV2 NS5 expressed in E. coli in high amounts and with preserved properties with regard to the native protein. Culture conditions were optimized in order to allow over-expression of NS5 as a soluble protein. The NS5 protein was then obtained with 98% purity degree after a two-step chromatography-based purification procedure with a final yield of 15 mg per liter of bacterial culture. In addition, the recombinant NS5 protein was shown to be recognized by a dengue-infected human serum. These results indicate that the recombinant NS5 protein preserves immunological properties with regard to the native NS5 and is suitable for immunization experiments aiming determination of the putative protective role the protein on the induce immune responses.
Reserch supported by FAPESP and CNPq grants.

Poster Session

VIR-PM2097 - Sensitivity improvement of pan-viral DNA array and high-throughput sequencing using propidium monoazide (PMA) or ethidium bromide monoazide (EMA) for the identification of viruses from tissue samples

Christian Bellehumeur[1], Brian Boyle[2], Josée Harel[1], Steve Charrette[2], Yvan L'Homme[3], Luke Masson[4], Carl A. Gagnon[2]

[1]Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Université de Montréal, St-Hyacinthe, Canada, [2]Institut de biologie intégrative et des systèmes (IBIS), Université Laval, Québec City, Canada, [3]St-Hyacinthe Laboratory, Canadian Food Inspection Agency (CFIA), St-Hyacinthe, Canada, [4]National Research Council Canada (NRC), Montréal, Canada

Pan-viral DNA array (PVDA) and high-throughput sequencing (HTS) are useful tools to identify novel virus of emerging diseases. Although PVDA and HTS work well with isolated virus, they are less sensitive to detect viruses in tissue samples. This is because of host genomic DNA (hgDNA) contaminating nucleic extract from tissue samples. Both propidium monoazide (PMA) and ethidium bromide monoazide (EMA) have the capacity to bind free RNA and DNA but are cell membrane-impermeable and thus are unable to bind protected RNA/DNA such as virion protected viral genomic material. DNA permanently linked to PMA or EMA following photolysis is not amplifiable by RNA/DNA polymerase. Thus a PMA or EMA treatment before nucleic extraction could lower hgDNA contamination. To validate this hypothesis, lung tissue homogenates were spiked with porcine reproductive and respiratory virus (PRRSV) and were processed with different combination of treatment: with/without ultracentrifugation and incubation with/without different concentration of EMA or PMA. Following each treatment, total DNA/RNA was extracted. Quantitative PCR (qPCR) was used to evaluate hgDNA contamination (beta-actin) and PRRSV presence in each DNA/RNA sample. Finally, PVDA and HTS were used to detect PRRSV in each DNA/RNA samples. Both EMA and PMA treatment increased beta-actin quantification at least by 11.40±0.52 Ct ($p<0.001$), indicating an important loss of hgDNA contamination in these samples. While EMA caused a dose-dependent decrease of PRRSV qPCR detection, no significant differences were seen in PRRSV qPCR quantification following PMA treatment. Ultracentrifugation pre-treatment (with/without PMA or EMA treatment) has no effect on hgDNA and PRRSV quantification. Negative results were obtained by PVDA with untreated samples or samples treated only by ultracentrifugation. However, PRRSV was detected in PMA and EMA treated samples. More PRRSV DNA probes were positive with PVDA following PMA treatment compared to EMA treatment. HTS experiments with PMA and EMA treated samples are in progress.

Poster Session

VIR-PM2099 - The generation of immune-modulatory gene knockout Orf virus recombinants for use in oncolytic virotherapy

Jacob P. van Vloten[1], Jondavid de Jong[1], Alexandra Rasiuk[1], Byram W. Bridle[1], Sarah K. Wootton[1]
[1]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada*

Parapoxvirus ovis (or OFRV) is the causative agent of the dermatological disease Orf, which primarily infects ungulates. To establish infection, ORFV requires an open wound to gain access to differentiating keratinocytes. During infection, viral virulence factors, including VEG-F, drive the differentiation of keratinocytes and promote vascularization, forming a microenvironment similar to that of solid state tumors. Initial interest in ORFV as an oncolytic virus stemmed from its natural ability to decrease tumor burden in mice. Additionally, as a member of the *Poxviridae* family, the ORFV genome is ~130kb of double-stranded DNA consisting of a conserved core flanked by variable regions. These variable regions are of considerable interest, as they contain multiple virulence factors and immune modulatory genes and represent target regions for gene insertion. Also, ORFV infection induces a strong immune response in sheep and mice, yet there is little evidence of neutralizing antibodies to prevent re-infection. In the context of an oncolytic, this makes repeat administration a reality that is further accentuated by a low level of circulating antibodies in the human population. To investigate the potential of ORFV as an oncolytic, a cloning system was devised to produce recombinant ORFV lacking viral VEG-F and viral Interleukin-10 by homologous recombination. Initially, ORFV from a symptomatic Ontario sheep was isolated and confirmed as ORFV by PCR and Western blot. Viral genomic DNA from passaged virus was purified, and used to PCR amplify 1kb flanking regions of both aforementioned genes. Amplicons were subsequently cloned into a shuttle vector under an ORFV promoter, flanked by Green Fluorescent Protein, and transfected into Sheep Skin Fibroblasts which were previously infected with wild-type ORFV. Recombinant plaques were selected by fluorescence, and propagated on Sheep Skin Fibroblasts. This represents a critical first step to in *vitro* and in *vivo* investigations of ORFV as an oncolytic.

Poster Session

VIR-PM2103 - MicroRNA profiling for the early detection of Hendra virus infection in horses

Cameron Stewart[1], Celine Deffrasnes[1], Chris Cowled[1], Glenn Marsh[1], Deborah Middleton[1], Andrew Bean[1]
[1]*CSIRO-AAHL, Geelong, Australia*

Hendra virus (HeV) occurs naturally in flying foxes (Pteropus spp), the reservoir host, with horses becoming infected by natural transmission from flying foxes. Although the HeV horse vaccine will reduce the risk of transmission from horses-to-humans, improved diagnostics is still an important component of HeV disease control. The aim of this project was to define diagnostic markers for early detection of HeV infection in horses. We have used deep sequencing, bioinformatics and molecular biology techniques to profile microRNAs associated with HeV infection in horses. Blood samples were obtained from infected and non-infected horses from a HeV infection trial conducted at BSL4. MicroRNAs were extracted and sent for deep sequencing and quantified by qPCR. Using miRDeep microRNA prediction software we identify 21 differentially regulated microRNAs on days 1-3-5-7 post-infection compared to uninfected horses. Interestingly, changes in microRNA profile preceded the detection of virus itself, or the onset of signs of virus infection. We also compared microRNA profiles in different biological samples such as urine, nasal swabs and blood and found differences in the microRNAs detected as well as differences in their expression pattern. Furthermore, this study identified potentially new horse microRNAs which are currently being investigated.

Poster Session

VIR-PM2105 - Novel recombinant vaccines based on replication-defective flavivirus vectors

Konstantin Pugachev[1], Maryann Giel-Moloney[1], Ana Goncalvez[1], Michael Vaine[1], Bradley Feilmeier[1], Teshome Mebatsion[1], Linong Zhang[1], Thorsten Vogel[1], Ray Oomen[1], Harry Kleanthous[1]
[1]Sanofi Pasteur, Cambridge, USA

The RepliVax® vaccine platform (RV) is based on flavivirus RNA genomes that are rationally attenuated by deletion and do not generate a productive infection similar to replication-competent virus. RV vectors are highly attenuated, capable of inducing robust antibody and T cell responses, and efficacious as shown for vaccine candidates against several flaviviruses, including Tick-borne encephalitis (TBE). A single dose of RV-TBE induced a robust neutralizing antibody response in monkeys which was more durable than 3 doses of an inactivated TBE vaccine control. RV-TBE induced similar genes and temporal expression patterns in macaques as has been observed for YF-17D vaccinated humans. In addition, to developing RV for flavivirus vaccine candidates we have engineered West Nile (WN)-based RV vectors to express non-flavivirus immunogens. The full length rabies virus G gene was cloned into the RV-WN genome and the chimeric virus replicated to high titers (8 logs) in helper cells and expression of RabG was stably maintained through multiple rounds of in vitro passaging. We evaluated RV-RabG in various animal models and the vaccine provided durable protection in mice and dogs induced protective levels of neutralizing antibodies in pigs. The technology is being applied to other non-flavivirus targets. Additionally, RV is typically administered as virus packaged in helper cells. Alternatively it can be given as nucleic acids (DNA or RNA). DNA immunization in mice with plasmids containing a RV genome under the CMV promoter induced specific neutralizing antibody responses similar to vaccination with packaged virus, and provided long term protection from challenge.

Poster Session

VIR-PM2107 - RepliVax®-TBE, a single dose tick-borne encephalitis vaccine based on a European TBE virus subtype, induces broadly cross-neutralizing antibodies in mice and monkeys and protects mice against heterologous TBEV subtypes

Ana P. Goncalvez[1], Liubov Kozlovskaya[2], Julia V. Rogova[2], John A. Catalan[1], Alexander A. Rumyantsev[1], Harold Kleanthous[1], Galina Karganova[2], Konstantin V. Pugachev[1]

[1]Sanofi Pasteur, Discovery-USA, Cambridge, USA, [2]FSBI Chumakov Institute of poliomyelitis and viral encephalitides RAMS, Russia

Tick-borne encephalitis (TBE) is one of the most serious neurological diseases in Europe and Asia. Inactivated virus vaccines (INV) are available, however multiple doses and periodic booster vaccinations are required to elicit and maintain protective imunoresponse. A single dose of a novel replication-defective RepliVax®-TBE vaccine candidate bearing the structural protein genes of a European subtype of TBEV (strain Hypr), was shown to be highly immunogenic in mice and monkeys. It protected immunized animals from homologous Hypr or surrogate Langat virus challenge (2013 PNAS 110:13103-8). Whether RepliVax®-TBE is also effective in protecting against heterologous Far Eastern and Siberian TBEV subtypes was addressed. A single dose of RepliVax®-TBE elicited efficient cross-protective responses in mice as shown by challenge with several Siberian, Far-Eastern and European TBEV strains. Sera from RepliVax®-TBE vaccinated Rhesus macaques neutralized the three TBEV subtypes in vitro, and mice passively immunized with the NHP sera were protected from challenge with Far Eastern and Siberian strains. Therefore, these data support the use of RepliVax®-TBE based on a European subtype in regions where TBEV of heterologous subtypes are prevalent.

Poster Session

VIR-PM2000 - Acylation of HCV core Cys184 is essential for its maturation

Sabrina Bossé[1], Nathalie Majeau[1], Denis Leclerc[1]
[1]*Université Laval, Québec, Canada*

Hepatitis C virus (HCV) core protein is the main structural protein involved in viral assembly and virus production. The maturation of core is achieved by successive processing by signal peptidase (sp), generating the immature form (191 aa), and by signal peptide peptidase (spp), generating the mature form (177 aa). We identified two acylation sites, Cys172 and Cys184, important for HCV assembly. Acylation of Cys172 is involved in the anchoring of mature core to the ER. However, the role of Cys184 acylation is still not well understood. This residue is one of the amino acids substituted in the spmt mutant, which is resistant to spp cleavage. Our hypothesis is that acylation of Cys184 is important for spp cleavage and core maturation. To determine if acylation of Cys184 is required for spp cleavage, we replaced Cys184 by a Ser. We expressed the C184S mutant in hepatic cells (Huh7.5) and examined core localization by immunofluorescence. Only fully processed protein can migrate to the lipid droplets (LD) in order to complete virus assembly; immature core stay to the ER membrane. We found that the C184S mutant showed a diffuse localization pattern consistent with ER localization. This dispersed pattern was also observed with IF176/7AL mutant, which is resistant to spp cleavage. Acylation inhibitors affected as well the association of WT core to LD. We also introduced the C184S mutation into Rluc-HCV virus and measured luciferase activity 72 h post-infection to evaluate the impact of Cys184 acylation on virus replication and production. While C184S mutant had no effect on replication, the amount of infectious virus released was drastically reduced compared to WT Rluc-HCV supernatant, which is relevant of impaired spp cleavage. Herein, we present evidence that Cys184 acylation is essential for spp cleavage and virus production. It provides important insight for understanding of HCV viral assembly.

Poster Session

VIR-PM2002 - Investigating the role of PCBP2 in the HCV life cycle

Lance Martin[1,2], Ryan Flynn[1], Robert Spitale[1], Peter Sarnow[2], Howard Chang[1], Selena Sagan[2,3]
[1]Department of Dermatology, Stanford University, Stanford, USA, [2]Department of Microbiology & Immunology, Stanford University, Stanford, USA, [3]Department of Microbiology & Immunology, McGill University, Montreal, Canada

Hepatitis C virus (HCV) infection is a global health problem with over 170 million people infected worldwide. Our previous work suggested a new model for the 5' end of the HCV genome in which a miR-122 molecule binds to the HCV genome across stem-loop 1 (SLI, nts 5-20). This interaction is predicted to stabilize SLI. Interestingly, SLI is required for viral RNA replication and hence we were curious whether stabilization of SLI by miR-122 provided a platform for recruitment of proteins required for viral replication. PCBP2, a nucleic acid binding protein with preference for C-rich motifs, binds to SL1. PCBP2 is induced by type I IFN and viral infection and have diverse roles in gene expression including RNA processing, translational control and mRNA stabilization. Depletion of PCBP2 results in a loss in HCV RNA and in vitro studies suggest that PCBP2 can circularize dsRNAs flanked by the HCV 5' and 3' UTRs. The proximity of SLI to the miR-122 binding sites prompted us to investigate whether PCBP2 and miR-122 physically interact in HCV-infected cells. To our surprise, PCBP2 IP'd miR-122 in both HCV-infected and uninfected cells. These results led us to hypothesize that PCBP proteins bind to the HCV genome as well as miR-122 and that these interactions facilitate RNA replication by circularizing the HCV genome. To identify PCBP binding sites in the HCV genome, we performed individual nucleotide-resolution crosslinking IP (iCLIP) analysis of PCBPs in HCV-infected cells. Conserved PCBP binding sites mapped to six distinct regions across the HCV genome including the poly-U/UC region. To investigate the roles of PCBPs in the HCV life cycle, we will combine mutagenesis studies with assays for viral translation, replication, genome circularization, and particle production. We anticipate that our mutational analyses will reveal the role of PCBPs in the HCV life cycle.

Poster Session

VIR-PM2004 - Molecular basis of influenza virus survival outside the host : potential role of the hemagglutinin.

Olivier Sawoo[1, 2], Amelie Dublineau[1], Christophe Batejat[1], Jean-Claude Manuguerra[1], India Leclercq[1,2]
[1]Institut pasteur ,Paris , France, [2]Dens Diderot University, Paris, France

Influenza A virus (IAV) causes millions of death through yearly epidemics and the threat of pandemic with new IAVs such as the H1N1(2009) virus, have stimulated numerous studies on their transmission mechanism. However the knowledge on how the agent or how environmental factors may impact on IAV persistence is still rudimentary. We had previously shown that not only IAV can persist for an extended period of time but also that the susceptibility of the virus to a given temperature or salinity in water over time were not due to genomic degradation. In the aim of finding the molecular determinants of IAV persistence in the environment, we use IAV pseudotypes based on lentiviral vectors which provide a way to study viral persistence and where the external structures of IAV could be easily targeted. In this study, we compared the pandemic H1N1(2009) virus against the seasonal H1N1(1999) virus in order to comprehend the impact of environmental factors on those viruses. As a result, IAV pseudotypes of the two H1N1 viruses were generated.The IAV pseudotypes were, then, subjected to various environmental parameters over time and tested for infectivity. In water, at no and medium salinity levels, no difference were observed between H1N1 2009 and 1999 pseudotypes as shown with their viral conterparts. Increasing temperature and salinity had a strong negative effect on the survival of the pseudotyped IAVs, a higher impact on H1N1 pdm pseudotypes than H1N1 seasonal pseudotypes. In addition, 15 pseudotyped mutants based on the intermolecular comparison of both viruses on the hemagglutinin (HA) gene were studied and several key-point mutations For a more comprehensive overview, HAs from other different sub-types such as H3N2 and highly pathogenic H5N1 were included in the study. We, hereby, show the potential role of the external structures in the survival of IAVs.

Poster Session

VIR-PM2006 - Prevalence and predictors of heterosubtypic antibodies to Influenza A virus in humans

Ines Kohler[1], Alexandra U. Scherrer[2], Osvaldo Zagordi[1], Matteo Bianchi[1], Arkadiusz Wyrzucki[1], Marco Steck[1], Bruno Ledergerber[2], Huldrych Grünthard[2], Lars Hangartner[1]

[1]Institute of Medical Virology, University of Zurich, Zurich, Switzerland, [2]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

Antibodies recognizing highly variable epitopes in the head of the hemagglutinin protein dominate the human neutralizing antibody response to influenza viruses. These antibodies interfere with receptor binding and are highly strain-specific. As a consequence, they only recognize viruses closely related to the immunizing strain. Rare heterosubtypic antibodies, i.e. antibodies that can neutralize more than one subtype or even genus of influenza virus, have been described, and their epitopes comprise interesting targets for the development of pan-influenza vaccines. However, to date it is not clear to which extent such antibodies are also present in humans and what are the factors that favor their elicitation. We therefore analyzed serum from 305 individuals for the prevalence and predictors of heterosubtypic antibodies. It was found that vaccination not only promoted higher binding and neutralizing antibody titers to homosubtypic influenza isolates but also increased heterosubtypic human immune responses. Both binding and neutralizing antibody titers in relation with age of the donors mirrored the course of the different influenza strain circulation during the last century. Advanced age appeared to be of advantage for both binding and neutralizing titers to most subtypes. In contrast, the first virus subtype encountered was found to imprint to some degree subsequent antibody responses. Antibodies to recent strains, however, primarily seemed to be promoted by vaccination. We provide evidence that repetitive vaccinations stimulate both homo and heterosubtypic immune responses not only in young and middle-aged, but also in more senior individuals. Our analyses suggest that repetitive influenza vaccinations not only prevent infection against currently circulating strains but can also stimulate broader humoral immune responses that potentially attenuate infections with zoonotic or antigenically shifted strains.

Poster Session

VIR-PM2008 - The effect of cholesterol on cytokine expression in human lung epithelial cell infected with 2009 pandemic influenza A virus

Shu Chen Hsu[1], Helene Minyi Liu[1], Yung Hung Chang[1], Hao Wei Wang[1], SY Chang[1,2], CL Kao[1], Chun Nan Lee[1,2]
[1]Graduate Institute of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, Taipei, Taiwan, [2]Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan

Background: Cholesterol is reported as an important factor associated with viral infection in many viruses. In 2009, pandemic H1N1 influenza virus (pH1N1) had caused world-wide panic. Most of the deaths were caused by cytokine storm and the effect of high concentration cholesterol environment on influenza virus infection is still unknown. In this study we intend to explore the role of cholesterol in 2009 pH1N1 infection in human lung epithelial cell. Materials and Methods: The pH1N1 virus strain used was isolated in the Virology Laboratory of the National Taiwan University Hospital in 2009 and propagated in MDCK cell in minimum essential medium (MEM) containing 2 μg/ml TPCK-Trypsin. Calu-3 cells were cultured in MEM supplemented with 1 mM sodium pyruvate. Cholesterol was dissolved in absolute ethanol before passing through 0.22 μm filter. Sialic acid expression was detected by immunofluorescence assay. The mRNA expression of cytokines was measured by real-time qPCR. Results: Virus growth curve in Calu-3 cells showed that no significant difference between the growth in culture medium containing 0.04 mM and 0.08 mM cholesterol. Cell toxicity assay showed that over 80% of Calu-3 cell survived after treating with high concentration of cholesterol. Cholesterol concentration in cell plasma membrane was significantly increased when cells were treated with cholesterol. The expression of alpha 2,3- and alpha 2,6-linked sialic acid on Calu-3 was higher at high concentration of cholesterol. We also observed the adsorption of virus slightly increased and higher mRNA expression of IL-6 in Calu-3 cell infected with pH1N1 at early stage of infection at high concentration of cholesterol. Conclusion: We demonstrated more expression of sialic acid in Calu-3 cells at high concentration of cholesterol and observed a significantly higher expression of cytokines at early stage of pH1N1 infection. It may provide a novel therapeutic strategy in influenza virus –infected Hypercholesterolemia patients.

Poster Session

VIR-PM2010 - The regulation and functional significance of GALNT3 expression during influenza A virus infection

Shoko Nakamura[1], Masayuki Horie[2], Mayo Yasugi[3], Tomo Daidoji[4], Atsushi Kuno[5], Daisuke Okuzaki[6], Tomoyuki Honda[1], Hisashi Narimatsu[5], Takaaki Nakaya[4], Keizo Tomonaga[1]
[1]Institute for Virus Research, Kyoto University, Kyoto, Japan, [2]Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan, [3]Department of Veterinary Environmental Sciences, Osaka Prefecture University, Osaka, Japan, [4]Department of Infectious Diseases, Kyoto Prefectural University of Medicine, Kyoto, Japan, [5]Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan, [6]Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Mucus is largely formed by mucins, which consist of a family of N- and O-linked glycoproteins, and produced on the surface of respiratory epithelial cells during the infections of respiratory viruses, such as influenza A virus (IAV). However, only a little is known about production and role of mucins in viral infections. Here we show the molecular mechanism underlying mucin production and its involvement in the regulation of IAV infection. At first, to investigate the mucin expression in IAV infection, we infected human upper respiratory epithelial cells with several IAV strains and demonstrated that mucins are markedly increased in the early stage of IAV infection. In addition, lectin microarray analysis showed that alteration of a variety of O- and N-glycosylation was observed in the cellular membrane fraction of IAV-infected cells. Furthermore, miRNA microarray analysis showed the downregulation of miR-17-3p and miR-221, which target GalNAc transferase 3 (GALNT3) encoding a glycosyltransferase that initiates mucin-type O-glycosylation, in IAV-infected cells. All these observations suggested that IAV infection induces GALNT3 expression through miRNA regulatory pathway, leading to the mucin production and O-linked glycosylation alteration in IAV-infected cells. Moreover, analyses using siRNA against GALNT3 and IAV minigenome system revealed that upregulation of GALNT3 may enhance IAV gene transcription and replication. Our results demonstrated that upregulation of GALNT3 via regulation of miR-17-3p and miR-221 may affect not only mucin production but also IAV transcription and replication. We are currently investigating the detailed function of GALNT3 in IAV infection by using Galnt3-knockout mice.

Poster Session

VIR-PM2012 - A large-scale analysis of interspecies transmission of influenza A viruses in pigs

Nipawit Karnbunchob[1], Keisuke Ueno[1], Manabu Igarashi[1], Kimihito Ito[1]
[1]*Bioinformatics, Sapporo, Japan*

Influenza A virus is a zoonotic pathogen that infects avian and mammalian hosts. Wild aquatic birds are natural reservoirs of all subtypes of the influenza A viruses. Pigs are potential intermediate hosts for viral genetic reassortment between human and avian viruses. This genetic reassortment can led to new viral strains that might cause a pandemic. Thus, monitoring influenza A viruses in pig population plays an important role to control the disease. To clarify viral circulation and transmission in avian, swine and human species, we compared nucleotide sequences of influenza viruses isolated from birds, pigs and humans. The nucleotide sequences were downloaded from National Center for Biotechnology Information. If two viruses isolated from different host species have almost identical sequences, we can consider interspecies transmission happened between these species. The search for similar pairs of sequences was conducted by using a bioinformatics technique called reciprocal best hits. Two sequences X and Y are said to be reciprocal best hits, if X is the most similar sequence to Y and Y is the most similar sequence to X. By looking for the reciprocal best hits among nucleotide sequences of influenza viruses isolated from different host species, we found a number of possible interspecies transmissions in the dataset. The most results of the transmission are consistent with the literature that determined those nucleotide sequences, suggesting that our method can correctly detect interspecies transmission. The prevention of the genetic reassortment between avian viruses and human viruses in pigs is an important way to reduce the risk of future pandemics of influenza A viruses. We anticipate that information obtained by our method can contribute to set potential strategies to block viral transmission.

Poster Session

VIR-PM2014 - Respiratory viruses detection by the multiplex PCR method in patients with upper and lower respiratory tract infections

Candan Cicek[1], Ayse Arslan[1], Eylem Ulas Saz[2], Husnu Pullukcu[3], Gursel Cok[4]
[1]Ege University Medical Faculty Dept. of Microbiology, Izmir, Turkey, [2]Ege University Medical Faculty Dept. of Pediatri, [3]Ege University Medical Faculty Dept. of Infectious Diseases and Clinical Microbiology, [4]Ege University Medical Faculty Dept. of Chest Diseases

Aim:In this study, we aimed to determine respiratory viruses in a short time by multiplex PCR in patients with the upper and lower respiratory tract infections. Materials and methods:Between January 2007 and August 2013, 1708 patients with the diagnosis of respiratory tract infections were enrolled in the study [45,1% female, 54.9% male, age range: 5 days-94 years (median: 6 year), 1220 children (median: 3 year), 488 adults (median: 46 year)]. Respiratory viruses were investigated by multiplex PCR in respiratory samples (nasopharyngeal swab, bronchoalveolar lavage, trans-tracheal aspiration) from these patients. Results:A total of 1708 patients, 620 (36.3%) patients who were positive for one or more respiratory virus and 1088 (63.7%) patients were negative. In 57 of these patients (3.3%) two virus and 6 (0.4%) patients revealed three virus. Most of multiple factors detected samples were belong to children (89.5%). One or more respiratory viruses were detected in 39.2% of pediatric patients and 29.1% of adult patients ($p <0, 000$). Respiratory specimens were obtained from 387 (22.7%) outpatients and 1321 inpatients (77.3%). In 48.5% outpatients and 32.7% hospitalized patients one or more respiratory viruses were detected ($p<0.000$). Distribution of viruses in positive cases was 12.1% pandemic influenza H1N1, 9.0% RSV, 3.7% rhinovirus, 3.0% influenza A virus, respectively. Conclusion:Respiratory viruses were positive 40% in patients with acute respiratory tract infections. Multiple viral infections were detected in approximately 4% patients. Almost 90% of these patients were found to be of the pediatric patients. If pandemic influenza H1N1 virus is kept separate, the three most common pathogen were RSV, rhinovirus and influenza A virus, respectively. There was no statistically different the rates being infected with viral respiratory pathogens between adult and pediatric patients. However, agent detection rate decreased in patients age grows. Respiratory viruses were detected more frequently in outpatient compared to hospitalized patients.

Poster Session

VIR-PM2016 - Generation of a highly pathogenic H7N6 subtype avian influenza virus from an avirulent isolate by serial intracerebral passage in quail and chickens

Tatsufumi Usui[1,2], Yukiko Uno[2], Ikuto Kato[2], Yoshikazu Fujimoto[2,3], Hiroshi Ito[1,2], Toshihiro Ito[1,2], Tsuyoshi Yamaguchi[1,2]

[1]Avian Zoonosis Research Center, Faculty of Agriculture, Tottori University, Tottori, Japan, [2]Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan, [3]Centre of Biomedical Research, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

The pathogenicity of H7 subtype avian influenza viruses increases with increasing number of basic amino acids at the cleavage site of the haemagglutinin (HA) glycoprotein and with the involvement of other viral factors. However, there is no direct evidence that low pathogenic H7 virus can mutate into a highly pathogenic virus in poultry. In 2009, H7N6 subtype low pathogenic avian influenza viruses (LPAIVs) were isolated from poultry quail in Japan. These isolates have three basic amino acids (K-R-R) at the HA cleavage site. In the present study, to evaluate the risk of the evolution of an H7N6 LPAIV into a highly pathogenic form, an isolate, A/quail/Aichi/1/2009(H7N6), was intracerebrally passaged in quail and chickens. The HA cleavage site was found to acquire an arginine residue (K-R-R-R) after eight passages in quail, and another arginine residue (K-R-R-R-R) after 16 passages in quail followed by one passage in a chick. The K-R-R-R-R sequence is known to be present in highly pathogenic strains. Another four passages in 1-day-old chicks induced an amino acid substitution (D44N) in the M2 protein. Three more passages in 1 to 3-week-old chickens resulted in four amino acid substitutions (L84R and N495D in HA, G34R in NA and K108N in NS1). Intravenous inoculation of the latter isolate caused 100% mortality in 4-week-old chickens, whereas the precursor virus with K-R-R-R-R in HA and D44N in M2 caused 0% mortality. These results show that a highly pathogenic H7N6 subtype avian influenza virus could be generated from an avirulent strain simply by serial passage in poultry birds, and that LPAI epidemics need to be controlled because LPAIV, particularly the H7 subtype, circulates in poultry as HPAIV precursors.

Poster Session

VIR-PM2018 - Disadvantageous deletion mutants retained in nucleopolyhedroviruses isolated from several saturniid wild silkworms

Kuni Sasaki[1], Yuan-jiao Huang[2], Mei-ling Shi[3], Xue-ying Wang[4], Zenta Kajiura[5], Jun Kobayashi[1]
[1]*Yamaguchi University, Yamaguchi, Japan,* [2]*Guangxi Medical University, Nanning, China,* [3]*Guangxi Academy of Sericulture Sciences, Nanning, China,* [4]*Shenyang Agricultural University, Shenyang, China,* [5]*Shinshu University, Ueda, Japan*

Nucleopolyhedroviruses (NPVs) of saturniid silkworms are important in both insect pathology and insect-based technology, because they cause severe damage to silk production in Asian countries and are also utilized as expression vectors for production of valuable proteins in wild silkworms larger than the domesticated silkworm, Bombyx mori. Comparative analyses of partial genomic DNA sequences of NPVs isolated from Antheraea pernyi (Liaoning, China), Samia cynthia ricini (Guanxi, China), and A. yamamai (Nagano, Japan) have revealed their overall high-homology, strongly indicating that they are variants of an identical NPV, and various mutations such as SNPs and INDELs. Among them, large deletions were found in two regions, one of which contains the ecdysteroid UDP-glucosyltransferase gene (egt) and the other contains the cathepsin and chitinase genes (v-cath and chiA). Several patterns of egt deletion were found in all of the three NPV isolates, while intact egt was detected only in the isolate from S. c. ricini. These egt deletions include most of the coding sequence, although 28 bp within the coding sequence as well as 5' and 3' UTRs remain intact, and have accelerated the speed of killing infected insects as reported for other NPVs, resulting in the decreased production of progeny viruses. Several patterns of deletion in the v-cath and chiA region were found only in the isolate from S. c. ricini. These deletions include the promoters and most of the coding regions of both v-cath and chiA, and have depressed degeneration of infected insects and release of polyhedral inclusion bodies, resulting in the decreased transmissibility of progeny viruses. Thus, the saturniid NPVs retain the disadvantageous deletions in the two regions for any reason. For a better understanding of the mutations, further studies on virus genome diversification and virus-host interactions are in progress.

Poster Session

VIR-PM2020 - Analysis of two genomes of Anticarsia gemmatalis MNPV clones with high variation on virulence

Briana Ferreira[1], Fernando Melo[2], Marlinda Souza[1], Bergmann Ribeiro[2]
[1]*Emprapa Genetic Resources and Biotechnology, Brazilia, Brazil,* [2]*University of Brazilia, Brazilia, Brazil*

Baculoviruses are pathogenic to insects and have been effective in controlling pests in agricultural and forestry areas. In Brazil, the baculovirus AgMNPV has been used as a biological insecticide since the early eighties to control the soybean caterpillar, Anticarsia gemmatalis. Viral clones, derived from wild type (Ag-79) were assayed in Anticarsia gemmatalis larvae. Two clones showed high difference in their virulence, Ag-01 was the most virulent (LC50= 75OB/mL) and Ag-16 showed lower virulence (LC50= 1301 OB/mL). This study aimed to analyze the genomic sequence of two AgMNPV clones with high difference on their virulence. The genomes of the viral clones analyzed showed the presence of a single copy of the pe38 and he65 genes which are in duplicate in the genome of the Ag-2D clone. Was observed that one of the viral clones (Ag-16), with lower virulence, has many variations in the ie-2 and pe-38 gene regions, which are responsible to transactivate early genes to start viral replication. Furthermore, other genes presented alterations like the odv-e56, and which have a essential role in the maturation and envelopment of the ODV, and bro-a and bro-b genes that are in fused in only one ORF. The hr region number was also different among clones (Ag-2D with nine, Ag-01 with eight and Ag-16 with seven hrs). Studies on the per os infectivity factors genes and factors associated with virulence of the host are important to the understanding of the viral population dynamics in natural conditions and can be valuable in the development of biological control programs.

Poster Session

VIR-PM2022 - Cytorhabdovirus P protein has RNA silencing suppressor activity in plants, but not in insect cells

Krin S. Mann[1], Karyn Johnson[2], Ralf G. Dietzgen[1]

[1]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Australia, [2]School of Biological Sciences, The University of Queensland, Brisbane, Australia

Plant rhabdoviruses cause economically important crop diseases and are commonly vectored by insects in which they also multiply. The type species of the genus Cytorhabdovirus is Lettuce necrotic yellows virus (LNYV). The LNYV genome consists of 12, 807 nucleotides and encodes six proteins in the order 3'– N – P – 4b – M – G – L –5'. However, little is known about the functional properties of these proteins. Given that most plant viruses possess activities to counteract the host's RNA silencing defences, the potential RNA silencing suppressor (RSS) activity of the LNYV proteins was identified and evaluated in both plant and insect systems. GFP-expressing Nicotiana benthamiana (16c) plants agroinfiltrated with LNYV protein expression constructs continued to express GFP only in the presence of the LNYV P protein, indicating local RSS activity. However, on a systemic level, the spread of silencing was unhindered as upper non-infiltrated leaves of 16c plants lost GFP expression. Analysis of the small RNA profiles indicated that LNYV P did not affect short-interfering (si-) RNA accumulation. This suggests the mode of action of LNYV P is different than the majority of plant viral RSS, which bind to siRNAs. In insect cells, using a suppressor-deficient, self-replicating Flock House virus (FHV) system, transfected Drosophila melanogaster S2 cells revealed that none of the LNYV proteins acted as RSS. In contrast, known RSS tombusvirus P19 and FHV B2 were able to suppress RNA silencing in this system. The RNA silencing pathways in plants include target amplification by host RNA-dependent RNA polymerases, which appear to be absent in insects. The observed lack of RSS activity in S2 cells, and inability to suppress systemic silencing and siRNA accumulation in plants suggests that LNYV P may act on the components involved in the amplification stages of the RNA silencing pathway.

Poster Session

VIR-PM2024 - Molecular epidemiology of human parainfluenza virus type 3 in Taiwan

Yung Hung Chang[1], SC Hsu[1], SY Ho[2], MY Liu[1], SY Chang[1,2], CL Kao[1], Chun Nan Lee[1,2]
[1]Graduate Institute of Clinical Lab. Sciences & Medical Biotech.,NTU, Taipei, Taiwan, [2]Deptartment of Laboratory Medicine, NTUH, Taipei, Taiwan

Human parainfluenza viruses (hPIVs) are one of the important causes of acute respiratory illness (ARI) among children under age 5. HPIV-3 was the major cause of ARI among all hPIV serotypes, but long term studies of molecular epidemiology related to hPIV-3 were still lacking in Asia. Of all the hPIV-3 genes, HN (hemagglutinin-neuraminidase) glycoprotein gene is commonly used to investigate molecular epidemiology by analyzing the phylogenetic relationships among different strains because it possesses high degree antigenic diversity. It could be distinguished into 3 major clusters, A, B, and C, based on the nucleotide sequence of the full-length HN gene. HPIV-3 of the clusters A and B circulated mostly in the last century, while cluster C circulated globally after 2000. In this study, we have collected hPIV-3-positive samples from National Taiwan University Hospital (NTUH) between 1988 and 2013 to investigate the molecular epidemiology of hPIV-3 in Taiwan. According to the record of virus isolation from 2000 to 2012, we found that more hPIV were isolated after 2006, and 95% of them were hPIV-3. Phylogenetic analysis of the full-length HN gene indicated that hPIV-3 isolates in Taiwan in late 1980s and middle 1990s belonged to the cluster C and clearly different from the hPIV-3 isolates from western countries in the same period. The hPIV-3 strains responsible for the increasing cases after 2006 in Taiwan were mainly belonged to clusters C1 and C 3 which caused the outbreaks around the world. This is the first report to indicate that hPIV-3 of cluster C has been prevalent in Asia since late 1980s.

Poster Session

VIR-PM2026 - Characterization of avian Paramyxoviruses isolated in Kazakhstan

Kobey Karamendin[1], Aidyn Kydyrmanov[1], Saule Asanova[1], Klara Daulbayeva[1], Aigerim Seidalina[1], Yermukhammet Kasymbekov[1], Elizaveta Khan[1], Marat Sayatov[1]
[1]Institute of Microbiology and Virology, Almaty, Kazakhstan

Large-scale ecological study of the circulation of APMVs in wild birds in the Central Asian region was conducted to isolate the urgent virus, define their ecological niches and phylogenetic relationships. Kazakhstan has an extensive territory which two large flyways of water birds cross – the Siberian-Black Sea–Eastern-African and the Central–Asian–Indian. It has numerous places of nesting, moulting and summer concentration of birds. There is little information about the circulation of APMVs among wild birds in Kazakhstan and wild avifauna. More than 5000 samples from wild birds representing 17 orders, 38 families and 155 species were tested. All in all 34 strains of APMVs were isolated and 25 from them were APMV-1 and two isolates belonged to APMV-4, 6 isolates were APMV-8 and one APMV-6. These obtained data indicate the prevalence of 0.5%. Analysis of virus-host interactions showed the prevalence of APMVs only within the order Anseriformes (ducks, swans and geese) and all the rest 16 orders were negative. Ratio between ducks, swans and geese as APMV carriers was 16:1:17 respectively. APMV-4 and APMV-1 strains were isolated in the middle and at the end of 2000s, APMV-6 and APMV-8 strains in 2013. Geographical distribution shows that APMV-1 strains from wild birds were isolated in Tengiz-Korgalzhyn lakes system in Central Kazakhstan and one isolate in Southern Kazakhstan, APMV-4 and APMV-6 in Southeastern Kazakhstan in Balkhash-Alakol lakes system and APMV-8 in Northern Kazakhstan. Phylogenetic studies of a fragment of F-gene of the most important APMV-1 serotype showed the divergence of them into two classes 1 and 2. Kazakhstan strains from wild birds formed a cluster of viruses inside the class 1, which include lentogenic viruses with low or apathogenic properties. Identity belong viruses was 98 %. Isolated APMV-1 stains had cleavage site SGGERQERLVG typical for lentogenic strains.

Poster Session

VIR-PM2028 - Paramyxovirus Sendai virus N protein plays a critical role in restricted production of copyback-type defective-interfering genomes to escape from detection by host innate immunity

Asuka Yoshida[1], Ryoko Kawabata[1], Takemasa Sakaguchi[1], Takashi Irie[1]
[1]Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan

One of the first defenses against infecting pathogens is the innate immune system activated by cellular recognition of pathogen-associated molecular patterns (PAMPs). Virus-derived RNA species are thought to serve as PAMPs for infection of RNA viruses. Many of the viruses have been shown to possess the ability to antagonize the innate immune system to escape from it. Here, we compared two Sendai virus (SeV) strains, Cantell and Z, showing extremely different phenotypes in IFN-β inducibility. Such difference was dependent on the presence of a massive amount of copyback-type defective-interfering (DI) genomes, strong inducers of IFN-β, even in the commercial stock of Cantell but not on deficiency in IFN-antagonistic accessory proteins C and V. Cloning analysis by sequential limiting dilutions revealed that the Cantell stock contained at least two types of viruses with high and low producibility of the copyback-type DI genomes. Serial passages of the DI-producing clone resulted in rapid accumulation of the DI genomes, paralleled with inducibility of IFN-β, but serial passages of the other clone had no such effect, indicating that the accumulation of DI genomes in the Cantell stock was due to emergence of the DI-producing virus. Surprisingly, such remarkable difference of these clones was proven to be virtually depending on a single amino acid substitution within the N protein by comparisons of their genome sequences and recombinant SeVs possessing N of these clones in the backbone of strain Z. N protein of the DI-producing clone resulted in lower density of nucleocapsids than that of the DI-non-producing clone, probably causing the different producibility of the DI genomes. The results indicate that the N protein of SeV plays a critical role in restricted production of DI genomes to avoid recognition of infection by host cells.

Poster Session

VIR-PM2030 - Respiratory viral infections in patients at the early phase after hematopoietic stem cell transplantation; prospective and comprehensive surveillance

Masayuki Saijo[1,2], Satsuki Kakiuchi[1,2], Masanori Tsuji[3], Hidekazu Nishimura[4], Mutsuyo Ito-Takayama[1], Chang-Kweng Lim[1], Yukie Yamaguchi[1], Masashi Mizuguchi[2], Akira Oka[2], Shuichi Taniguchi[3]

[1]National Institute of Infectious Diseases, Tokyo, Japan, [2]The Uniersity of Tokyo, Tokyo, Japan, [3]Toranomon Hospital, Tokyo, Japan, [4]Sendai Medical Center, Sendai, Japan

Background: Patients, who receive hematopoietic stem cell transplantation (HSCT), are vulnerable to infectious diseases including respiratory viral infections (RVIs) due to severely impaired immunity. However, not a few RVIs remain undiagnosed due to lack of clinically available diagnostic methods. Therefore, there are few prospective and comprehensive studies on this issue. Objective: The objective of the present study is to prospectively survey the outbreak of RVIs in the patients, who received HSCT and to clarify the morbidity in such patients. Patients and methods: HSCT patients in Toranomon Hospital, Tokyo, Japan, were enrolled in the study from Jun 2010 to May 2012. Oropharyngeal (OP) swab or bronchoalveolar lavage samples were collected weekly regardless of symptoms during the period of -6 and 100 days of HSCT. Viruses were isolated using Vero, HEL, HEp-2 and MDCK cells. For predominantly isolated parainfluenza virus 3 (PIV-3) strains, the nucleotide sequence of the hemmaglutinin-neuraminidase (HN) gene was determined and the phylogenetic analysis was conducted to estimate the infection route. Results: Among 268 post-transplant occasions, RVIs were isolated from 64 patients; 3 asymptomatic, 21 upper respiratory tract, and 40 lower respiratory tract infections. The RVIs were caused by PIV-3, PIV-2, PIV-1, influenza A, respiratory syncytial virus, and enterovirus in 51, 5, 2, 2, 2, and 1 occasions, respectively. Relatively large outbreak of respiratory infections due to PIV-3 was demonstrated every year. Most PIV-3 isolates in each season aggregated in a single cluster in phylogenetic analysis, suggesting that the PIV-3 respiratory infections occurred as a form of nosocomial infection. Conclusion: PIV-3 is one of the most important RVI pathogens in patients with HSCT, because it easily spread as a form of nosocomial infection. For prevention of nosocomial PIV-3 infections, virological diagnosis is necessary, indicating the importance of the development of rapid diagnostic kits for PIV-3.

Poster Session

VIR-PM2032 - Nipah virus nonstructural C protein has a novel nuclear export signal

Ryo Horie[1], Misako Yoneda[1], Chieko Kai[1]
[1]*Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan*

Nipah virus (NiV) is a member of the genus Henipavirus, which emerged in 1998. NiV causes severe encephalitis in human with highly mortality. We had previously reported that NiV nonstructural C protein played key roles in NiV pathogenicity, but its detailed mechanism has been still unknown. In the present study, we analyzed the localization of C protein to elucidate its function. We observed that NiV C protein expressed from a transfected plasmid was mainly localized to the cytoplasm, but partially to the nucleus. We showed that the N-terminal and the C-terminal regions were necessary for nuclear accumulation of C protein. The N-terminal region fused with EGFP distributed in the cytoplasm, whereas the C-terminal region fused with EGFP distributed in both the cytoplasm and the nucleus. Further, alanine scanning mutagenesis identified the amino acid residues of NiV C protein required for the localization to the cytoplasm. The sequence of the region did not contain previously reported NES like motif. The intranuclear accumulation of NiV C protein was not enhanced after leptomycin B treatment. These results suggest that NiV C protein has the ability to shuttle between the nucleus and the cytoplasm and the nuclear export pathway of C protein is distinct from the CRM1-dependent pathway.

Poster Session

VIR-PM2034 - Structure of the Nipah virus phosphoprotein oligomerization domain and its function in viral replication

Misako Yoneda[1], Kazutaka Murayama[2], Ryo Horie[1], Tomomitsu Doi[1], Mikako Shirouzu[2], Kouichiro Syoji[1], Tomoko Fujiyuki[1], Hiroki Sato[1], Shigeyuki Yokoyama[2], Chieko Kai[1]
[1]Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]Systems and Structural Biology Center, RIKEN, Yokohama, Japan

Nipah virus (NiV) is a recently emerged severe human pathogen. The NiV phosphoprotein (P) is important for efficient encapsidation of viral RNA and plays important role as polymerase cofactor. The crystal structure of the tetramerization domain (residues 442-584) was determined by the multiple isomorphous replacement method. The NiV-P structure consists of three α-helices, which are stabilized by interactions with the neighboring helices, including three hydrogen bonds. Further, to elucidate the critical part of the interface that maintains the tetramer formation, site-directed mutational analysis was performed. Some mutants adversely affected minigenome replication. These results showed that C-terminal half of the third α-helix must significantly contribute to the stabilization of the coiled-coil tetramer of NiV-P.

Poster Session

VIR-PM2036 - Measles genotypes associated with outbreaks and sporadic cases in the United States during the post-elimination era

Raydel Anderson[1], Rebecca McNall[1], Jennifer Rota[1], Elena Lopareva[1], Pierre Rivailler[1], Marcus Collins[1], Greg Wallance[1], Susan Redd[1], William Bellini[1], Paul Rota[1]
[1]*Centers for Disease Control and Prevention, Atlanta, USA*

Genetic characterization of wild-type measles viruses is a critical component of laboratory surveillance for measles because this information helps to identify the source of the virus and can help verify the absence of endemic transmission of virus in countries with measles elimination goals. Measles elimination was verified in the United States in 2001, and this report describes virologic surveillance that was conducted to monitor the maintenance of elimination. Between 2001 and 2013, sequence data from clinical specimens and viral isolates were obtained from confirmed measles cases in the United States. Sequences representing 95 outbreaks and 115 sporadic cases indicated the presence of 11 of the 24 recognized measles genotypes. The most common genotypes detected were genotype D4, usually identified from imported cases from European countries, and genotype D8, associated with importations from numerous countries including Thailand, India, Romania and the UK. A number of viruses belonging to genotype B3 were imported from India and Africa as well as genotype H1 from China. Multiple genotypes were detected from cases in which the source of infection was unknown suggesting that these cases had multiple sources. The diversity of the measles virus genotypes observed in the in the United States during the post elimination era was consistent with the epidemiological data showing sustained lack of endemic transmission, and the multiple genotypes detected reflected the various imported sources of virus.

Poster Session

VIR-PM2038 - Sequence analysis of Ferret badger Rabies virus from Taiwan outbreaks in 2013

Chun-hsien Tseng[1], Hsiang-Jung Tsai[1], Shu-Hwae Lee[1], Hsin-Fu Liu[2], Yu-Hau Shih[3], Yu-Ju Lin[3], Tien-Shine Huang[3], Ai-Ping Hsu[3]

[1]Animal Health Research Institute, Council of Agriculture, New Taipei City, Taiwan, [2]Mackay Memorial Hospital, Department of Medical Research, New Taipei City, Taiwan, [3]Animal Health Research Institute, Council of Agriculture, New Taipei City, Taiwan

Taiwan has been rabies-free since 1959. However, in middle of 2013, ongoing rabies outbreaks among Ferret badgers have happened in Taiwan. From the genome sequencing and phylogenetic analysis of 10 Ferret badger Rabies virus (RABV) isolated in 2013, Taiwan Ferret badger RABV could be divided into 3 groups those are significantly different in geographical distributions—TWI (locating in east part of Taiwan), TWII (locating in south part of Taiwan), and TWIII (locating in central part of Taiwan). In the N, P, M, G, L gene segments, TWII and TWIII showed nucleotide divergence about 5%, and it is lower than both compared with TWI. In Taiwan, the most cases were restricted in Ferret badger, but only one dog outbreak was due to a rabid Ferret badger biting. RNA copy number detection based on real-time RT-qPCR showed 40,000-fold higher RABV RNA copy numbers from rabid Ferret badger brain sample, compared to rabid dog brain. This RABV RNA copy number detection result may indicate cross-species transmission of Ferret badger RABV with a lower replication capacity in a non-original host. Commissioned MCC tree analysis by Mackay Memorial Hospital showed obviously Ferret badger RABV of Taiwan and China were descended from a common ancestor, and two groups were divided apart in 1883. Branched phylogenetic tree pattern of Taiwan Ferret badger RABV occurred at least 66 years ago, and it could be inferred these rabies virus groups have been latent in Taiwan for a long time.

Poster Session

VIR-PM2040 - Impact of enterovirus isolation protocol on perception of Nonpolio enterovirus species C diversity

Temitope Faleye[1], Johnson Adeniji[1]
[1]University of Ibadan, Ibadan, Nigeria

INTRODUCTION: The Nigerian vaccine-derived poliovirus serotype 2 (VDPV2) outbreak has been the largest globally with almost all the isolates recovered being recombinants with nonstructural region of nonpolio enterovirus species C (NPESC) origin. Despite this, there has been under-reporting of NPESC members in the country, consequent of the cell lines included in previous isolation protocols. Therefore, in this study we examined the impact of including other cell lines in the isolation protocol on the recovery and diversity of NPESC members. METHODOLOGY: Fifteen environmental samples previously concentrated and analysed as part of the poliovirus environmental surveillance program in Nigeria were randomly selected and inoculated into MCF 7 and LLC-MK2 cell lines. Isolates were typed as enteroviruses and species C (EC) members using different RT-PCR assays. Afterwards, confirmed EC isolates were inoculated into L20B cell line to detect polioviruses. Subsequently, partial VP1 and 3D genes of species C isolates were amplified and sequenced. Finally, isolates were genotyped and analysed for recombination. RESULTS: Forty-eight (48) isolates were recovered from the fifteen samples, 47 (97.9%) of which were enteroviruses. Of the enteroviruses, 32 (68.1%) were ECs, 19 (40.4%) were polioviruses and 13 (27.7%) were NPESC members. All 13 NPESC isolates were recovered on MCF 7 and include CVA13 (clusters A and C) and CVA20. Also, phylograms showed evidence of recombination between Nigerian CVA13, CVA20 and OPV2 to make the circulating VDPV2 isolates. Furthermore, NPESC isolates were recovered from 3 concentrates that had been previously reported negative for enteroviuses when analysed on RD and L20B cell lines. CONCLUSIONS: NPESCs are circulating in Nigeria and their under-reporting was due to the combination of cell lines used for enterovirus isolation in previous reports. Also, CVA13 and CVA20 isolates circulating in Nigeria might have provided the nonstructural region for the characterised circulating VDPV2s recovered in the outbreak.

Poster Session

VIR-PM2042 - ER stress-induced apoptosis by enterovirus 71 via phosphorylating IRE1 and JNK2 to facilitate viral shedding

Jim-Tong Horng[1], Chih-Shan Chen[1], Jia-Rong Jheng[1], Jin-Yuan Ho[1]
[1]Department of Biochemistry, Chang Gung University, Kweishan, Taoyuan, Taiwan

Enterovirus 71 (EV71) is a single-stranded RNA virus belonging to the Picornaviridae family. EV71 release after completing replication has been suggested by the caspase-induced apoptosis, but the defined mechanism(s) involved are not fully understood. In this study, we addressed the role of endoplasmic reticulum (ER) stress-induced apoptosis in EV71 release after one round of replication. We demonstrated that EV71 infection enhanced the phosphorylatation of IRE1 (Inositol-requiring enzyme 1) and its downstream target c-Jun N-terminal protein kinase (JNK). We observed that knockdown of IRE1 and JNK2, but not JNK1, results in a decreased level of virus titer, which was due to the attenuated viral protein expression and viral RNA replication. This was further ratified by the treatment of a JNK inhibitor SP600125 in the early stage. Addition of SP600125 and a caspase inhibitor Q-VD-OPh at a later stage without influencing virus replication resulted in reduction of viral particle release. Silencing IRE1 and JNK2 but not JNK1 abolished caspase and PARP cleavage, supporting our current hypothesis that cellular IRE1-JNK2 pathway is involved in EV71-induced apoptosis, which in turn facilitates virus release.

Poster Session

VIR-PM2044 - Cardiac glycosides act beyond the modification of intracellular Na+/K+ milieu to potently inhibit enteroviruses

Kanxing Wu[1], Justin Jang-Hann Chu[1]
[1]Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Enteroviruses are positive-sense, single-stranded RNA viruses that can cause a variety of human diseases ranging from acute flaccid paralysis, meningitis to hand, foot and mouth disease (HFMD). In recent years, enterovirus 71 (EV71) has emerged as a clinically significant enterovirus due to its strong association with neurological complications in HFMD outbreaks. Given the lack of therapeutic options, there is a need to identify novel inhibitors of EV71. Cardiac glycosides (CG) are well-established inhibitors of the Na+/K+ ATPase that disrupt the cellular milieu of Na+ and K+. From the screening of a natural products library, we report the identification of several CGs (including peruvoside, ouabain and bufalin) as non-toxic potent inhibitors of EV71 at nanomolar concentrations. Similar inhibition was found against coxsackievirus A16 (CA16) and echovirus 7 suggesting a pan-entero antiviral activity. In vivo efficacy was demonstrated in a 1 week-old Balb/c mouse model for EV71. Strand-specific RT PCR and a bicistronic IRES luciferase reporter assay revealed that viral RNA replication was inhibited by peruvoside while EV71 IRES-driven translation was not. Using small molecule inhibitors, we identified calcium influx, calcium store depletion and Src kinase signaling as critical processes in peruvoside's anti-EV71 activities. siRNA-knockdown of genes in these pathways (RYR1, ITPR3 and Src) also led to a reduction in peruvoside's anti-EV71 potency. In addition, immunofluorescence microscopy revealed a disassembly of Golgi stacks into perinuclear vesicles by peruvoside treatment that is distinct from the Golgi haze in brefeldin A (BFA) treatment. The phosphorylation of GBF1 in response to a calcium/kinase signal during mitosis has been reported to cause a similar phenomenon. To this end, we noted increased levels of GBF1 phosphorylation in peruvoside-treated cells. These findings suggest that CGs may inhibit enteroviruses by modifying host factors needed for viral replication and potentially lead to new therapies for diseases caused by enteroviruses.

Poster Session

VIR-PM2046 - Viral distributions of the duck Hepatitis A virus in experimentally infected ducklings

Yu-hua Shih[1,2], Hsu Ai-ping[1], Chou Chung-Hsi[2], Tseng Chun-hsein[1]
[1]Animal Health Research Institute Council of Agriculture, Executive Yuan, New Taipei City, Taiwan, [2]School of Veterinary Medicine National Taiwan University,Taipei City, Taiwan

This study try the one-step real-time reverse transcription polymerase chain reaction (one-step rRT-PCR) to detecting and distinguishing between duck hepatitis A virus type 1 (DHAV-1) and type 2 (DHAV-2).Applying the rRT-PCR for DHAV-1 and DHAV-2 virus distribution test.Thirty DHAV-1 and DHAV-2 antibodies free 1-day -old ducklings were inoculated oral with 0.5 ml of viral suspension containing 1.45 LD50 with DHAV-1 strain (04D). Thirty ducklings were inoculated oral with 0.5 ml of viral suspension containing 0.43LD50 with DHAV-2 strain (04G). Three ducklings were euthanatized at 2, 4, 8, 12, 16, 20, 24, 28, 38,36h post-inoculation(PI).Applying the rRT-PCR for DHAV-1 and DHAV-2 virus distribution test, the DHAV-1 viral nucleic acids can be detected from lung, liver and intestine at 24th hour post inoculation of 1-day-old ducklings with DHAV-1, and the DHAV-2 viral nucleic acids can be detected from lung, pancreas, liver and intestine at 32nd hour post inoculation of 1-day-old ducklings with DHAV-2. The ducklings began death at 30th hour PI with DHAV-1 and at 36th hour PI with DHAV-2. These results suggest that ducklings infect DHAV as fast as 24th hour virus would distribute to other organs.

Poster Session

VIR-PM2048 - Xylem vessel analysis in Turnip mosaic virus infected plants

Daniel Garcia Cabanillas[1], Juan Wan[1], Jun Jiang[1], Jean-François Laliberté[1]
[1]*INRS, Montreal, Canada*

Turnip mosaic virus (TuMV) is a positive single stranded RNA virus and belongs to the genus Potyvirus. Its genome (10 kb) encodes a polyprotein that is proteolytically processed into 11 viral proteins. TuMV infection induces the formation of endoplasmic reticulum-derived vesicles that are involved in viral replication and intercellular movement. The viral protein responsible for vesicle formation is a 6 kDa membrane protein known as 6K2. 6K2-induced vesicles can be observed by confocal microscopy following infection of Nicotiana benthamiana plants with a cDNA clone of TuMV that produces 6K2 as a red or green fluorescent protein fusion. Longitudinal histological sections of infected stem tissues were made and 6K2 vesicles were observed in xylem vessels. Xylem sap was collected and was found to be infectious. Electron microscopy negative staining showed the presence of viral particles in the sap. Western blot analyses on xylem sap confirmed the presence of viral proteins, and also host proteins, such as the initiation eukaryotic initiation factor 4E. The presence of viral proteins and particles raises new questions on systemic infection through the xylem by plant viruses.

Poster Session

VIR-PM2050 - Infectivity of Potato virus Y (PVY) to potato cultivars having extreme resistance gene, Rychc

Ohki Takehiro[1], Kenji Asano[1], Kazuyoshi Hosaka[2], Toshiya Igarashi[3], Tetsuji Ogawa[4], Kazusato Ohshima[5], Tetsuo Maoka[1]

[1]NARO Hokkaido Agricultural Research Center. Sapporo, Hokkaido, [2]Obihiro University of Agriculture and Veterinary Medicine. Obihiro, Hokkaido, [3]Potato research institute, Calbee Potato Co. Ltd. Kawanishi, Hokkaido, [4]Agriculture and Forestry Technical Development Center, Nagasaki. Unzen, Nagasaki, [5]Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Honjo-machi, Saga

The resistance gene to Potato virus Y (PVY), Rychc is known to confer extreme resistance on potato plants; PVY is unable to multiply or multiplies at a very low level in the cultivars with the gene. To revalue their effect, PVY-O, PVY-N and a new strain PVY-NTN which occurred since 1990s were mechanically inoculated with carborundum to the potato cultivar "Konafubuki" having Rychc, and symptoms were compared with two potato cultivars "Alwara" and "NY121" having similar extreme resistance genes, Rysto and Ryadg, respectively. The three PVY strains induced a few small necrotic spots only on Konafubuki leaves but not on NY121 or Alwara three weeks after inoculation. The three cultivars did not show systemic symptoms six weeks after inoculation; however, the PVY strains were all detected from some of both inoculated leaves and upper leaves of the potato cultivars by RT-PCR and RT-PCR-MPH (Microplate Hybridization) although TAS-ELISA failed to detect PVY. Transmission to new tubers was not confirmed. We further examined the resistance mechanism to PVY-NTN, using Konafubuki. Potato plants rarely developed systemic symptoms (necrosis) when cultivated for a long term (more than two months after inoculation). Necrotic spots on inoculated leaves and necrosis on upper leaves were cut off and used to inoculate tobacco leaves “Nicotiana tabacum cv X-nc”. Infection of PVY-NTN was confirmed on some tobacco plants, suggesting that PVY-NTN was present on necrotic spots and necrosis on Konafubuki leaves. Infected tobacco plants were, then, grafted to Konafubuki potato to find clear stem necrosis around the grafted part on a few potato plants. In conclusion, Rychc gene incited extreme resistance reactions in which the three PVY strains were able to infect slightly but failed to transmit to new tubers. The new strain PVY-NTN induced the same symptoms as did PVY-O and PVY-N.

Poster Session

VIR-PM2052 - Evidence for in vitro and in vivo cleavage of C-terminal region of the tomato ringspot virus NTP-binding protein by the membrane-associated signal peptidase

Ting Wei[1], Helene Sanfacon[2]
[1]Dept of Botany, University of British Columbia, Vancouver, Canada, [2]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, Canada

Tomato ringspot virus (ToRSV, a nepovirus) replicates in association with endoplasmic reticulum (ER)-derived membranes. The ToRSV NTP-binding protein (NTB) and the NTB-VPg polyprotein, which includes the VPg domain, are integral membrane proteins associated with ER-bound replication complexes. Membrane association of NTB is directed by an N-terminal amphipathic helix and two C-terminal hydrophobic helices separated by a few amino acids. The first C-terminal hydrophobic helix directs the translocation of the C-terminal region of NTB-VPg in the ER lumen, resulting in the recognition of an N-glycosylation site in the VPg. A putative signal peptidase (SPase) cleavage site was identified in the region separating the two C-terminal hydrophobic domains, and cleavage at this site was confirmed in vitro using canine microsomal membranes and a construct (cNTB-VPg) containing the C-terminal region of NTB and the VPg. Ectopic expression of cNTV-VPg in plants allowed the identification of cleavage fragments that were consistent in size with processing at the predicted SPase site. Variations in the C-terminal region of NTB-VPg are observed among ToRSV isolates. The VPg N-glycosylation site is conserved in the ToRSV-Rasp2 and ToRSV-GYV isolates, but not in the ToRSV-Rasp1 isolate. The second hydrophobic domain is only weakly predicted in ToRSV-Rasp1 due to the deletion of two hydrophobic amino acids. Finally, the region of the predicted SPase cleavage site contains several point mutations in ToRSV-Rasp1. These variations correlated with reduced cleavage of the ToRSV-Rasp1 cNTB-VPg protein at the predicted SPase site in vitro. Similarly, release of protein fragments corresponding to the predicted SPase cleavage was not observed in planta for this isolate. Taken together, these results suggest that the C-terminal region of ToRSV NTB-VPg is cleaved by the signal peptidase in plants, but the efficiency of cleavage varies with the isolate.

Poster Session

VIR-PM2054 - Host factors involved in potyvirus replication

Rudolf Sägesser[1], Anne Marie Haeberle[1], Charikleia Polioudaki[2], Annette Nassuth[1], K. Rusanov[1], Efthimia Mina Tsagris[1,2]

[1]Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology Hellas (F.O.R.T.H.), Heraklion, GREECE, [2]Dept of Biology. University of Crete, Heraklion, Greece

Replication proteins of known RNA viruses share highly conserved motifs, such as helicase and polymerase motifs, suggesting a common evolutionary ancestor. The plant potyviruses express their replication proteins as functional cluster (cI-6k-Nia-Nib) on an approximately 340 kD polyprotein with Nib as the catalytic subunit of the viral replicase (RNA-dependent RNA-polymerase or RdRp). Nevertheless, there is increasing evidence that the viral replicase (RDRP) is composed of both viral and host proteins. This is also supported by the trans-activity of the Nib protein which may be recruited to the replication complex through protein-protein interaction with viral or host factors or through RNA-protein interaction. We are investigating host proteins for their possible role in the viral replicase complex of the plum pox potyvirus (PPV). To address this, an expression library of Nicotiana benthamiana (kindly provided by Prof. Bol, Gorlaeus lab, Leiden, Netherlands) was screened for proteins that interacted with the Nib from PPV in a yeast two-hybrid system (2HS) under the control of three reporter genes. 12 independent clones significantly interacted with Nib, but were negative against all non- related baits tested. Amongst the 12 putative positives, the majority could be subdivided into a class representing nuclear-encoded chloroplast proteins and a class consisting of putative RNA-binding proteins. We believe that the large number of positives found in the 2HS is due to the multifunctional domains of the NIb protein. Further discrimination tests resulted in 3 independent survivor clones showing protein-protein interaction by the use of heterologous RdRp probes. Supported by contract number BIO4-CT97-2300.

Poster Session

VIR-PM2056 - Investigating defense responses of Nicotiana benthamiana involving the 14-3-3 gene family in virus-induced effector-triggered immunity

Jennifer Spencer[1], Soha Sobhanian[1], Melanie Sacco[1]
[1]*California State University, Fullerton, USA*

Plants possess multiple tiers of immunity, the most specific of which involves resistance proteins that provide effector-triggered immunity (ETI). ETI is often associated with the hypersensitive response (HR), a type of programmed cell death. The 14-3-3 gene family is highly conserved across eukaryotes and acts in myriad cellular processes, including plant immunity involving resistance (R) proteins that mediate ETI. Using co-immunoprecipitation and mass spectrometry, we identified a 14-3-3 isoform from Nicotiana benthamiana interacting with the amino-terminal, coiled-coil domain of the R protein Tm2-2 from Solanum lycopersicum (tomato), which confers resistance against Tobacco mosaic virus (TMV) through recognition of the virus 30K movement protein (MP). By silencing N. benthamiana 14-3-3 homologs using virus-induced gene silencing (VIGS), essential roles in plant defenses and the HR-like response are being investigated. Eleven14-3-3 homologs identified from the N. benthamiana genome database were cloned into the Tobacco rattle virus vector pTV:00, transformed into Agrobacterium tumefaciens and agroinfiltrated into N. benthamiana leaves to induce 14-3-3 gene silencing. To detect roles for 14-3-3 proteins in plant resistance responses, N. benthamiana silenced for different 14-3-3 genes were examined for loss of HR-like responses when challenged with the Polerovirus P0 protein from Sugarcane yellow leaf virus (SCYLV), which induces cell death when expressed in N. benthamiana through an unknown R protein, or with the TMV MP in the presence of Tm2-2; the degree and specificity of silencing was assessed by RT-PCR. At the same time, interactions between the ten 14-3-3 genes described from tomato (TFTs) and Tm2-2 or the TMV MP were examined by co-immunoprecipitation, as well as the effect of TFT overexpression on R protein-mediated HR. Data from these studies will be presented and the potential of a biological role for 14-3-3 proteins in TMV MP- or SCYLV P0-induced immune responses will be discussed.

Poster Session

VIR-PM2058 - Unravelling the story behind the recruitment of HSP70 as a novel strategy for uncoating of Cucumber necrosis virus (CNV)

Syed Benazir Alam[1], D'Ann Rochon[1,2]
[1]University of British Columbia, Faculty of Land and Food Systems, Vancouver, Canada, [2]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Center, Summerland, Canada

Uncoating of a virus particle to release its nucleic acid is a critical aspect of the viral multiplication cycle, i.e., the establishment of infection. We are interested in studying the role of HSP70 homologs in the uncoating process of CNV. Identification of such host components could assist in the development of host-directed therapeutics which could have a great impact on virus disease management. We have found that HSP70 protein levels increase significantly during CNV infection in *Nicotiana benthamiana*. HSP70 has been found to coimmunoprecipitate with the CNV coat protein (CP) and low levels also copurify with CNV particles. CNV/HSc70 interaction was observed in virus overlay assays. To examine whether HSP70 could potentially participate in CNV disassembly, HSP70 was overexpressed via heat shock (HS) treatment in the local lesion host *Chenopodium quinoa* and leaves were then inoculated with virus particles. We observed a statistically significant increase in the number of local lesions in HS versus non-HS plants. However, a significant increase was not observed when virion RNA was used as an inoculum suggesting that HSP70 may play a role in CNV disassembly. A CNV CP mutant lacking the hydrophobic β-region in the arm domain did not show a statistically significant difference in the number of local lesions on HS versus non-HS plants suggesting that the β-region may interact with HSP70. We have also found that incubating HSP70/HSc70 with virus *in vitro* renders the particles more sensitive to trypsin digestion suggesting a role in conformational change of virions. CNV CP contains a transit peptide near the amino terminus which includes the β-region and most chloroplast transit peptides have been shown to have HSP70 binding sites, we are hypothesizing that during disassembly the particles expand and the β-region of the arm domain externalizes allowing HSP70 recruitment followed by disassembly of the particle.

Poster Session

VIR-PM2060 - Broad-spectrum transgenic resistance against distinct tospovirus species at the genus level

Jui-Chu Peng[1,2], Tsung-Chi Chen[3], Joseph A. J. Raja[4,5], Ching-Fu Yang[1,4], Wan-Chu Chien[5], Chen-Hsuan Lin[1], Fang-Lin Liu[1], Hui-Wen Wu[1], Shyi-Dong Yeh[1,4]

[1]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, [2]Divsion of Crop Enviroment, Tainan District Agriculture Research and Extension Station, Tainan, Taiwan, [3]Department of Biotechnology, Asia Univerity, Taichung, Taiwan, [4]Agricultural Biotechnology Center, National Chung Hsing University, Taichung, Taiwan, [5]NCHU-UCD Plant and Food Biotechnology Center, National Chung Hsing University, Taichung, Taiwan

Thrips-borne tospoviruses cause severe damages in economic crops worldwide. In this investigation, transgenic tobacco plants carrying individual constructs containing the RNA-dependent RNA polymerase motifs within the L gene (WLm) of Watermelon silver mottle virus (WSMoV) were generated by Agrobacterium-mediated transformation. These constructs included: (i) translatable WLm in a sense orientation; (ii) untranslatable WLmt with two stop codons; (iii) untranslatable WLmts with stop codons and frame-shift; (iv) untranslatable antisense WLmAs; and (v) WLmds with an untranslatable inverted repeat of WLm containing a tospoviral 3' terminal consensus sequence (ATTGCTCT) and an NcoI site as a linker. A total of 46.7-70.0% transgenic tobacco lines derived from individual constructs, with WLmds as the best, showed resistance to the homologous WSMoV, and 35.7-100% plants of which exhibited broad-spectrum resistance against four other serologically unrelated tospoviruses of Tomato spotted wilt virus (TSWV) serogroup, Groundnut yellow spot virus (GYSV) serogroup and Impatiens necrotic spot virus (INSV) serotype. Moreover, the selected transgenic tobacco lines also exhibited broad-spectrum resistance against five additional Asia-type tospoviruses of WSMoV and Iris yellow spot virus (IYSV) serogroups. However, the transgenic lines were not resistant to plant viruses out of the genus Tospovirus. Northern analyses indicated that the broad-spectrum resistance is mediated by RNA silencing. To validate the L conserved region resistance in vegetable crops, all the constructs were also transferred to transgenic tomato lines, which also confer high levels of resistance against WSMoV and other distinct tospoviruses. Thus, here we demonstrate that our approach generates broad-spectrum resistance against tospoviruses at the genus level.

Poster Session

VIR-PM2062 - Potyvirus-induced RNA granules at a point of convergence between viral replication and translation

Anders Hafrén[1, 2], Andres Lõhmus[1], Kristiina Mäkinen[1]
[1]University of Helsinki, Helsinki, Finland, [2]Current: Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

Potato virus A (PVA; genus Potyvirus) has a positive-stranded RNA genome of 9565 bp. PVA replicates within viral replication complexes (VRCs) in association with cytoplasmic membranes of the host cell. Viral RNA (vRNA) trafficking within an infected cell is a tightly coordinated process, which includes many pathways, such as movement, translation/replication, RNA degradation, and encapsidation, that compete for the vRNA produced in VRCs. The precise regulation of viral genome utilization for different purposes, however, is not well understood, but the process could involve cellular mRNA pathways. Interestingly, PVA infection induces the formation of vRNA containing granule structures (PGs), which share some properties with other plant RNA granules and contain some RNA granule proteins described also in virus-infected animal cells. Our results show that VRCs and PGs are distinct structures, which can, however, spatially associate during infection. Viral genome-linked protein VPg can target PVA RNA efficiently to translation in synergy with PG components P0 and eIF(iso)4E (Hafren et al., 2013). PGs can't be detected during VPg-activated viral translation proposing that the existence of PGs and PVA translation are linked and that vRNA is allocated either to translation or to PGs in an interdependent manner. [Hafren, A., Eskelin, K., and Mäkinen K. (2013) "Ribosomal protein P0 promotes Potato virus A infection and functions in viral translation together with VPg and eIF(iso)4E" Journal of Virology,87, 4302–4312.]

Poster Session

VIR-PM2064 - The role of phosphorylation of the ribosomal protein S6 in 40S-60S ribosomal subunit joining and translation of the Cauliflower mosaic virus 35S polycistronic RNA

Eder Mancera-Martínez[1], Lyuba Ryabova[1]
[1]*IBMP-CNRS, University of Strasbourg, France*

Initiation restart after long ORF translation is normally restricted by unknown pathways. The plant viral reinitiation factor transactivator–viroplasmin (TAV) exceptionally promotes translation of the Cauliflower mosaic virus 35S polycistronic RNA through a mechanism called transactivation, which involves retention on polysomes and reuse of eIF3 and the reinitiation supporting protein (RISP) to regenerate reinitiation-competent-ribosomal complexes. RISP, TAV and the 60S ribosomal subunit are co-localized in epidermal cells of infected plants, and eIF3-TAV-RISP-60S rpL24 (L24) complex formation can be shown in vitro. The crystal structure of yeast 80S revealed that the L24 N-terminal domain— the TAV-binding site—is bound to the interface of 60S, while its C-terminal alfa-helix—the RISP binding site—protrudes out of 60S towards to 40S. The group of Yusupov suggested that L24 and rpS6 (S6) may form a bridge between 40S and 60S. S6 has long been known as a target of rapamycin (TOR), but the functional role of S6 phosphorylation remains unknown. Significantly, TAV can trigger TOR signalling activation, followed by RISP phosphorylation in a rapamycin-sensitive manner. When phosphorylated, RISP binds TAV and stimulates TAV function in reinitiation. Thus, we investigated the possible interaction between S6 and L24, and the role of RISP in 40S-60S joining. We failed to reveal a direct link between L24 and S6, while phospho-RISP, has the capacity to interact not only with L24, but also with S6 in Arabidopsis. The Arabidopsis S6 C-terminal helix contains 5 serine residues that are predicted to be phosphorylated in a TOR-sensitive manner. Our preliminary results suggest a role for S6 phosphorylation in binding to RISP and CaMV transactivation. Accordingly, S6-knockout Arabidopsis plants are more resistant to CaMV, and less efficient in TAV-mediated transactivation. Our results indicate that the RISP-mediated link between 40S and 60S is sensitive to TOR and may play a role in transactivation.

Poster Session

VIR-PM2066 - Continuous replication of European mountain ash ringspot associated virus (EMARaV) in in-vitro cultures of Sorbus aucuparia

Hans-Peter Muehlbach[1], Dagmar Svensson[1], Rashida Perveen[2], Claudia Hirschfeld[1], Jenny Robel[3], Hanny Tantau[1]
[1]Biocentre Klein Flottbek, University of Hamburg, Hamburg, Germany, [2]Bahauddin Zakariya University, Multan, Pakistan, [3]Division Phytomedicine, Humboldt University Berlin, Berlin, Germany

The European mountain ash ringspot-associated virus (EMARaV) is the eponymous species of the genus Emaravirus. Presently, four virus species are allocated into this genus, namely, in addition to EMARaV, fig mosaic virus (FMV), rose rosette virus (RRV) and raspberry leaf blotch virus (RLBV), including several geographic variants. Emaraviruses are negative-strand RNA viruses with a divided genome of four up to probably eight RNA molecules. EMARaV causes the serious ringspot disease in Sorbus aucuparia L.. Since up to now the virus could not be mechanically transmitted to herbaceous host plants, studies on its replication and gene expression strategies are hampered. Therefore, tissue cultures were established from shoots of EMARaV-infected Sorbus aucuparia, and long-term replication of EMARaV was analyzed by detection of viral RNA3 through RT-PCR. EMARaV infection was continuously detectable even after 18 months of in vitro culture, indicating that callus and cell suspension cultures sustain long-term replication of the virus. Interestingly, growth characteristics and morphological appearance of calli and suspended cells were not affected by EMARaV-infection. These cultured cells should overcome the difficulties of virus isolation that probably originate from the high content of phenolics in the mountain ash leaves, thus facilitating isolation and analysis of virus particles, which was not achieved yet from Sorbus aucuparia leaf tissue. Finally, cultured cells of Sorbus aucuparia, either uninfected or EMARaV-infected, provide a suitable source of protoplasts for transfection or transformation with viral and other foreign genes.

Poster Session

VIR-PM2068 - Penetration of pollen tubes with accumulated RBDV into stigmas is essential in causing the first viral infection in the stigma to lead to systemic infection

Masamichi Isogai[1], Tetu Yoshida[1], Nobuyuki Yoshikawa[1], Chiaki Nakanowatari[1]
[1]*Faculty of Agriculture, Iwate University, Morioka, Japan*

To understand horizontal transmission of Raspberry bushy dwarf virus (RBDV) by pollen, Torenia plants (Torenia fournieri in the family Scrophulariaceae) were hand-pollinated with pollen grains from RBDV-infected raspberry plants (Rubus idaeus in the family Rosaceae). Interestingly, six of the eighteen pollinated plants were infected with the virus. When raspberry pollen grains were pollinated on Torenia stigmas, these pollen grains germinated on Torenia stigmas, and then the pollen tubes penetrated into the stigma, even though the pollen tubes were arrested in the styles. In whole-mount in situ hybridization analysis of germinating pollen grains from infected raspberry plants, the virus accumulated in the germinating apertures and the tips of the pollen tubes. Furthermore, tissue blot hybridization analysis of Torenia plants pollinated with infected raspberry pollen grains revealed that the first virus infection site leading to systemic infection is the stigma. In contrast, when infected raspberry pollen grains that had lost germination capacity were used for pollination on Torenia stigmas, the virus did not cause stigma infection and horizontal transmission to a mother plant body. These results indicate that penetration of pollen tubes with accumulated RBDV into stigmas is essential in causing the first viral infection in the stigma to lead to systemic infection. Pollen is the only method of field transmission of RBDV from infected raspberry plants to healthy raspberry plants. In this study, we demonstrated that horizontal transmission of RBDV by pollen occurs in cross-incompatibility combination. Therefore, it might be possible that RBDV spreads its infection in its host plant species beyond the plant family level by pollen through wind and/or pollinating insects, if the virus infection was established at stigmas by penetration of infected pollen tubes into these stigmas.

Poster Session

VIR-PM2070 - Development of screening and application method of antiviral agents against to the TMV and PepMoV-infected plant

Eun-Gyeong Song[1], Bo-Ram Moon[1], Byoung-Eun Min[1], Ji-Yeon Kwon[1], Ki-Hyun Ryu[1]
[1]Seoul Women's University, Seoul, South Korea

Many antiviral chemicals derived from microorganisms, plants and other organisms as well as synthetic organic chemicals have been tested for antiviral efficiency against to the plant viruses. However, antiviral screening methods have disadvantages for checking activity because they need much time and space for growing host plant and simultaneously screening many antiviral chemicals. In this study, the developing antiviral screening system using plant virus-infected leaf discs was designed. Green fluorescent protein (GFP)- fusion plant viruses (TMV-GFP and PepMoV-Vb1/GFP) infected leaf discs were soaked with antiviral agent in the 24-well plate and virus reduction was simply checked with GFP signal under the UV light as well as by using traditional methods of RT-PCR and western blot. Several antiviral agents derived from bacteria, fungi and plant cells were tested with this system and it showed dramatic antiviral activity against to both TMV and PepMoV infected plants. This antiviral screening system could be efficiently utilized for speedy test of antiviral agents against to GFP-tagging plant virus or other organism-infected plant.

Poster Session

VIR-PM2072 - Involvement of coilin/Cajal bodies in tobravirus infection

Natalia Kalinina[1], Svetlana Makarova[2], Jane Shaw[3], Andrew Love[3], Maria Nartova[2], Valentin Makarov[1], Michael Taliansky[3]

[1]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia, [2]Biological Department, Lomonosov Moscow State University, Moscow, Russia, [3]The James Hutton Institute, Dundee, UK

Coilin/Cajal body deficiency in transgenic Nicotiana benthamiana plants, in which the expression of the coilin gene was inhibited by RNAi, intensifies infection with tobacco rattle virus (TRV, tobravirus) resulting in persistent severe systemic symptoms. Cajal bodies (CBs) are dynamic subnuclear compartments involved in the biogenesis of ribonucleoproteins. CBs are linked with the nucleolus, both on physical and functional levels. Coilin is a major structural scaffolding protein necessary for CB formation, composition and activity. We demonstrated that the viral 16K protein, a suppressor of post-transcriptional gene silencing, directly interacts with plant coilin in vitro. The sites of interaction were mapped within molecules of the viral and cellular proteins using a Far-Western assay. The 16K protein consists of two structural parts: an ordered N-terminal region containing two putative "zinc fingers", and a C-terminal region with two bipartite independent nuclear localization signals (NLSs) (Ghazala et al., 2008). The site of the interaction was mapped to a region within the structured N-terminal part of the 16K protein (amino acid residues 40-60). On the contrary, in the case of plant coilin, the interaction site was mapped within a region in the central, fully-intrinsically disordered domain of coilin from 194 to 211 amino acid residues. This region contains a putative nucleolar localization signal (NoLS) (Hebert, Matera, 2000). An increasing number of reports reveal that CBs are involved in the cellular response to different types of stress including viral infections (Boulon et al., 2010). Our data suggest that coilin/Cajal bodies could take part in plant defence mechanisms in the response to tobravirus infection and direct the interaction between coilin and the viral 16K protein, thus triggering a plant response that suppresses host susceptibility to the virus. This work was supported by RFBR, the grant 13-04-01467a.

Poster Session

VIR-PM2074 - Translation repression and P-body components are involved in induced defenses against viruses in plants

Peter Moffett[1], Louis-Valentin Meteignier[1], Ji Zhou[2], Silke Robatzek[2], Saikat Bhattacharjee[3], Mohamed El Oirdi[1], Chantal Brosseau[1]

[1]Département de Biologie, Université de Sherbrooke, Québec, Canada, [2]The Sainsbury Laboratory, Norwich Research Park, Norwich,UK, [3]Regional Centre for Biotechnology, 180, Udyog Vihar Phase I, Gurgaon, India

One branch of plant immunity is conferred through NB-LRR proteins, which recognize specific proteins encoded by pathogens, including viruses. NB-LRR proteins show structural and functional similarities to mammalian NOD-like receptors (NLRs) and their activation results in the induction of multiple signaling pathways. Despite a great deal of investigation, little is known about how these signaling pathways result in the clearing of pathogens, particularly viruses. To address this, we have studied the fate of Potato virus X (PVX) RNAs upon NB-LRR activation using a transient and inducible expression system. Using reporter genes and polysome analysis we have shown that NB-LRR responses lead to viral RNAs being precluded from interacting with ribosomes. However, viral RNAs are not immediately degraded and translation in the host cells is not affected globally, indicating a specific repression of viral RNAs at the level of translation. RNAs repressed at the translational level are often shunted to RNA processing bodies (PBs) where, in many cases, they are eventually degraded. We have used a YFP-tagged version of the PB component Decapping1 (YFP-DCP1) to investigate PB dynamics upon NB-LRR activation. Using a microscopy-based PB quantification assay, we find that NB-LRR activation induces a robust increase in the number of PBs present in the cell, but only in the presence of virus, suggesting that PB formation is induced in response to large amounts of translationally repressed viral RNA. At the same time, we also show that knocking down the expression of PB components results in an enhanced NB-LRR-mediated response. Consistent with this, we find that PB knock-down results in higher expression levels of several defense-related genes, suggesting that these genes' translation may be constitutively repressed. Together, our results suggest that translational repression and PBs may play roles at multiple levels in defense against viruses.

Poster Session

VIR-PM2076 - Flavivirus replication and peroxisome biogenesis: implications for antiviral signaling

Shangmei Hou[1], Jae H. You[1], Natasha Malik[2], Lori Frappier[2], Tom C. Hobman[1]
[1]Department of Cell Biology, University of Alberta, Edmonton, Canada, [2]Department of Molecular Genetics, University of Toronto, Toronto, Canada

Flaviviruses comprise a large family of positive sense, single-stranded RNA viruses that are transmitted mainly through arthropods. Every year they infect hundreds of millions of people resulting in enormous economic and health burden. Unfortunately, there are very few vaccine and therapeutic options for these viruses. Our laboratory studies virus-host interactions at the cellular level with the goal of identifying novel anti-viral targets. Much of our research has focused on flavivirus capsid proteins, which are small structural components of the virus functioning to protect the viral genome. Recent studies indicate that these proteins also perform essential nonstructural functions. A proteomic screen identified the peroxisome biogenesis factor, Pex19p as a host protein that interacts with the capsid proteins of West Nile virus (WNV) and Dengue virus (DENV). Peroxisomes are well known for their roles in lipid metabolism but recent evidence indicates that these organelles also serve as signaling platforms for early antiviral defense. We hypothesize that capsid-mediated interference with peroxisome function is important for efficient virus replication. Our microscopic analysis shows that infection of cells with WNV or DENV leads to a significant reduction in peroxisome numbers. This could be a result of interference in the peroxisome biogenesis pathways and/or acceleration in the peroxisome degradation process. To explore the former possibility, we examined the localization of several Pex19p-dependent peroxisomal member proteins such as Pex3p and Pex16p and determine whether the interaction between Pex19p and capsid proteins affect the function of this host factor. In addition, we are investigating whether flavivirus infection and/or capsid expression affects the turnover of peroxisomes, through autophagy. In short, our study will provide insight into how flavivirus employ novel countermeasures against host cell antiviral signaling and/or exploitation of cellular energy sources.

Poster Session

VIR-PM2078 - The phylogeny and common structural features of the right-hand-shaped polymerases

Heli Mönttinen[1], Janne Ravantti[1], Minna Poranen[1]
[1]*University of Helsinki, Helsinki, Finland*

Polymerases are essential for life via the preservation of genetic information by replication and repair processes as well as the expression of genes by catalyzing transcription. Polymerases having a right-hand-shaped fold and the catalytic site structurally similar to Pol I of Escherichia coli are classified into three DNA-dependent DNA polymerase families, RNA-dependent RNA polymerases, single-subunit DNA-dependent RNA polymerases and reverse transcriptases. These polymerases are found from archaea, bacteria, eukaryotes and their viruses. We have applied a novel bioinformatics tool (Homologous Structure Finder; HSF) to identify structurally common features and phylogenetic relationships among this diverse superfamily of polymerases. The polymerases were automatically clustered based on the structural alignments. We identified common structural cores for all right-handed polymerases as well as for four main clusters of polymerases. Moreover, based on these cores, we constructed the phylogenetic trees separately for each main clusters.

Poster Session

VIR-PM2080 - Development of universal vaccines against H5N1 influenza based on different forms of M2 vaccines and their combinations with HA vaccines

Bojian Zheng[1], Ho-Chuen Leung[1], Kwok-Yung Yuen[1]
[1]*Department of Microbiology, the University of Hong Kong, Hong Kong*

In this study, we aims to develop universal vaccine based on different forms of M2 vaccine candidates and their combinations with HA against H5N1 infection. We designed and prepared mM2e (synthetic monomer M2e peptide), M2e-MAP (tetra-branched multiple antigen peptide), fusion protein M2e-ASP-1 (1 M2e fused with ASP-1) and M2e3-ASP1 (3 M2e fused with ASP-1), DNA-M2, rAAV-M2, DNA-HA, rAAV-HA, protein HA and inactivated virus vaccine. We tested immune responses and protective effects of these vaccine candidates, as well as effects of different aduvants. Our results showed that (1) M2e-MAP, M2e-SAP-1 and M2e3-ASP-1 elicited much stronger M2e-specific antibody responses than M2e, DNA-M2 and rAAV-M2; (2) M2e-MAP and M2e3-ASP-1 provided better protection and cross-protection against viral challenges of different clades/strains of H5N1 virus and H1N1 virus than M2e-ASP-1, whereas mM2e, DNA-M2 and rAAV-M2 did not show obvious protection against viral challenge of H5N1 virus; (3) consistently, the mice vaccinated with M2e-MAP and M2e3-ASP-1 showed much lower viral load detected and less inflammation found in lung tissues; (4) cross-protective effect of M2e-based vaccines might be related to the identity of the M2e sequence between the vaccine and the H5N1 virus; (5) rAAV-HA, DNA-HA and inactivated virus vaccine provided effective protection against infection of homologous H5N1 virus, whereas these vaccine candidates and protein HA provided poor cross-protections against heterologous H5N1 viral infection; and (6) combination vaccinations with M2e-based and HA-based vaccine candidates, particularly vaccination of both M2e-MAP and inactivated virus vaccine, provided potent cross-protection against challenges with heterologous strains of H5N1 virus. Taken together, this study has demonstrated that both M2e-MAP and M2e3-ASP-1 can provide potent cross-protection against infections of some heterologous strains of H5N1 virus and H1N1 virus, while the strategy of combination vaccinations with both HA-based and M2e-based vaccines may be able to improve the cross-protection against heterologous H5N1 virus infection.

Poster Session

VIR-PM2082 - Induction of robust immunity by the emulsification of recombinant lipoproteins

Hsin-Wei Chen[1, 2], Chen-Yi Chiang[1], Ming-Hsi Huang[1], Chien-Hsiung Pan[1, 2], Chun-Hsiang Hsieh[1], Mei-Yu Chen[1], Hsueh-Hung Liu[1], Shih-Jen Liu[1, 2], Pele Chong[1, 2], Chih-Hsiang Leng[1, 2]

[1]*National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli, Taiwan, ROC.,*
[2]*Graduate Institute of Immunology, China Medical University, Taichung, Taiwan, ROC.*

Improving vaccine efficacy is a key goal in the development of modern subunit vaccines. Many attempts have focused on the use of either immunomodulators or antigen delivery systems to achieve this goal. Here, we report a novel approach that can enhance antigen association and induce robust immunity. We expressed either a recombinant lipidated dengue-1 envelope protein domain III (LD1ED III) or a recombinant non-lipidated dengue-1 envelope protein domain III (D1ED III) in an E. coli system. The LD1ED III contains a bacterial lipid moiety, which is a potent immunomodulator. We demonstrated that LD1ED III possesses an inherent immunostimulation ability that can activate RAW 264.7 macrophage cells by up-regulating their expression of CD40, CD80, CD83, CD86 and MHC II, whereas D1ED III could not induce the up-regulation of these molecules. Moreover, combining LD1ED III with a multiphase emulsion system (called PELC) increased the antigen association more than either combining D1ED III with PELC or the antigen alone. Enhanced antigen association has been shown to correlate with stronger T cell responses, greater antibody avidity and improved neutralizing capacity. Our results demonstrate that combining recombinant lipoproteins with PELC improved both the intensity and the quality of the immune response. This approach is a promising strategy for the development of subunit vaccines that induce robust immunity.

Poster Session

VIR-PM2084 - Development of mammalian orthoreovirus as a viral vaccine vector to display a conserved neutralizing epitope of Influenza Virus Hemagglutinin

Sarah Katen[1], Jason Iskarpatyoti[1], Mine Ikizler[1], Karl Boehme[2], Terence Dermody[1]
[1]Vanderbilt University School of Medicine, Nashville, USA, [2]University of Arkansas for Medical Sciences, Little Rock, USA

Vaccine prophylaxis is the most effective method of combating viral diseases. However, many viral pathogens have been refractory to the development of successful vaccines. Human influenza, responsible for 250,000 deaths annually, does not have a universal, single-dose vaccine. The influenza hemagglutinin (HA), which functions in viral attachment and fusion, is the primary binding site of neutralizing antibodies. Current influenza vaccines elicit responses to the highly variable head domain (HA1) of HA, necessitating repeated vaccination. However, antibodies to the conserved stalk domain (HA2) are broadly neutralizing across many influenza subtypes, making this an ideal target for a universal vaccine-induced immune response. Neutralizing stalk antibodies bind to a short alpha-helical segment of HA2 and make contacts with HA1 and conserved residues in the HA fusion loop. We have successfully recapitulated the broadly neutralizing HA epitope into a replication-competent reovirus vector to serve as a potential vaccine to induce broadly neutralizing immune responses. The conserved HA2 epitope, consisting of HA residues 40-57, has been inserted into both the alpha-helical tail and beta-spiral body domains of reovirus attachment protein sigma1. The incorporation of an arginine-to-tryptophan mutation at residue 43 of the HA epitope replicated the fusion loop contact, allowing stalk-specific anti-HA antibodies to bind the epitope. The insertions have minimal effects on reovirus replication and are stable over ten passages. This vector successfully displays the target epitope for a universal influenza vaccine and is currently being tested in mice for generation of influenza-specific immune responses. Funding: NIH R01 AI076983 ChIRP T32 AI095202

Poster Session

VIR-PM2086 - Optimizing foreign gene expression in Fowl Adenovirus 9 (FAdV-9) vectors

James Ackford[1], Peter Krell[2], Gregoy Bedecarrats[3], Eva Nagy[1]
[1]Department of Pathobiology, University of Guelph, Guelph, Canada, [2]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Canada, [3]Department of Animal and Poultry Science, University of Guelph, Guelph, Canada

Fowl adenoviruses (FAdVs) are ubiquitous amongst poultry and have been implicated in diseases including inclusion body hepatitis (IBH). These viruses demonstrate various pathogenicity and contain large dsDNA genomes that are capable of accommodating transgenes, making FAdVs excellent vectors for viral gene expression. We are currently developing recombinant FAdVs for use in vaccine delivery and gene therapy, specifically by using a non-pathogenic FAdV-9 strain. Previously, open reading frames at the left end of the FAdV-9 genome were studied identifying a 2.4 kb non-essential region that can be used to generate recombinant FAdVs (recFAdVs). However, while experiments have demonstrated that transgene specific antibodies (Ab) are produced following intramuscular vaccination of chickens with recFAdVs, optimizing the host immune response generated by these recFAdVs remains a priority. One way to improve Ab production induced by the recFAdVs is to increase foreign gene expression and protein production by optimizing the expression cassette. Therefore this study aims to test various high expression promoters and enhancer elements to improve levels of transgene expression in recFAdVs both in vitro and in vivo. Expression constructs were made using the vector pCI-Neo. Five promoters are studied: the immediate early cytomegalovirus (CMV) promoter, the artificial chicken β-actin/CMV early enhancer (CAG) promoter, the human elongation factor 1α (EF-1α), the chicken β-actin promoter, and fowlpox derived L2R promoter. Promoter activity is compared by measuring transient expression of enhanced green fluorescent protein (EGFP) in transfected chicken liver (CH-SAH) cells at different times post-transfection. Data suggests that the CMV and CAG promoters provided the highest levels of expression in CH-SAH cells compared to the EF-1α, chicken β-actin, and L2R promoters.

Poster Session

VIR-PM2088 - Successful mass production of artificial virus-like particle vaccine in the pupae of Bombyx mori: The design of c-DNA of avian influenza H5 hemagglutinin

Kuniaki Nerome[1], Shigeo Sugita[2], Kazumichi Kuroda[3], Toshiharu Hirose[1], Sayaka Matsuda[1], Kei Majima[4], Kazunori Kawasaki[5], Okti Nadia Poetri[6], Retno D Sorjoedono[6], Reiko Nerome[1]

[1]The Institute of Biological Resources (IBR), Okinawa, Japan, [2]Equine Research Institute, Japan Racing Association, Tochigi, Japan, [3]Division of Microbioogy, Nihon University, School of Medicine, Tokyo, Japan, [4]Baculotechnologies Co.,Ltd., Kagawa, Japan, [5]National Institute of Advanced Science and Technology (AIST), Osaka, Japan, [6]Faculty of Veterinary Medicine, Bogor Agicultural University IPB, Bogor, Indonesia

Highly pathogenic avian influenza virus H5N1 strain is still one of the great health threats to human. In order to develop the efficient vaccine to prevent the threat, we have established an epochal vaccine production system. We selected A/tufted duck/Fukushima/16/2011as a vaccine strain, which belonged to subclade 2.3. As the viruses in the clade spread out form one country to another, and it should be important target for vaccine production.. A recombinant baculovirus containing synthetic chimera H5N1 HA gene optimized for codon usage in Bombyx mori was inoculated to pupae. At 4 days after inoculation, HA titer per pupa was reached approx. one million, indicating 100 times higher than that of an embryonated chicken egg infected with influenza virus. In the homogenates of pupae, the existence of polymorphic virus-like particles (VLP), ranging from approximately 30 nm to 300 nm in diameter, was observed by electron microscope. VLPs were encircled with high dense 14 nm-long which spikes apparently similar to HA spikes of authentic influenza virus. It was the first report showing that sole HA gene could produce larger amounts of VLPs. It was also evident that VLPs consisted of phospholipid and hemagglutinin molecules. To examine the immunogenicity of the HA proteins, a chicken was immunized with the homogenate of the pupae and HI titer in its serum were determined. The titer was reached 8,192 after one month immunization. The efficient formation of VLPs should be the cause of the high immune response. The resultant HI antibody reacted with epizootic H5N1 viruses and also inhibited plaque formation by avian influenza virus. On the basis of low protein content per 1 HA unit and its conspicuous production, present study strongly suggests that high quality influenza VLP vaccine can be provided at low cost in near future.

Poster Session

VIR-PM2090 - Papaya mosaic virus-like particles harboring a fusion of a peptide epitope from the ectodomain of Influenza A matrix protein 2 as a universal flu vaccine.

Damien Carignan[1], Geneviève Paquet[1], Gervais Rioux[1], Marie-Eve Laliberté-Gagné[1], Marilène Bolduc[1], Denis Leclerc[1]

[1]*Infectiology Research Centre, Quebec, Canada*

The recent emergence of highly virulent influenza strains and the consequent risks of pandemics call for an urgent need of influenza vaccines that are broadly cross-protective. The ectodomain of matrix protein 2 (M2e) is highly conserved amongst all human influenza strains and could be used as a universal antigen. To overcome its low immunogenicity we have fused a peptide epitope from M2e to the N-terminus of papaya mosaic virus coat protein that were assembled around a single stranded RNA into virus-like particles (PapMV-M2e). A single immunization with PapMV-M2e was sufficient to mount appreciable levels of serum M2e specific IgG and IgG2a antibody responses. A booster immunization is thought as likely to generate protective levels of anti-M2e antibodies. PapMV-M2e, when co-immunized with trivalent inactivated flu vaccine (TIV), substantially increased TIV-specific IgG and IgG2a antibody titers to levels identical to those obtained with PapMV, indicating that PapMV-M2e can also act as an adjuvant to seasonal influenza vaccines. These results show that PapMV-M2e virus-like particles are able to induce M2e specific antibody responses and improve seasonal influenza vaccines by providing them a universal antigen. It is the first demonstration that a PapMV-based vaccine platform can also act as an adjuvant. PapMV-M2e could thus be an important component of a universal influenza vaccine.

Poster Session

VIR-PM2092 - Virus-like partcles as vaccine for Paramyxoviruses

Pramila Walpita[1], Lisa Johns[1], Brian Gilbert[2]
[1]University of Hawaii Schoold of Medicine, Honolulu, USA, [2]Baylor College of Medicine, Houston, USA

Human use vaccines are available for only two of the many highly pathogenic paramyxoviruses, namely for measles and mumps. For several others, such as human respiratory syncytial virus (hRSV), human metapneumovirus (hMPV), human parainfluenza virus 3 (hPIV3), and the highly lethal zoonotic Nipah virus (NiV), vaccines are much needed. We are evaluating VLP based vaccines for two of these paramyxoviruses, NiV and RSV. We have generated NiV virus-like particles (NiV-VLPs) and RSV VLPs in mammalian cells, each composed of the two surface glycoproteins G and F, and the matrix protein M, all retaining their native properties. We have previously reported the vaccine potential of NiV VLPs (Walpita et al., PLoS One, 2011). Evaluation of its protective efficacy in the ferret model of NiV disease is currently in progress. Likewise, we have tested the vaccine potential of RSV VLPs in vitro. The results of Western blot analysis and immunogold-labelling technique confirmed 1), that all the three proteins, i.e., the two surface glycoproteins and the matrix proteins were incorporated in the VLPs; 2), that the recombinantly expressed F protein was cleaved intracellularly, similarly to the virus expressed F protein, to generate fusion competent F1, and F2 subunits; and 3), that the VLPs were functionally assembled and immunoreactive. The protective efficacy of the RSV VLPs was tested in the cotton rat model of RSV disease. Results showed protective immunity in the respiratory tract of the animals.

Poster Session

VIR-PM2094 - Generation and characterization of live attenuated influenza A(H7N9) candidate vaccine viruses

Svetlana Shcherbik[1,2], Nicholas Pearce[1,2], Amanda Balish[1], Charles T Davis[1], David Cureton[1,3], Guaniri Mateu-Petit[1,3], Li-Mei Chen[1], Melissa B Pearce[1], Terrence Tumpey[1], Julie Villanueva[1], Tatiana Bousse[1]
[1]Centers for Disease Control and Prevention, Atlanta, USA, [2]Battelle, Atlanta, USA, [3]Atlanta Research and Education Foundation, Atlanta, USA

Avian influenza A (H7N9) virus has emerged recently and it continues to cause severe disease with a high mortality rate in humans, prompting the development of candidate vaccines viruses. Live attenuated influenza vaccines (LAIV) are 6:2 reassortant viruses containing the HA and NA gene segments from wild type influenza viruses to induce protective immune responses and the six internal gene segments from Master Donor Viruses (MDV), to provide temperature sensitive, cold-adapted and attenuated phenotypes. LAIVs based on the Russian MDV strain, A/Leningrad/134/17/57 (H2N2), have been generated for distribution to vaccine manufacturers in developing countries, according to WHO recommendations. A/Anhui/1/2013(H7N9)-CDC-LAIV7A and H7N9-RG-LAIV candidate viruses were generated by classical re-assortment in eggs and by reverse genetics, respectively. Vaccine candidates produced by both methods retained MDV temperature-sensitive and cold-adapted phenotypes. The sequence analysis of A/Anhui/1/2013(H7N9)-CDC-LAIV7A revealed two egg-adapted amino acid substitutions in HA - N123D and N149D (H7 numbering) and one substitution in NA (T10I). These changes enhanced the replicative capacity of the virus in eggs, but did not alter virus antigenicity, as ferret antiserum to this vaccine candidate inhibited hemagglutination by homologous H7N9 virus efficiently. Safety studies in ferrets confirmed that A/Anhui/1/2013 (H7N9)-CDC-LAIV7A was attenuated compared to wild-type A/Anhui/1/2013. In addition, the genetic stability of this vaccine candidate was examined in eggs and ferrets by monitoring sequence changes acquired during virus replication in the two host models. No changes in the viral genome were detected after five passages in eggs. However, after ten passages, additional mutations were detected in the HA gene. The vaccine candidate was shown to be stable in a ferret model; post-vaccination sequence data analysis showed no changes in viruses collected in nasal washes present at day 5 or day 7. The data indicate that the A/Anhui/1/2013(H7N9)-CDC-LAIV7A reassortant virus is a safe and stable candidate vaccine virus.

Poster Session

VIR-PM2096 - Identification of new viroid-like circular RNAs from grapevine and apple plants by deep sequencing

Zhixiang Zhang[1], Shuishui Qi[2], Shanshan Chen[1], Shouwei Ding[3], Qingfa Wu[2], Shifang Li[1]
[1]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, [2]University of Science and Technology of China, Hefei, China, [3]Institute for Integrative Genome Biology, University of California, Riverside, USA

Identification of pathogens by assembly of small RNAs of deep sequencing has become a routine diagnostic tool in plant pathology. This strategy is mainly dependent on sequence information. Thus, discovery of pathogens independent of known sequences is still a challenging task. In previous study, a computation program, PFOR (progressive filtering of overlapping small RNAs), has been developed to identify circular RNA including viroids by deep sequencing independent of homology. Here, we developed an improved version (PFOR2) and validated it using small RNAs from a grapevine and apple trees. Using this improved program with 3~8 timers higher speed on the tested data, we successful identified two viroid-like RNAs from grapevine and apple trees. The circular RNA from grapevine may be a new species of the genus Apscaviroid because of the following characteristics: i) it forms a predicted rod-like secondary structure and the metastable structures hairpin I and II; ii) it has the central conserved and the terminal conserved regions, the characteristic of members of the genus Apscaviroid; iii) it only shows a maximum of 79% nucleotide sequence identity with other kown viroids, which is far below the main species demarcation limit of 90%. The sencond circular RNA detected in apple plant consists of 434 nucleotides. It has conserved nucleotides of hammer head ribozyme and can self-cleave in both plus and minus strand, indicating presence of a new viroid of Avsunviroidae or a new satellite RNA. Furthermore, Identification of the new circular RNA in apple confirmed the presence of ASSD-RNA-2 purified from apple with apple scar skin disease thirty years ago. In a word, PFOR2 is a powerful tool to further understand the world of circular RNAs which is proving to play key roles in regulation of gene expression.

Poster Session

VIR-PM2098 - Human mammary tumor virus (HMTV) sequences in human milk

Teiko Nartey[1], Stella Melana[1], Heberth Moran[1], Tania Marin[1], Kathleen Arcaro[2], Douglas Anderton[3], Polly Etkind[1], James F. Holland[1], Beatriz GT Pogo[1]

[1]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai Hospital, New York, USA, [2]Department of Veterinary and Animal Sciences, University of Massachusetts at Amherst, Amherst, USA, [3]Social and Demographic Research Department, University of Massachsetts at Amherst, Amherst, USA

Retroviral sequences 90-95% homologous to the mouse mammary virus (MMTV) were detected in 38% of American women's breast cancers (BC) but were not detectable in non-BC tissue from the same breast. The sequences of the entire provirus structure and viral particles have been isolated and designated as human mammary tumor virus (HMTV). Hormone response elements are present in HMTV-LTR suggesting an association between HMTV and hormonally-responsive tissues. The incidence of HMTV sequences is higher in gestational BC which is associated with hormonal fluxes. Epithelial cells from milk are also under hormonal regulation and therefore provide excellent specimens to search for HMTV sequences. Milk samples from 92 healthy women (Reference Group-RG) and from 73 women who had breast biopsies (Biopsy Group-BG), were studied. HMTV-env gene sequences were detected by PCR in milk genomic-DNA of 7.6% women of the RG and in 20.6% of the BG (p: 0.015). The sequences were 90-95% homologous to MMTV. HMTV-env gene and HMTVenv-LTR junction sequences were detected in high-speed pellet RNA, implying the presence of HMTV viral particles. PCR assays to detect murine mitochondrial cytochrome oxidase gene and intracisternal A-type particle sequences were performed to rule out murine DNA contamination. Eight women in the BG had BC and the breast tumor of one of these eight women contained HMTV sequences. In the remaining 65 women of the BG, HMTV was detected in three times the number of women as compared to the RG (21.5% versus 7.6%; p: 0.016). The significance of HMTV in milk from healthy women, the greater frequency in the milk of women in the BG and its possible infectivity for infants are important questions under study. The similarity between HMTV and MMTV is striking and suggests one possible avenue for viral transmission.

Poster Session

VIR-PM2100 - An efficient method for extracting nucleic acids from marine microbial communities

Jaclyn Mueller[1], Alexander Culley[2], Martyne Audet[2], Grieg Steward[1]
[1]University of Hawaii at Manoa, Honolulu, USA, [2]Université Laval, Québec, Canada

Anodisc aluminum oxide (AAO) filters have high porosity and can be manufactured with a pore size that is small enough to quantitatively capture viruses. These properties make the filters potentially useful for harvesting total microbial communities from water samples for molecular analyses, but their performance for DNA and RNA extraction has not been systematically or quantitatively evaluated. In this study, we used isolates (a virus, a bacterium, and a protist) and natural seawater samples to test variables that we expected would influence the efficiency with which nucleic acids are recovered from nanoporous (0.02µm) AAO filters (Anotop, Whatman). Extraction chemistry had a significant effect on DNA yield, and back flushing the filters during extraction was found to improve yields of high molecular weight DNA. Using the preferred protocol, the mass of DNA recovered from microorganisms collected on AAO filters was ≥ 100% of that extracted from pellets of cells and viruses, and 93 (±10)% of that obtained by direct extraction of a liquid bacterial culture. These latter values are minimum estimates of the relative recovery of microbial DNA and RNA, since liquid cultures include dissolved nucleic acids that are retained inefficiently by the filter. In conclusion, we demonstrate that DNA and RNA can be extracted from microorganisms on AAO filters with similar efficiency to that achievable by direct extraction of microbes in suspension or in pellets. These filters are therefore a convenient means by which to harvest total microbial communities from multiple aqueous samples in parallel for subsequent molecular analyses.

Poster Session

VIR-PM2102 - RING finger protein 43(RNF43), a novel interactor of Influenza A virus Nucleoprotein, abates NP driven P53 mediated apoptosis and increases cell survival

Himani Nailwal[1], Shipra Sharma[1], Adarsh Kumar Mayank[1], Komal Kamra[2], Sunil Kumar Lal[1]
[1]International Centre for Genetic Engineering and Biotechnology, Delhi, India, [2]Department of Zoology, Delhi University, Delhi, India

Influenza A virus (IAV) is known to induce apoptosis in infected cells for efficient replication and pathogenesis. Recent developments have indicated that the virus strategizes stabilization and accumulation of p53 through Nucleoprotein (NP) in the infected host cell and hence causes cell death. Deciphering the host proteins that are of importance to influenza A virus for carrying out p53 mediated apoptosis can provide insights into the mechanistic details of this process. In this study, using yeast two hybrid technique, we have identified RING finger protein 43 (RNF 43) as a novel putative interacting partner for NP. This interaction was further confirmed with co-immunoprecipitation of NP with RNF43 in IAV infected epithelial cells, A549. Also, through Immunofluorescence assay, RNF43 and NP were found to co-localize in the nucleus at different time points after infection. In the present study, we have demonstrated that the RNF43 transcripts and its respective protein levels were progressively reduced with increase in time duration of IAV infection and dose in A549 cells. Moreover, ectopic expression of RNF43 in HEK293T cells led to a decrease in NP mRNA and vRNA levels. Importantly, we found that RNF43, which interacts with p53, abates NP driven increase in P53 transcriptional activity and accumulation by decreasing its half life in the cells which finally leads to a decline of Bax and PUMA, downstream effectors of p53 mediated apoptosis, in both IAV and NP microenvironment in A549 cells. Furthermore, a radical decline in cellular apoptosis analyzed through FACS based Annexin V and 7AAD staining substantiates the anti apoptotic role of RNF43 in IAV infected and NP transfected A549 cells. Collectively, our study explains the mechanism by which NP targets the host protein, RNF43, to exploit p53 mediated apoptosis pathway for efficient viral replication.

Poster Session

VIR-PM2104 - HCV Hijack the Nuclear Transport Mechanism to Form a Selective Barrier in the Membranous Web

Aviad Levin[1,2,3], Christopher J. Neufeldt[1,3], Daniel Pang[1,2], Kristen Wilson[1,2], Michael A. Joyce[1,2], Richard W. Wozniak[1,3], D. Lorne J. Tyrrell[1,2]

[1]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Canada, [2]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada, [3]Department of Cell Biology, University of Alberta, Edmonton, Canada

Background: Hepatitis C virus (HCV) is a positive strand RNA virus of the flaviviridae family and a blood borne pathogen with 170-200 million chronic carriers worldwide. It is a major cause of liver disease. HCV known to replicate in the cytoplasm of infected cells, and it is able to rearrange the endoplasmic reticulum (ER) and other host cell membranes into a structure called the membranous web. This membranous web rearrangement was recently proposed to be a virally encoded organelle which is a separate compartment from the cytoplasm. Recently we reported that HCV recruits nuclear pore proteins to the viral replication site [1]. These observations had led to this study which shows that HCV proteins utilize the nuclear transport system for selective transport to the membranous web, the site of virus replication and assembly. Results: We identified and characterized 8 potential nuclear localization signals (NLS) and 3 nuclear export signals (NES) in HCV proteins. We demonstrated that these sequences are able to bind specific nuclear transport factors and are sufficient to mediate transport of a reporter protein into the nucleus and mediate selective transport of the reporter into the membranous web of HCV-infected cells. We have also showed that the HCV proteins containing these sequences are able to interact with nuclear transport factors in-vitro and in-vivo. Moreover, we illustrate the time points at which these interactions are actively involved in the HCV life cycle. Conclusion: We propose that the HCV, a virus that replicate in the cytoplasm, hijacks the nuclear transport mechanisms to allow selective transport into the membranous web. Reference: 1. Neufeldt CJ, Joyce MA, Levin A, Steenbergen RH, Pang D, et al. (2013) Hepatitis C virus-induced cytoplasmic organelles use the nuclear transport machinery to establish an environment conducive to virus replication. PLoS Pathog 9: e1003744.

Poster Session

VIR-PM2106 - Sequential Immunization induces broad cross-neutralizing antibody responses across HRV types

Tim Tibbitts[1], Yanhua Yan[1], Wen Cheng[1], Lihua Shen[1], Victor Costa[1], Jacqueline Cieszynski[1], Raymond Oomen[1], Jeffry Almond[1], Harry Kleanthous[1], Kirill Kalnin[1]

[1]*Sanofi Pasteur, Cambridge, USA*

Although there is accumulating evidence suggesting that as many as 40 to 60% of acute exacerbations of asthma and COPD are associated with infection with Rhinovirus types ABC (HRV-ABC),the development of universal vaccines against HRV is not considered feasible due to hypervariability of the major neutralizing domains (NIms) s. Re-targeting of the pre-existing immune response from the NIms to more conserved, broadly cross-protective determinants (CPD) following sequential immunization with several antigenically distinct HRV types might be a strategy for reducing vaccine valency. Here we report that sequential immunization of guinea pigs with the three antigenically distinct HRVB serotypes 3, 37 and 92 elicit broadly cross-neutralizing responses in serum across a great proportion of the HRV B serogroup. Epitope mapping of the P1 structural domain revealed that serum from sequentially immunized animals recognized several CPD epitopes which mapped to the capsid surface of a modeled Rhinovirus VLP representing HPV86. Preliminary screening of sera from normal healthy adults demonstrated similar anti-HRV neutralizing responses as induced through sequential immunization in rodents. Rationalizing antigen design to make broadly cross-neutralizing vaccines will be discussed.

Poster Session

VIR-PM2108 - Seroconversion and seroprotection after hepatitis-B vaccination administered via subcutaneous route in patients with haemophilia

KS Mhamime[1], Naresh Gupta[1]
[1]*Maulana Azad Medical College, New Delhi, India*

Primary vaccination against hepatitis-B virus administered by the standard intramuscularly may produce local haematoma in patients with bleeding disorders like haemophilia. Objective This study assesses the efficacy of subcutaneous route for safer vaccination in patients with haemophilia. Methods Thirty HBsAg-ve male patients with moderate/ severe haemophilia were administered three standard doses of recombinant hepatitis-B vaccine subcutaneously on day 0, 30, and 180 and serum levels of anti-HBs, anti-HBc(total), and ALT were assayed one month after each dose of vaccine in this study over 14 months period. Thirty controls (non-bleeding) received them via standard intramuscular route. Seroconversion and seroprotection were defined by anti-HBs titres, more than ≥1mIU/ml and ≥10mIU/ml respectively. Results Age of the cases ranged 1½ -52 years, mean 12.85 ± 10.6 amongst 30 haemophilia patients, 28 haemophilia-A and 2 haemophilia-B. Five of the cases received two doses only. Seroconversion rates one month after 1st, 2nd, and 3rd dose were 76%, 100%, and 100% respectively as against the rate of 100% after first dose itself in control group. Corresponding seroprotection rates were 56%, 95%, and 100% amongst the cases versus 40%, 97%, and 100% amongst the controls respectively. Geometric mean titres (GMT) of anti-HBc were not statistically different between the cases and control during the three anti-HBc assays. All 25 cases and 30 controls achieved 100% seroprotection after 3rd dose, with anti-HBc GMT of 706 and 650 mIU/ml respectively. None of the cases or controls reported any side effects except one case died in road accident after two doses. Conclusions Subcutaneous route of hepatitis-B vaccination in haemophilia using standard dose recombinant vaccine achieves full seroconversion and full seroprotection, and is similar to the conventional intramuscular route. Subcutaneous route is safe in patients of bleeding disorders.

Workshop Sessions

BAM-WK100.01 - New Insights into pathogenomics of the tuberculosis agent

Roland Brosch[1]
[1]*Institut Pasteur, Unit for Integrated Mycobacterial Pathogenomics, Paris, France*

Global spread and restricted genetic variation are hallmarks of Mycobacterium tuberculosis, the agent of human tuberculosis. In contrast, Mycobacterium canettii strains that also cause human tuberculosis and exhibit unusual smooth colony morphology are very rare and almost exclusively found in patiens from or with contact to the geographical region of the Horn of Africa. We recently sequenced and analyzed the genomes of several representative strains of smooth tubercle bacilli. This analysis showed that these M. canettii isolates are evolutionarily early branching and recombinogenic, with larger genome sizes, higher rates of genetic variation, fewer molecular scars and distinct CRISPR-Cas systems and prophages relative to M. tuberculosis. Mouse infection experiments showed that M. canettii strains were less persistent and less virulent than M. tuberculosis, which is in line with the overall epidemiological tuberculosis situation. Taken together, our data suggest that M. tuberculosis emerged from an ancestral M. canettii-like pool of mycobacteria in a geographical restricted area by gain of persistence and virulence mechanisms. Despite the differences, we also found that the tuberculosis-causing mycobacteria share a highly conserved core genome. Among the concerned gene clusters, the genomic locus encoding the ESX-1/type VII secretion system is absent from the attenuated BCG and Mycobacterium microti vaccine strains. This system and its secreted proteins, many of which carry conserved T-cell epitopes, are involved in the rupture of the phagosomal membrane of host-phagocytes and thus influence numerous cell biological parameters during infection. The presence or absence of ESX-I from certain strains determines the virulence and the immunological properties of a given strain. This knowledge is of importance for the search of potential virulence factors of M. tuberculosis and for the construction of better recombinant vaccines and diagnostic tools.

Workshop Sessions

BAM-WK100.02 - The role of epistasis in the evolution and epidemiology of multidrug-resistant tuberculosis

Sebastien Gagneux[1]
[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Multidrug-resistant tuberculosis (MDR-TB) is a global public health emergency. Although much is known about the mechanisms of resistance to the different anti-TB drugs, little is known on the evolutionary trajectory of Mycobacterium tuberculosis exposed to prolonged drug pressure and its impact on the spread of MDR-TB. Recent studies by our group and others suggest that this evolutionary trajectory is more complex than previously thought. In particular, epistatic interactions between drug resistance-conferring mutations, compensatory mutations and different strain genetic backgrounds seem to play an important role. For example, we recently identified a set of mutations in rpoA and rpoC of RNA polymerase of M. tuberculosis resistant to rifampicin. These mutations compensate for the fitness cost associated with the resistance causing mutations in rpoB. Moreover, using molecular epidemiological approaches, we and others have found that these compensatory mutations were associated with ongoing transmission of MDR-TB, indicating that compensatory evolution contributes to the success of highly drug-resistant strains of M. tuberculosis in countries with a high burden of MDR-TB. Similarly, using an M. smegmatis model, we observed sign epistasis between rpoB mutations causing resistance to rifampicin and mutations in gyrA conferring resistance to fluoroquinolones, indicating that mutations causing resistance to different drugs can compensate for each other's fitness costs. Intriguingly, the particular combinations of rpoB and gyrA mutations exhibiting the highest in vitro fitness were also the more common in clinical strains. Taken together, our findings suggest that epistasis is an important driver of MDR-TB epidemics, and that it should be considered in the development and deployment of new anti-TB drug regimens.

Workshop Sessions

BAM-WK100.03 - Disarming *Mycobacterium tuberculosis*: mechanisms of attenuation and protection of MTBVAC, a live attenuated vaccine in clinical trials

Carlos Martin[1]
[1]*University of Zaragoza, Spain*

Due to the failure of the current vaccine BCG to protect against the respiratory form of the disease, the development of a new tuberculosis vaccine is an urgent need. Live attenuated vaccine candidate MTBVAC is an attenuated Mycobacterium tuberculosis (MTB) genetically engineered to fulfil the Geneva consensus requirements to enter human clinical trials. We selected a MTB clinical isolate belonging to Euro-American lineage to generate two independent deletions without antibiotic-resistance markers. One is the phoP, essential for virulence in MTB where it controls expression of approximately 2% of the genes, including those for the ESX-1 secretion apparatus, a major virulence determinant and the secretion of the immunodominant Ag85 complex. Deletion in phoP gene lead to compromised production of pathogen-specific cell wall components and attenuation both ex vivo and in vivo. Deletion in fadD26, inactivate phthiocerol dimycocerosates (DIM) operon which, codes for the production of one of the major MTB virulence factors. MTBVAC vaccines are inefficient in inducing apoptosis and colonizing new cells, correlating with the strong attenuation profile of these strains previously observed in vitro and in vivo. MTBVAC exhibits safety and biodistribution profiles similar to BCG and confers superior protection in preclinical studies. A major difference between MTBVAC and BCG, which is derived from the cattle pathogen Mycobacterium bovis, is that BCG has many deletions in its genome when compared to MTB, and during the attenuation process BCG had lost over a hundred additional genes from its genome including immunodominant antigens. MTBVAC is the first live attenuated vaccine to enter clinical evaluation in Lausanne (Switzerland) with Prof Spertini as Principal Investigator and sponsored by Biofabri and TBVI (ClinicalTrials.gov: NCT02013245).

Workshop Sessions

BAM-WK100.04 - Elucidating cholesterol catabolism by mycobacterium tuberculosis

Israel Casabon[1], Kirstin Brown[1], Jie Liu[1], Adam Crowe[1], Jason Rogalski[1], Leonard Foster[1], Lindsay Eltis[1]
[1]The University of British Columbia, Vancouver, Canada

Mycobacterium tuberculosis (Mtb) remains a global threat. Understanding the pathogen's physiology will facilitate the development of urgently needed novel therapeutic strategies. A key aspect of Mtb's physiology is its ability to catabolize cholesterol, a major constituent of the granuloma and a critical nutrient during chronic infection. Although cholesterol catabolism is a potential target for novel therapeutics, many aspects of this catabolism remain unknown. In Mtb and related actinobacteria such as Rhodococcus jostii RHA1 (RHA1), the side chain and rings A/B of cholesterol are degraded to yield 3aα-H-4α(3'-propanoate)-7aβ-methylhexahydro-1,5-indanedione (HIP), which contains rings C/D. HIP catabolism appears to be largely specified by the KstR2 regulon, which includes fadD3, ipdAB, and Rv3553. These genes encode a HIP-CoA synthetase, a predicted CoA transferase and a flavoenzyme, respectively. All three enzymes are essential for cholesterol utilization by Mtb, but only ipdA appears to be essential for survival in infection models. In RHA1, deletion of ipdAB and ro04649, the ortholog of Rv3553, abrogated growth on HIP. Similar results were obtained by deleting two other KstR2 regulon genes (i.e. fadA6 and the ortholog of Rv3548c) in M. smegmatis. A ΔipdAB mutant of Mtb was similarly unable to use cholesterol or to grow on glycerol in the presence of cholesterol. Furthermore, ΔipdAB and Δro04649 mutants of RHA1 were unable to grow on pyruvate in the presence of HIP, suggesting that these strains accumulated bacterio-toxic metabolites derived from HIP. These phenotypes were complemented using the Mtb homologs. Using metabolite profiling, we identified a number of previously undescribed cholesterol-derived CoA thioesters in deletion mutants, including several ring C-opened metabolites. The metabolite profiles further indicated that Rv3553 acts upstream of IpdAB. Based on these results, we propose a model for HIP degradation in Mtb and related Actinobacteria.

Workshop Sessions

BAM-WK100.05 - Deciphering mycobacterial cell division: A new interaction partner of FtsZ in Mycobacterium smegmatis and M. tuberculosis

Miguel Vicente[1], Thomas Munder[2], Stefano Casonato[3], Riccardo Manganelli[3], Susanne Gola[1]
[1]Spanish National Biotechnology Centre CNB - CSIC, Madrid, Spain, [2]University of Applied Sciences, Jena, Germany, [3]University of Padua, Padua, Italy

We identified the SepF orthologue of Mycobacterium tuberculosis (Mtb) as a new interaction partner of FtsZMtb. Combined results from controlled overproduction, cellular localisation and analysis of conditional mutants suggest that SepF is essential for mycobacterial cell division and exerts its role through interaction with FtsZ. Bacterial division is initiated by the assembly of several proteins at the division site where they form a ring-like structure, the divisome. Divisome assembly depends on the correct placement of the essential FtsZ protein. FtsZ is the target of several inhibitory compounds, which proves the idea that bacterial division and, specifically, components of the divisome are druggable and that blocking this molecular structure can prevent proliferation. To identify mycobacterium-relevant components of the divisome, a Y2H screen was performed with FtsZMtb. One hit was identified as SepF, a protein involved in the division in other bacteria. The presence of the carboxyl terminus of FtsZMtb was critical for the interaction and cross-reactivity was found between FtsZMtb and SepFMsm. Depending on the presence of FtsZ, SepF-GFP fusions formed ring-like structures at potential division sites in M. tuberculosis and M. smegmatis. Alteration of sepF expression in M. smegmatis led to filamentous cells, indicating a division defect. Depletion of SepF resulted in a complete block of division and filaments lacking SepF did not contain septa. SepFMtb could complement the phenotype, suggesting that the proteins from both species are functionally equivalent. Divisome components are sufficiently conserved between slow and fast growing mycobacterial species and in this study we show that systematic analysis in M. smegmatis is useful to obtain valuable insights on the process of Mycobacteria division which could help to find new ways to block proliferation of the pathogen.

Workshop Sessions

BAM-WK101.01 - A journey from the genome to a broadly protective vaccine against meningococcus B

Mariagrazia Pizza[1]
[1]*Novartis Vaccines, Siena, Italy*

Most of the vaccines available today, although very effective, have been developed using conventional technologies. The vaccinology field is evolving very rapidly and the new available technologies have opened alternative ways in designing improved vaccines or novel vaccines against infections for which preventive measures do not exist. In this context, a new approach named “Reverse Vaccinology”, and based on the identification of novel antigens through bioinformatics analysis of a bacterial genome, has been instrumental to the identification of a vaccine against Neisseria meningitidis serogroup B, a bacterium causing a devastating disease characterized by meningitis and sepsis. The most promising antigens identified by this revolutionary technology are the basis for the first broadly protective meningococcal serogroup B vaccine, which has been recently approved in Europe, Australia and Canada. The development of a novel vaccine based on previously unknown antigens has opened scientific questions on their function, immunogenicity, and ability to be effective targets for antibody recognition in different strains representing the meningococcal genetic diversity. These studies have provided many insights in the mechanism of virulence and pathogenesis of meningococcus, serving as basis of a new epidemiological tool to evaluate vaccine coverage. The new MenB vaccine is expected to reduce the incidence of meningococcal disease, especially in infants, providing added public health benefits.

Workshop Sessions

BAM-WK101.02 - Can phages prevail over Staphylococcus aureus cells?

Sylvain Moineau[1]
[1]*Université Laval, Québec, Canada*

Despite being found on the skin and in mucous membranes of healthy carriers, Staphylococcus aureus is responsible for a wide range of diseases. In fact, S. aureus is one of the main causes of hospital associated infection and foodborne contamination. Methicillin-resistant S. aureus (MRSA) are also often found in hospital or community outbreaks and their emergence is becoming a global concern. In addition, some S. aureus strains cause food poisoning, which results from the ingestion of staphylococcal enterotoxins secreted during growth in foods. To combat these detrimental effects, polyvalent virulent phages are being investigated to increase our list of antimicrobials. Of particular interest are the staphylococcal phages belonging to the Myoviridae family (dsDNA genome and contractile tail) known for their broad host range, including their ability to infect staphylococci of animal and human origin, including MRSA. Comparative genomics showed that many of these myophages are virulent (devoided of a lysogenic module) and safe (no gene coding for virulence factors). We recently showed that these myophages can also be safely produced in large quantities on a coagulase-negative staphylococcal species, namely Staphylococcus xylosus. This generally-recognized as safe species has long been used as an industrial starter culture in Greek, Spanish, Italian and other fermented sausage processes. Finally, the selected phages must be able to resist and kill the targeted bacterial strains in particular ecosystems or environmental conditions. As a proof of concept, we show that a cocktail of three staphylococcal phages efficiently eradicated the S. aureus population after 14 days of cheese ripening at 4ºC. The use of such phage cocktail also prevented enterotoxin C production. Taken altogether, virulent phages appear to be able to control S. aureus.

Workshop Sessions

BAM-WK101.03 - Microbial natural products remain promising sources for the discovery of novel antibiotics

Olga Genilloud[1]
[1]*Fundación MEDINA, Health Sciences Technology Park, Granada, Spain*

Infectious diseases are one of the leading causes of death worldwide and the emergence of multi-drug-resistant bacteria, and the lack of new antibiotics in development with new modes of action are today widely accepted as one of the major clinical concerns that require to be addressed from the public and private sectors. One of major clinical needs concern MDR Gram-negative bacteria hospital-acquired infections, frequently associated with high mortality rates and few treatment options. Very limited options have been developed to date against Gram negative, and even last resorts antibiotics such as colistin and polymixin B are facing resistances. Historically microbial natural products (NPs) have been one of the most prolific sources of new leads for the discovery of novel antibiotics, with a large number of compounds and analogs successfully introduced in the market in the past decades and still today in clinical practice. NPs present a unique chemical space and architectural complexity, and their potency and selectivity is the result of an extended evolutionary selection to create biologically active molecules with the required properties to interact and potentially inhibit bacterial targets. MEDINA is a non-profit research center focused on the discovery of novel antibiotics from NPs with more than 50-years of inherited experience in drug discovery from the former MSD Spain Basic Research Center. MEDINA harbors one of the richest and most diverse NPs collections that are at the origin of our collaborative research programs focused on the discovery of new antibiotics. As a result of these screening programs we have identified a novel family of compounds with interesting new chemistry and biological activities currently in development that will be discussed in the context of current antibiotic discovery efforts in the academia and the pharma sector.

Workshop Sessions

BAM-WK101.04 - New technologies in using recombinant attenuated *Salmonella* vaccine vectors

Roy Curtiss III[1]
[1]*Arizona State University, The Biodesign Institute, Tempe, USA*

We have genetically engineered *Salmonella* serovars Typhimurium, Paratyphi A and Typhi vaccines for regulated delayed attenuation in vivo, regulated delayed in vivo synthesis of protective antigens specified by codon-optimized DNA sequences and regulated delayed lysis in vivo such that strains can be grown under conditions that enable the vaccines to display after oral immunization the capabilities of a wild-type strain to survive host defense stresses and efficiently colonize lymphoid effector tissues before manifesting attenuation to preclude causing disease symptoms and synthesizing protein antigens to induce protective immune responses. We have engineered strains to eliminate or decrease synthesis of serotype-specific LPS O-antigen and flagellar antigens (to enable reuse of the vaccine vector), to expose conserved LPS core and over-expressed immunologically cross-reactive surface outer membrane protein antigens needed for the acquisition of essential iron and manganese ions, to diminish induction of gastroenteritis symptoms while retaining abilities to recruit innate immunity, and to exhibit biological containment by cell lysis to preclude persistence in vivo or survival if excreted. Recent work focuses on improving delivery of DNA vaccines to prevent viral and parasite infections. Strains are totally safe at high doses to newborn, pregnant, protein malnourished and immunocompromised mice. We are using these technologies to develop vaccines to prevent infections of newborns with *Streptococcus pneumoniae*, *Mycobacterium tuberculosis* and a diversity of enteric bacterial pathogens causing diarrheal disease and fever. Vaccines are also being developed against pathogens of agriculturally important animals, especially to enhance food safety by preventing infections by *Salmonella, E. coli* pathovars and *Campylobacter jejuni* that can be passed through the food chain to humans.

Workshop Sessions

BAM-WK102.01 - Defeating the iron deficit: the role of siderophores in extra-intestinal pathogenic E. coli virulence

Charles Dozois[1]
[1]*INRS-Institut Armand-Frappier, Laval, Canada*

Iron is an essential element and co-factor required for a number of metabolic processes in most bacteria and animals. In response to infection, a cascade of host signals leads to increased sequestration of iron. Bacterial pathogens have therefore evolved different mechanisms to sequester iron from the host during infection and siderophore-mediated iron acquisition is a key aspect of virulence of enterobacteria such as *E. coli* , *Salmonella*, and *Klebsiella pneumoniae*. By using different strategies, enterobacterial pathogens such as extra-intestinal *E. coli* (ExPEC) are able to retrieve iron sequestered by host proteins. One of these strategies is the use of siderophores, which are small secreted molecules with high affinity for iron. ExPEC are known to synthesize up to four different types of siderophores: enterobactin, salmochelins, yersiniabactin and aerobactin. Steps required for iron acquisition by siderophores include (1) siderophore synthesis in the cytoplasm, (2) siderophore secretion, (3) ferri-siderophore reception, (4) ferri-siderophore internalization and (5) iron release in the cytoplasm. The importance of different genes involved in steps of siderophore synthesis, release, and utilisation are investigated and compared using animal host models, as well investigation of the fate of siderophores as determined by chemical analyses through mass spectrometry. These studies confirm that ,despite redundancy, abrogation of specific systems for export or utilisation of siderophores, as opposed to siderophore synthesis, result in decreased virulence and could represent potential targets against this important group of bacterial pathogens.

Workshop Sessions

BAM-WK102.02 - Microbes, metals and electron flow: extracellular electron transport (EET) in action

Kenneth Nealson[1]
[1]*University of Southern California, Los Angeles, USA*

Redox chemistry dominates much of microbial metabolism, primarily by the establishment of membrane potentials via electron flow to electron acceptors for respiration. With the exception of a few key quinones, this electron flow involves metals, which are defined as elements that can rapidly and efficiently donate or accept electrons. While the predominant and most familiar redox reactions occur at the cell membrane, and involve soluble electron donors and acceptors, it is now clear that another class of redox reactions involves insoluble reactants that are oxidized or reduced outside the cell membrane by a process called extracellular electron transport, or EET. The primary biochemical problem that must be solved by EET-capable microbes is the movement of electrons from the cytoplasm to the cell exterior. In Shewanella oneidensis MR-1, this is accomplished via a series of multi-heme cytochromes (Mtr proteins) that can transport electrons across the membrane to other multi-heme cytochromes located on the outer membrane, where they are transferred to insoluble extracellular substrates via several different mechanisms. While EET was first noted during the study of metal-oxide reducing microbes, it is now clear that it is involved with the donation and uptake of electrons to a wide variety of compounds, including several minerals and even electrodes poised at the proper potentials. The interaction of microbes with these charged surfaces can lead to attachment, growth, biofilm formation, and electron flow (in or out of the cell). In addition, attached cells respond at the transcriptional level to changes in surface charge. Clearly, the role of surface potential is a key factor to consider in many aspects of microbial ecology and metal metabolism.

Workshop Sessions

BAM-WK102.03 - The dark matter of the genome: mapping the targetome of an iron-responsive small RNA

David Lalaouna[1], Marie-Claude Carrier[1], Eric Massé[1]
[1]University of Sherbrooke, Sherbrooke, Canada

Bacterial small RNAs (sRNAs) are crucial regulators of cellular functions by modulating gene expression in response to various environmental changes. Many bacterial species were shown to express hundreds of sRNAs. These sRNAs regulate target mRNAs by direct base-pairing to positively or negatively affect their translation and stability. In E. coli and other Enterobacteriaceae, the sRNA RyhB regulates iron homeostasis. Under iron-rich conditions, the transcriptional repressor Fur blocks ryhB transcription. During conditions of iron starvation however, Fur becomes inactive and relieves repression of ryhB. Under these conditions, RyhB directly regulates approximately 20 different mRNAs encoding iron-using proteins. By binding to those mRNAs, RyhB shuts down translation and stimulate their rapid degradation through the action of the endoribonuclease E (RNase E). RyhB contributes actively to increase the levels of free intracellular Fe2+ (iron sparing) under conditions of iron starvation by reducing the expression of iron-using proteins and by stimulating siderophore synthesis. Surprisingly, although we have used microarrays to successfully determine the effect of RyhB on the transcriptome of E. coli, this approach was insufficient to determine the regulation of undetected but important mRNAs. Thus sRNAs can significantly modulate many target mRNAs without interfering with their levels, which unfortunately prevents target detection by classical techniques. To address this challenge, we developed a method based on affinity purification and RNA sequencing, which could identify any RNA molecule interacting with sRNAs, regardless of the regulation. Thus, we used sRNAs tagged with the bacteriophage MS2 RNA stemloops, which are bound with high specificity by the MS2 coat protein. We then analysed all bound target RNAs by RNA sequencing. We will present new targets that suggest an unexpected but important mechanism for bacterial cells.

Workshop Sessions

BAM-WK102.04 - Biotechnological approach to selenite detoxification through the formation of Se0 nanoparticles by means of a Bacillus mycoides strain isolated from the rhizosphere of Astragalus bisulcatus

Emanuele Zonaro[1], Silvia Lampis[1], Giovanni Vallini[1]
[1]*University of Verona, Verona, Italy*

Selenium is a trace element commonly found in the earth's crust. It belongs to the Group 16 (chalcogens) of the Periodic Table and occurs in a variety of oxidation states in the environment. In particular, the predominant Se species in oxic conditions are the oxyanions selenite (SeO32-) and selenate (SeO42-), with the former exerting the highest toxicity. Interestingly, the ability to reduce SeO32- into the non-toxic elemental form is widespread among microorganisms. The present work investigates on the reduction mechanisms of selenite to zero valent selenium nanoparticles by Bacillus mycoides SeITE01, a bacterial strain isolated from the rhizosphere of the Se-hyperaccumulator legume Astragalus bisulcatus. The strain SeITE01 exhibits resistance to SeO32- up to 25 mM and is capable of complete reduction of 0.5 and 2.0 mM SeO32- within 12 and 24 hours, respectively. SeITE01 also demonstrated to convert 91% of the selenite initially added to the growth medium into elemental selenium, with cultures developing a deep red color characteristic of crystalline monoclinic Se0. However, Se0 production was delayed respect to selenite depletion in the culture medium. Characterization of red Se0 precipitate by using transmission electron microscopy, scanning electron microscopy and UV-Vis spectroscopy revealed the presence of extracellular spherical nanoparticles. In few cases, also intracellular nanoparticles were detected. Size of such selenium nanoparticles range from 50 to 400 nm in diameter, according to the different incubation times. SeITE01 protein fractions were assayed for selenite reduction activity, which can be associated to membrane proteins and spent culture medium after NADH addition. On the basis of the results gained so far, two different mechanisms for the synthesis of selenium nanoparticles have been proposed. The involvement of proteins/peptides able to extracellularly reduce selenite and a possible reduction of selenite by membrane reductases.

Workshop Sessions

BAM-WK102.05 - Metabolic alterations induced by metal toxicity in xenobiotic degrading bacterial cultures

Sean Booth[1], Aalim Weljie[2], Raymond Turner[1]
[1]University of Calgary, Calgary, Canada, [2]University of Pennsylvania Translational Research Center, Philadelphia, USA

Human industrial activities have introduced huge quantities of toxic pollutants into the environment. Through the process of bioremediation, bacteria are able to metabolize many of these organic pollutants into non-toxic end-products. Many polluted sites are co-contaminated with metals in addition to organic pollutants, which are toxic to bacteria and interfere with the degradation process. While some specific mechanisms of metal toxicity have been elucidated they are by no means comprehensive nor explicit to the context of xenobiotic degradation. Understanding how metal exposure specifically affects bacteria during organic pollutant metabolism will allow for improved bioremediation strategies to be developed for co-contaminated sites. To this end, metabolomic profiles of Pseudomonas pseudoalcaligenes KF707 grown either on succinate or biphenyl in the presence of aluminium or copper were compared. It was hypothesized that if metal exposure affects the metabolism of an organic pollutant (biphenyl) differently than a simple carbon source (succinate) then the metabolic profiles of cultures grown on each carbon source would differ and provide insight into the alternate mechanisms of toxicity. Gas-chromatography mass-spectrometry (GC-MS) was used to identify and quantify metabolites both from cells as well as the spent media from cultures. Multiple chemical classes of metabolites were found including sugars, amino and carboxylic acids, nucleotides and biphenyl degradation intermediates. Concentrations of these metabolites were subject to multivariate analysis to determine whether they differed significantly between culture types. Depending on both the carbon source and metal, metabolic profiles of both cells and media supernatants were perturbed differently. While the metabolic profiles of the control cells were expected to be different depending on the medium, they were not observed to converge upon exposure to the same toxic metal. The specific metabolites that were altered allow us to conclude that bacteria respond uniquely to metal exposure when metabolizing xenobiotic compounds.

Workshop Sessions

BAM-WK103.01 - Building new bacterial strains for bioplastics production using functional metagenomics and chromosome engineering

Trevor Charles[1], Jiujun Cheng[1], Ricardo Nordeste[1], Tam Tran[1], John Heil[1]
[1]University of Waterloo, Waterloo, Canada

Bacterial production of polyhydroxyalkanoate (PHA) bioplastics has potential as a key part of the vegetable oil biorefinery. Production of PHA polymers with desirable characteristics must be optimized in order for bioplastics to compete with fossil fuel-derived plastics. We have developed functional metagenomic screens for novel PHA synthesis pathway genes. These screens are performed in Sinorhizobium meliloti and Pseudomonas putida, using broad host range cosmid soil metagenomic libraries that are available from the Canadian MetaMicroBiome Library (http://cm2bl.org). DNA sequence analysis indicates that a range of Class I and Class II PHA synthase encoding genes can be isolated using this approach. Expression of these genes in different host backgrounds such as S. meliloti, P. putida or Escherichia coli, facilitated by ΦC31-mediated chromosome engineering methods, results in differing quality and quantity of PHA product. We are also investigating the effects of carbon source and culture conditions on the final end product, with a focus on cultivation using oilseed derived feedstock. As a result of this work, we intend to be able to contribute towards the range and variety of PHA bioplastics that can be reliably produced.

Workshop Sessions

BAM-WK103.02 - Identification of polyketide synthase pathways from a large-insert soil metagenomic library

Mark Liles[1], Jinglie Zhou[1], Rosa Ye[2], Svetlana Jasinovica[2], Megan Niebauer[2], Amanda Krerowicz[2], Ronald Godiska[2], ChengCang Wu[2], Blaine Pfeifer[3], David Mead[2]

[1]Auburn University, Auburn, USA, [2]Lucigen Corporation, Middleton, USA, [3]SUNY-Buffalo, Buffalo, USA

Polyketides are a group of secondary metabolites with a wide diversity of structures and bioactivities. Polyketide synthases (PKS) can classified as type I, type II, or type III. Type I PKS pathways usually contain a multidomain architecture, usually encoded by large genetic regions of > 40 kb. In this study we constructed a large-insert soil metagenomic clone library (19,200 clones of ~110kb each) from an agricultural soil (Cullars Rotation, Auburn, AL) using a broad host range shuttle BAC vector, pSmartBAC-S. A phylogenetic analysis of the metagenomic library, based on 16S rRNA gene sequences, indicated a very diverse assemblage of microbial genomes, representing nine bacterial phyla. Degenerate PCR primers targeting a conserved KS domain were used to amplify this domain from the BAC clone library. The resulting KS domain amplicons exhibited only 32.3% - 82.7% amino acid identity with known KS domains in GenBank. These unique KS domains were used to design probes and primers to identify BAC clones that contain a Type I PKS pathway. The Type I PKS BACs were transformed into an E.coli BTRA strain engineered for PKS expression. The transformed clones were screened for synthesis of antibacterial compounds by bioassays against the pathogens Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae. Notably, several clones expressed antibacterial activities. Next-Gen sequencing of the BAC clones revealed complete or nearly complete Type I PKS pathways, with limited homology to known pathways. Clones that express antimicrobial activity will be further characterized by LC/MS analysis. These results demonstrate the recovery of complete and novel PKS pathways from a large-insert soil metagenomic library and expression of these pathways in a heterologous host.

Workshop Sessions

BAM-WK103.03 - Expanding the bioinformatics toolbox for the analysis of genomes and metagenomes

Luis Rodriguez-R[1], Chengwei Luo[1], Konstantinos Konstantinidis[1]
[1]*Georgia Institute of Technology, Atlanta, USA*

The advent of high-throughput sequencing revolutionized microbiological studies during the last decade, and provided new insights into the diversity and metabolic potential of microbial communities and populations. Both the availability of large collections of genomes and metagenomes, and the accessibility to low cost sequencing, offer new possibilities in almost all areas of microbiologic research. However, the tools to analyze genomic and metagenomic data are clearly lagging behind the developments in sequencing technologies and the archived data. Our overarching objective is to develop quantitative whole-genome approaches that address several practical challenges of contemporary genomic and metagenomic research such as assessing the fraction of the community captured by a metagenomic dataset (Nonpareil), estimating the sequencing effort required in study design (Nonpareil), determining the taxonomic affiliation of (meta)genomic sequences (MyTaxa), binning assembled contigs into population genomes based on time-series metagenomes (BinGeR), and identifying the overall similarity between genomes (ANI and AAI). Our approaches rely both on statistical theory as well as newly emerging concepts such as the existence of sequence-discrete populations in natural communities. We have applied and validated our tools based on in-silico generated datasets of known composition as well as publicly available metagenomes, and obtained important new insights into various microbiomes. For example, we were able to rank microbial communities in terms of their complexity and identify novel species that are abundant in the human gut but have no sequenced representatives and hence, should be targets of future isolation or single-cell efforts. Our tools are publicly available through http://www.enve-omics.gatech.edu/, allowing external users with a wide range of expertise in computational biology to perform online analysis of their data as well as download and integrate our tools into their own workflows.

Workshop Sessions

BAM-WK103.04 - Understanding and manipulating the wheat microbiome for improved productivity

Etienne Yergeau[1], Myriam Fernandez[2], Brenda Haug[3], Rong Li[3], Matthew Links[4], Luke Masson[1], Luke McCarthy[3], Curtis Pozniak[5], Sean Hemmingsen[3], Charles Greer[1]

[1]National Research Council, Montreal, Canada, [2]Agriculture and Agri-Food, Swift Current, Canada, [3]National Research Council, Saskatoon, Canada, [4]Agriculture and Agri-Food, Saskatoon, Canada, [5]University of Saskatchewan, Saskatoon, Canada

Canada is a major wheat producing nation and wheat contributes $11B annually to Canada's economy. With that in mind, the National Research Council Canada, along with its partners, created the Canadian Wheat Alliance (http://canadianwheatalliance.ca/) to increase wheat yields and reduce losses from drought, heat, cold and diseases through plant and microbial based approaches, including modifying the wheat microbiome. Understanding and manipulating the wheat microbiome has the potential to reduce the incidence of plant disease, increase agricultural production, reduce chemical inputs and reduce emissions of greenhouse gases, resulting in more sustainable agricultural practices. To understand the wheat microbiome and its relationship to environmental factors, two field experiments were analyzed: 1) bulk soil was sampled at three different depths for two consecutive years in plots with or without glyphosate, with different crop rotations and subjected or not to tillage; 2) bulk and rhizosphere soil and plant roots were sampled from various wheat varieties that were grown under low fertilization. DNA was extracted and bacterial and fungal communities were scrutinized by amplicon sequencing using Ion Torrent and Illumina MiSeq platforms. For the first experiment, Bacteria appeared to be mainly affected by sampling depth, while Fungi were mainly affected by crop rotation, indicating that different subsets of the wheat microbiome will respond differently to changes in the environment and to any microbiome engineering efforts. More detailed analyses of the Fusarium and AMF communities are ongoing. The second experiment is currently being analyzed and results will be presented and discussed. Future work will involve more detailed shotgun metagenomic analyses, the isolation of tailored beneficial microorganisms and targeted wheat microbiome engineering efforts.

Workshop Sessions

BAM-WK103.05 - Implementing single-cell approaches for the elucidation of marine natural compound producers in marine sponges

Tetsushi Mori[1], Micheal C. Wilson[2], Rimi Miyaoka[1], Masahiro Ando[3], Hiro-o Hamaguchi[3], Shigeki Matsunaga[4], Jörn Piel[2], Haruko Takeyama[1]

[1]Faculty of Science and Engineering, Waseda University Center for Advanced Biomedical Sciences, Tokyo, Japan, [2]Institute of Microbiology, Eidgenössische Technische Hochschule (ETH) Zurich, Zurich, Switzerland, [3]Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, Tokyo, Japan, [4]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

Marine sponges are known to produce a vast range of pharmaceutically and industrially important natural compounds. Recent speculations however, suggest that such natural compounds may be highly associated to the bacterial symbionts living within them. In recent years, studies on the metagenomes of the microbial flora within sponges have further supported this by the identification of numerous secondary metabolite biosynthetic gene clusters including those related to polyketides (PKs) and non-ribosomal peptides (NRPs). Nevertheless, many of the producers of such natural compounds are still to be determined. Thus, our goal in this study was to identify the producers of such metabolites in marine sponges by implementing the single-cell approach. The yellow chemotype of the marine sponge, *Theonella swinhoei*, were collected from Hachijo island and were processed to attain bacterial fractions. The bacterial fractions were analysed by flow cytometry, single-cells were sorted and subjected to genomic DNA amplification and nested-PCR for identification of the target metabolites and its producers. To further support our discovery, other single-cell analytical tools such as Raman microspectroscopy etc. were also used. In this work, we first targeted to identify the producer of the compound, onnamide A, found highly abundant within *T. swinhoei*. Thus far, in addition to the onnamide A biosynthetic gene cluster, we were successful in identifying a repertoire of biosynthetic gene clusters relevant to nazumamide, polytheonamide, keramamide and cyclotheonamide within genomic DNA samples of wells positive for a single filament bacterium termed *Candidatus* Entotheonella factor TSY1. We show here that the implementation of the single-cell approach was useful in allowing us to verify the producers of marine natural compounds.

Workshop Sessions

BAM-WK103.06 - Multiple drug resistance identified from a soil microbiome using functional metagenomics

Markus Oggenfuss[1], Pierre Schneeberger[1], Cosima Pelludat[1], Jürg E Frey[1], Fiona Walsh[1]
[1] *Agroscope, Waedenswil, Switzerland*

A streptomycin treated orchard soil from Switzerland was investigated for the presence of novel and characterized resistance mechanisms using functional metagenomics. This is a well characterized soil microbiota, for which the bacterial phylogeny (using 16S rRNA meta-sequencing) and the relative quantity of streptomycin and tetracycline resistance genes have been described by this group. The aim of this study was to investigate if novel antibiotic resistance genes were present in the soil microbiota and if functional metagenomics could be used to isolate known antibiotic resistance genes. The functional metagenome was created using fosmid libraries. The fosmid libraries contained clones with phenotypic resistance to rifampicin, ampicillin, amikacin, kanamycin, gentamycin, tetracycline, colistin and nalidixic acid. Multi-drug resistance was frequently identified using phenotypic methods. Phenotypic efflux tests identified that efflux played a role in resistance mechanism of selected isolates within each class of antibiotic resistance. The fosmids were sequenced, analyzed and compared to the gene databanks within NCBI and Comprehensive Antibiotic Resistance Database (CARD). However, only the kanamycin resistance gene aphA1 was identified with >90% sequence similarity. The remaining genes identified within the fosmids are of lower sequence similarity to known genes. The function of the remaining fosmid genes will be further investigated in order to elucidate the single and multi-drug resistance genes, which are responsible for the resistance phenotype.

Workshop Sessions

BAM-WK104.01 - Broad-spectrum compounds active against bacterial toxins, viruses and parasites

Neetu Gupta[1], Aurelien Michau[1], Karen Hinsinger[1], Hajer Abdelkafi[1], Romain Noel[1], Valerie Pons[1], Robert Davey[2], Jean-Christophe Cintrat[1], Julien Barbier[1], Daniel Gillet[1]

[1]Institute of Biology and Technology of Saclay, CEA, Gif Sur Yvette, France, [2]Texas Biomedical Research Institute, San Antonio, USA

We used a cell-based high-throughput screening to identify chemical inhibitors of cell intoxication by ricin. Two molecules named Retro-1 and Retro-2 were found (Stechmann et al., Cell 2010), which protect cells also against Shiga-like toxins produced by uro-hemolytic E. coli strains and cholera toxin. Retro-1 and Retro-2 selectively block retrograde toxin trafficking at the early endosomes-TGN interface, without affecting compartment morphology or other trafficking steps. They do not affect endogenous retrograde cargos, demonstrating an unexpected degree of selectivity and lack of toxicity. Retro-2 clearly protects mice from lethal nasal exposure to ricin (1 LD90). Retro-2 optimization led to a molecule 500 to 1000 fold more active against ricin and Shigatoxins with an IC50 around 25-50 nM. The most active enantiomer was determined. Another molecule named #20 protected cells against diphtheria toxin in addition to ricin, which translocation takes place at the level of the early endosome-endocytic carrier vesicle interface, these vesicles trafficking from early endosomes to late endosomes/lysosomes. This suggests that #20 acts along this pathway. Accordingly, #20 and Retro-1 or Retro-2 show additive effect on cell protection assay against ricin. Finally, #20 also gives some protection against ricin in mice. Escalating doses of these molecules on cells or in mice did not show toxicity. Interestingly, these compounds are active against other pathogens. #20 was able to protect cells against 3 viruses. Retro-2 was shown by others to have anti-leishmania activity on cells and in vivo in mice (Canton and Kima, Am. J. Pathol., 2012) and anti-polyoma viruses activity on cells (Nelson et al., mBio, 2013). We identified molecules targeting intracellular trafficking subsets that display broad-spectrum activities against pathogens (plant and bacterial toxins, viruses and parasites).

Workshop Sessions

BAM-WK104.02 - Identification and characterization of a novel anti-staphylococcal antimicrobial from the cystic fibrosis isolate, Pantoea agglomerans Tx10

Lucas Robinson[1], John Stavrinides[1]
[1]University of Regina, Regina, Canada

Pantoea is a Gram-negative enteric bacterium that is ubiquitous in the general environment, both aquatic and terrestrial. Pantoea has been used as a biocontrol agent for the treatment Erwinia amylovoa, which is the causative agent of fireblight (a disease that afflicts apple and pear trees). Previously identified Pantoea antibiotics Pantocins, Herbiculins and Microcins have all shown to inhibit the growth of E. amylovora. An isolate found in the sputum of a cystic fibrosis patient, Pantoea agglomerans Tx10 (Tx10), produces a novel antibiotic that exhibits inhibition of E. amylovora, but also human pathogenic Staphylococcus aureus and Streptococcus mutans, and other strains of Pantoea. The Tx10 isolate could therefore be a potential therapeutic for cystic fibrosis respiratory infections and other clinical pathogens. A Tn5 transposon mutagenesis conducted upon the strain in order to generate a library of knockout mutants. Mutants were selected using a screen that identified antibiotic deficient stains that can no longer inhibit the growth of S. aureus. Comparative analysis of the mutant knock out genes identified a coregulated six-gene cluster that is likely involved in the production of the antibiotic. The mutants however were shown to still have antibiotic activity against E. amylovora and a selected population of Pantoea strains, indicating that there are other antibiotic(s) being producted in the Tx10 strain. Pantoea represents an untapped source of novel antibiotics having clinical potential.

Workshop Sessions

BAM-WK104.03 - Evaluation of a glycosyl triazole library as inhibitors of bacterial N-acetylglucosaminidases

Eimear Black[1], Helene Kuhn[2], Amit Basu[2], Christopher Reid[1]
[1]Department of Science and Technology, Bryant University, Smithfield, USA, [2]Department of Chemistry, Brown University, Providence, USA

Peptidoglycan is a polymer consisting of sugars and amino acids that form a mesh-like layer outside the plasma membrane of bacteria, forming the cell wall. The sugar component of peptidoglycan consists of alternating residues of beta-(1,4) linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). N-acetylglucosaminidases (GlcNAcases) are involved in catalyzing the cleavage of the glycosidic bond between GlcNAc and MurNAc. These GlcNAcases have been implicated in processes such as cell elongation and division. The goal of our research is to synthesize small molecule inhibitors that can be accessed using the Ugi-4CCR and the 'click' reaction. The main aim is to identify small molecules that can be an impulsive for new scaffolds for bioactive compounds. A small library of GlcNAc triazole (GNT) based compounds were screened as potential inhibitors for GlcNAcases in whole cell assays against a panel of Gram-positive organisms. We have identified two GNTs with bacteriostatic activity against Bacillus subtilis and Bacillus cereus with MIC values of 60 μM. Incubation of B. subtilis cells with the synthetic substrate beta-p-nitrophenyl GlcNAc (pNPGlcNAc) in the presence of the GNT resulted in an inhibition of nitrophenol release, confirming that the biochemical target is an N-acetyl glucosaminidase. Treatment of B. subtilis with the GNT at a concentration below the MIC resulted in the formation of bacterial cells with elongated morphology, indicating impaired cell division and suggesting that the GNT targets an enzyme that is a critical component of the cell division machinery.

Workshop Sessions

BAM-WK104.04 - *Streptomyces* – a screening tool for bacterial cell division inhibitors

Charul Jani[1], Scott McAuley[2], Arryn Craney[1], Justin Nodwell[1,2]
[1]*Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario,* [2]*Department of Biochemistry, University of Toronto, Toronto, Ontario*

Cell division is essential for spore formation but not viability in the filamentous streptomycetes bacteria. Failure to complete cell division blocks spore formation, a phenotype that can be visualized by the absence of gray (in *Streptomyces coelicolor*) and green (in *Streptomyces venezuelae*) spore-associated pigmentation. Despite this unique phenotype, the streptomycetes cell division machinery, or divisome, is similar to that of other prokaryotes. Therefore, we hypothesized that chemical inhibitors of sporulation in model streptomycetes might interfere with cell division in other bacteria. To test this, we investigated 196 compounds that inhibit sporulation in *Streptomyces coelicolor* and focus on three (Fil-1, 2, and 3) that were found to confer cell division defects on B. subtilis. In addition, the compounds were found to inhibit the formation of heat resistant endospores in *B. subtilis*, a mechanism that also requires a fully functioning divisome. These results suggest that the streptomycetes life cycle is a powerful tool for identifying chemical inhibitors of cell division. Continued development of the assays, such as the introduction of a luminescent reporter in conjunction with a screen for growth inhibition of *B. subtilis*, could provide a high-throughput method for screening compounds that inhibit cell division and provide novel tools for further investigations into the cell division machinery or potential new antibiotics.

Workshop Sessions

BAM-WK104.05 - The effect of a novel antimicrobial peptide on survival and infection of acid-stressed Citrobacter rodentium in a mouse infection model

Tracy Lackraj[1], Kathene Johnson-Henry[2], Philip Sherman[2], Steve Goodman[3], Anca Segall[4], Debora Barnett Foster[1]
[1]Department of Chemistry and Biology, Ryerson University, Toronto, Canada, [2]Cell Biology Department, Research Institute, Hospital for Sick Children, Toronto, Canada, [3]Division of Biomedial Sciences, Herman Ostrow School of Dentistry of the University of Southern California, USA, [4]Department of Biology, Center for Microbial Sciences and Molecular Biology Institute, San Diego State University, USA

Introduction and Objectives: Citrobacter rodentium is a gram-negative, murine-specific enteric pathogen that infects epithelial cells in the colon. It is closely related to the clinically relevant human pathogen, enterohemorrhagic Escherichia coli (EHEC), a leading cause of hemorrhagic colitis and hemolytic uremic syndrome. Consequently, the C. rodentium murine model of enteric infection can serve as an important tool in enhancing our understanding of EHEC infection and in assessing the value of promising antimicrobials against infection. We previously reported that a novel antimicrobial peptide, wrwycr, inhibits bacterial DNA repair and significantly reduces the survival of acid-stressed EHEC. This study examines the impact of peptide pre-treatment on survival and infection of the closely related murine pathogen, C. rodentium in vitro and in vivo. Methods and Results: When acid exposure (pH 3.5) is preceded by a brief pre-treatment with the peptide, there is a marked and significant enhancement of acid-induced killing of planktonic C. rodentium in a peptide-dose- and time- dependent manner. Pre-treatment of existing C. rodentium biofilms with the peptide followed by acid stress does not cause a significant enhancement of pathogen killing, suggesting a protective role of the biofilm against peptide treatment. In the mouse model of infection, pre-treatment of 10^8 CFU C. rodentium with 50μM peptide for 5 minutes prior to orogastric gavage significantly reduces bacterial counts in fecal pellets to below detectable limits and also reduces crypt hyperplasia, indicative of pathogen-induced infection. Negative controls include peptide alone and a placebo peptide. Conclusions and Significance: These results demonstrate that peptide pre-treatment of C. rodentium significantly enhances acid-induced killing of this pathogen both in vitro and in vivo, and are consistent with the response of the related human pathogen, EHEC, to a similar treatment. These findings support a role for this peptide as a prevention strategy against infection by enteric pathogens.

Monday, 28 July 2014 **16:45 - 17:00 Room 519 B**

Workshop Sessions

BAM-WK104.06 - Novel antimicrobial properties of maple syrup polyphenolic extracts against uropathogenic bacteria

Vimal Maisuria[1], Nathalie Tufenkji[1]

[1]Department of Chemical Engineering, McGill University, Montreal, Canada

Catheter-associated urinary tract infections (CAUTIs) represent the most frequently encountered hospital acquired infection, corresponding to significant morbidity and health care costs. Empirical therapy with antibiotics is a standard management approach for uncomplicated CAUTIs. Due to current rising prevalence of antibiotic resistance, interest has grown in the need to develop alternate antimicrobial options for combating infection. The retreat of the pharmaceutical sector from new antibiotic development has exacerbated the challenge of widespread resistance and signals a critical need for innovation. Naturally occurring antimicrobial compounds are successful in inactivating a wide variety of pathogenic microorganisms as they often target multiple bacterial functions and as such, are less prone to development of resistance compared with antibiotics. North American maple syrup produced by concentrating the sap collected from certain maple (genus, Acer) tree species, contains a vast number of natural and process-derived phytochemicals among which phenolics constitute the majority. We found that the combination of antibiotics (either carbenicillin or ciprofloxacin) and polyphenolic rich maple syrup extracts demonstrate synergy for growth inhibition of different clinical isolates (Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa) associated with CAUTIs. Antimicrobial effects of polyphenolic rich maple syrup extracts was assessed against different uropathogens cultured either planktonically or in biofilms. The polyphenolic rich maple syrup extracts demonstrated antimicrobial activity against planktonic cells and also efficiently inhibited biofilm formation. Moreover, the effects of polyphenolic rich maple syrup extracts on expression of different virulence associated genes (e.g., genes for motility and biofilm formation) of these uropathogens were analyzed. Transcriptional level analyses showed that the polyphenolic rich maple syrup extracts affected different genes important for motility and biofilm formation, which was corroborated by reduction in biofilm formation by different uropathogens. The discovery of beneficial properties of this polyphenolic rich maple syrup extract may offer clinical advantage in combating infection.

Workshop Sessions

BAM-WK105.01 - Major Genetic events in the evolution of Vibrio cholerae O1 for the last two decades at Kolkata, India

Asish Mukhopadhyay[1], P Ghosh[1], A Naha[1], T Ramamurthy[1], S Shinoda[2]
[1]National Institute of Cholera and Enteric Diseases, Division of Bacteriology, Kolkata, India, [2]Collaborative Research Center of Okayama University for Infectious Diseases at NICED, Kolkata, India

Vibrio cholerae O1, the causative agent of devastating cholera, has two biotypes, namely, classical and El Tor based on certain phenotypic and genetic traits. From the recorded history, the world has experienced seven cholera pandemics. Among these, the first six pandemics are believed to be caused by the classical biotype whereas the ongoing seventh pandemic is caused by the El Tor biotype. Chronological study from Kolkata indicated that classical ctxB emerged in 1990 and V. cholerae O1 strains from 1995 onwards were found to carry classical ctxB, which totally replaced the El Tor ctxB. These strains were termed as El Tor variant. Studies from Kolkata predicted that some of the 1992 Kolkata O1 strains might have acted as progenitors for Mozambique outbreak strains in 2004. The amount of cholera toxin produced by El Tor variant strains was more or less equivalent to that produced by classical strains. Our Study revealed that the Haitian ctxB containing a unique mutation at the 58th nucleotide first appeared in Kolkata during April, 2006. Study also depicted that Haitian variant TcpA containing a novel mutation at the 64th amino acid position first appeared in Kolkata during 2003 and after that all El Tor tcpA was replaced by this new tcpA allele. It has been assumed that certain traits of the Haitian variant strains may have originated from this part of the world. These new El Tor variant strains replaced the prototype El Tor strains in Asia and Africa. Moreover, the severity of the disease appears to be intensifying, and recent cholera outbreaks in various places, including Zimbabwe and Haiti, have followed protracted period. An active holistic surveillance system is needed to tract the dissemination of these new El Tor variant strains, as these strains possess all the potentialities and foundation for a new pandemic.

Workshop Sessions

BAM-WK105.02 - Heterogeneity among Mycobacterium ulcerans from French Guiana revealed by multilocus variable number tandem repeat analysis (MLVA)

Yann Reynaud[1], Julie Millet[2], David Couvin[2], Nalin Rastogi[2], Pierre Couppié[3], Eric Legrand[1]
[1]Institut Pasteur, Cayenne, French Guiana, [2]Institut Pasteur, Pointe à Pitre, Guadeloupe, [3]Centre Hospitalier André Rosemon, Cayenne, French Guiana

Buruli ulcer is an emerging and neglected tropical disease due to M. ulcerans. It has been reported in 34 countries and is endemic in some part of Southeast Asia, Australia and especially in West and Central Africa where it represents today a major health problem. In the Americas, only few cases were reported for decades in Peru, Mexico, Surinam and French Guiana, but this last country is the only endemic area of the continent. Around 200 cases were reported in French Guiana since sixties, with todays between 5 to 10 cases each year. Such atypical incidence could probably be explained partly by the efficacy of Buruli diagnosis in this French region, but we can also hypothesize that M. ulcerans isolates have genetic characteristics distinguishing them from other countries, and that have to be explored. Our goal in the present study was then to examine the genetic diversity of M. ulcerans strains in this region by MLVA. A total of 25 DNA were purified from ulcer biopsies. MLVA were performed using six previously-described VNTR markers. A total of four allelic combinations were characterized in our study: genotype I has already been described before, genotype III and IV are very close to genotype I while genotype II appears totally different. When focusing on sequences variations, new ones were here identified. Globally, such a genetic diversity appears uncommon for that species which is usually characterized by a very low level of genetic variability and which can be considered as “monomorphic”. Further research based on complete genome sequencing of strains belonging to each genotype will lead to a better understanding of genetic specificities of M. ulcerans strains from French Guiana.

Workshop Sessions

BAM-WK105.03 - Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome

Hao Xu[1], Jieling Yang[1], Wenqing Gao[1], Feng Shao[1]
[1]*National Institute of Biological Science, Beijing, China*

Cytosolic inflammasome complexes mediated by a pattern recognition receptor (PRR) defend against pathogen infection by activating caspase 1. Pyrin, a candidate PRR, can bind to the inflammasome adaptor ASC to form a caspase 1-activating complex. Mutations in the Pyrin-encoding gene, MEFV, cause a human autoinflammatory disease known as familial Mediterranean fever. Despite important roles in immunity and disease, the physiological function of Pyrin remains unknown. Here we show that Pyrin mediates caspase 1 inflammasome activation in response to Rho-glucosylation of cytotoxin TcdB,a major virulence factor of Clostridium difficile, which causes most cases of nosocomial diarrhoea. The glucosyltransferase-inactive TcdB mutant loses the inflammasome-stimulating activity. Other Rho-inactivating toxins, including FIC-domain adenylyltransferases (Vibrio parahaemolyticus VopS andHistophilussomni IbpA) and Clostridium botulinum ADP-ribosylating C3 toxin, can also bio-chemically activate the Pyrin inflammasome in their enzymatic activity-dependent manner. These toxins all target the Rho subfamily and modify a switch-I residue. We further demonstrate that Burkholderia cenocepacia inactivates RHOA by deamidating Asn 41, also in the switch-I region,and thereby triggers Pyrin inflammasome activation, both of which require the bacterial type VI secretion system (T6SS). Loss of the Pyrin inflammasome causes elevated intra-macrophage growth of B. cenocepacia and diminished lung inflammation in mice. Thus, Pyrin functions to sense pathogen modification and inactivation of Rho GTPases, representing a new paradigm in mammalian innate immunity.

Workshop Sessions

BAM-WK105.04 - Bile salts enhance resistance of enterohemorrhagic *Escherichia coli* O157:H7 to human defensin HD-5

Crystal Gadishaw-Lue[1], Aju-Sue Francis[1], Debora Barnett Foster[1]
[1]*Department of Chemistry and Biology, Ryerson University, Toronto, Canada*

Introduction and Objectives: Enterohemorrhagic *Escherichia coli* (EHEC) causes a severe food and water-borne illness associated with diarrhea, hemorrhagic colitis (HC), and hemolytic-uremic syndrome (HUS). EHEC successfully survives transit through numerous stresses upon ingestion including bile salts and antimicrobial peptides in the duodenum of the small intestines. Previously we reported that bile salt treatment of EHEC upregulates genes encoding a two component signal transduction system (*basRS*) and a lipid A modification pathway (*arnBCADTEF*). Bile salt treatment also enhanced resistance to the cationic antimicrobial protein, polymyxin B, in an *arnT* and *BasRS* dependent manner. The current study examines the effect of bile salt treatment on EHEC resistance to two human cationic antimicrobials, HD-5 and LL-37. Methods and Results: Radial diffusion assays show a significant increase in resistance to HD-5 when EHEC are pre-treated with physiologically relevant concentrations of a bile salt mix as compared to untreated EHEC. The resistance is dose dependent with respect to both HD-5 and bile salts. The bile salt-induced resistance phenotype is lost in each of the *arnT* and *basS* mutants. Bile salt treatment does not affect resistance to the cathelicidin, LL-37. A similar bile salt induced resistance phenotype is observed in the related pathogen, enteropathogenic E. coli (EPEC) to polymyxin B. Conclusions and Significance: The results of this study provide evidence that bile salts serve as an environmental cue for EHEC by triggering changes that result in protective modifications of the bacterial outer membrane, thereby increasing resistance to the human defensin, HD-5 also encountered in the small intestine. This resistance is specific to HD-5 and is dependent on the modification of lipid A of lipopolysaccharide. A similar resistance phenotype is also induced in the related pathogen, EPEC.

Workshop Sessions

BAM-WK105.05 - Role of Fibroblast Growth Factor1 in interaction of Neisseria meningitidis with Human Brain Microvascular Endothelial Cells

Sheyda Azimi[1], Lee. M Wheldon[1], Neil.J Oldfield[1], Dlawer Ala'Aldeen[1], Karl.G Wooldridge[1]

[1]*Molecular Bacteriology Immunology Group (MBIG), Centre of Biomolecular Sciences, School of Life Sciences, University of Nottingham, Nottingham, UK*

Neisseria meningitidis (meningococcus) is an obligate human commensal bacterium that can cause meningitis and sepsis. Crossing the Blood-Brain Barrier (BBB) is a crucial step in the development of meningitis, but the mechanisms used by the meningococcus to achieve this are not fully understood. The aim of this study was to investigate the role of the Fibroblast Growth Factor1-IIIc isoform (FGFR1-IIIc) in the attachment to, and invasion of, Human Brain Microvascular endothelial cells (HBMECs) by N. meningitidis. Confocal microscopy showed that micro-colonies of adhered N. meningitidis recruit activated FGFR1. Direct interaction between meningococci and the extracellular domain of FGFR1-IIIc was demonstrated by ELISA confirming the ability of this bacterium to bind FGFR1-IIIc. Other bacterial meningeal pathogens, including Streptococcus pneumoniae and Haemophilus influenzae, were unable to bind to this receptor confirmed specificity. This study identified a novel receptor for meningococci, FGFR1, which may play an important role in the pathogenesis of this pathogen, and may constitute a new therapeutic and prevention target for disease caused by these bacteria

Workshop Sessions

BAM-WK105.06 - Structural analyses of Legionella LepB reveal a new GAP fold that catalytically mimics eukaryotic RasGAP and a Kinase like domain that inhibit Golgi trafficking

Qin Yu[1,2], Liyan Hu[2], Qing Yao[2], Yongqun Zhu[3], Dong Na[2], Da-Cheng Wang[1], Feng Shao[2]
[1]National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China, [2]National Institute of Biological Sciences, Beijing, China, [3]Life Sciences Institute, Zhejiang University, Hangzhou, China

Rab GTPases are emerging targets of diverse bacterial pathogens. Here, we perform biochemical and structural analyses of LepB, a Rab GTPase-activating protein (GAP) effector from Legionella pneumophila. We map LepB GAP domain to residues 313-618 and show that the GAP domain is Rab1 specific with a catalytic activity higher than the canonical eukaryotic TBC GAP and the newly identified VirA/EspG family of bacterial RabGAP effectors. Exhaustive mutation analyses identify Arg444 as the arginine finger, but no catalytically essential glutamine residues. Crystal structures of LepB 313-618 alone and the GAP domain of Legionella drancourtii LepB in complex with Rab1-GDP-AlF3 support the catalytic role of Arg444, and also further reveal a 3D architecture and a GTPase-binding mode distinct from all known GAPs. Glu449, structurally equivalent to TBC RabGAP glutamine finger in apo-LepB, undergoes a drastic movement upon Rab1 binding, which induces Rab1 Gln70 side-chain flipping towards GDP-AlF3 through a strong ionic interaction. This conformationally rearranged Gln70 acts as the catalytic cis-glutamine, therefore uncovering an unexpected RasGAP-like catalytic mechanism for LepB. In additon to the GAP domain, LepB N-terminal domain (LepB_NTD, 1-311aa) has the ability to inhibit yeast growth and four single mutants can lead to the completely loss of function. LepB_NTD has structure similarity with kinase but the catalytic center are not well conserved. Immunostaining with lots of endomembrane markers directed the finding of Golgi disruption especially the medial and trans Golgi. Overexpression of LepB_NTD but not the loss-of-function mutants in mammalian cells severely blocks host secretory pathway and inhibit protein transport through the Golgi with the aggregation of secreted protein on the cis Golgi. Our studies highlight an extraordinary structural and catalytic diversity of RabGAPs, particularly those from bacterial pathogens but the exact function of LepB_NTD in blocking of protein transport through Golgi and the target remain to be clarified.

Workshop Sessions

BAM-WK105.07 - Streptococcus pneumoniae infection increases blood-brain barrier permeability in a human cell culture model

Stavros Panagiotou[1], Michael Griffiths[1], Tom Solomon[1,2], Aras Kadioglu[1], Adjanie Patabendige[1]
[1]Institute of Infection & Global Health, University of Liverpool, Liverpool, UK, [2]The Walton Center NHS Foundation Trust, Liverpool, UK

Streptococcus pneumoniae is the biggest cause of bacterial meningitis in the UK and Ireland. Bacterial meningitis disrupts the normal functions of the blood-brain barrier (BBB) and causes inflammation of the meninges. The BBB is the protective barrier formed by brain endothelial cells that prevents the entry of pathogens and toxins into the brain. However, the exact mechanisms of central nervous system penetration of Streptococcus pneumoniae across the BBB are not fully understood. To better understand these mechanisms, we used a human BBB model comprising immortalised human brain endothelial cells co-cultured with primary human astrocytes. Cells were infected with D39, 6B or the mutated plnA¯ Streptococcus pneumoniae strain and the pneumococcal toxin Pneumolysin (PLY). Adhered and invasive bacteria were significantly low in number (~1.5 – 3.0 log CFU/ml) compared to the initial bacterial load (7 log CFU/ml). The bacterial infection caused a significant drop in the transendothelial electrical resistance (TEER, a measurement of BBB tightness) when 6B meningitis strain was used ($p<0.03$), indicating that even small numbers of bacteria were sufficient to increase BBB permeability. PLY application on the luminal (blood) side of the model led to a significant reduction in brain endothelial cell viability compared to the control ($p<0.001$). Co-incubation of PLY with plnA¯ increased BBB permeability and, led to an increase in transmigration of bacteria from the luminal to the abluminal (brain) side ($p<0.005$). However, incubation of plnA¯ alone showed no significant changes in TEER compared to the control ($p>0.05$). Our results suggest that even small numbers of bacteria are sufficient to increase BBB permeability. We also demonstrate that PLY was necessary for the bacteria to increase BBB permeability, and transmigrate across the BBB. Understanding the role of the BBB during Streptococcus pneumoniae will aid the identification of novel therapeutic strategies.

Workshop Sessions

BAM-WK105.08 - Vaccine approaches targeting colonization by Streptococcus pyogenes

Joseph J. Zeppa[1], Katherine J. Kasper[1], Delfina M. Mazzuca[1], S. M. Mansour Haeryfar[1], John K. McCormick[1]
[1]*Western University, London, Canada*

Streptococcus pyogenes is a globally important bacterial pathogen that only infects humans, and is causative in a range of diseases from pharyngitis to necrotizing fasciitis, as well as post-infection autoimmunity such as rheumatic fever. Superantigens are toxins produced by S. pyogenes that unconventionally activate up to 20% of an individual's T cells by crosslinking T cell receptor (TCR) β-chains with major histocompatibility complex (MHC) II molecules. Our laboratory has elucidated the importance of these toxins in acute nasopharyngeal infection by S. pyogenes. Infection of C57Bl/6 mice by S. pyogenes required mouse expression of MHC II human leukocyte antigens (HLA-mice) and this phenotype was dependent on expression of the streptococcal pyrogenic exotoxin A (SpeA) superantigen. We thus hypothesized that vaccination of HLA-mice with superantigens would prevent S. pyogenes infection through antibody-mediated neutralization. To test this, HLA-mice were vaccinated with either wild-type SpeA, or a MHC II binding mutant (SpeAY100A), and nasally inoculated with S. pyogenes. HLA-mice vaccinated with either form of SpeA showed a significant decrease in nasopharyngeal infection compared to sham vaccinated mice; however, only mice vaccinated with SpeAY100A demonstrated consistent detectable levels of anti-SpeA antibodies. Interestingly, mice vaccinated with wild-type staphylococcal enterotoxin B (SEB), a superantigen produced by Staphylococcus aureus, also showed a significant decrease in S. pyogenes infectivity. Immunization with this superantigen did not generate anti-SpeA cross-reactive antibodies, but rendered the same T cell populations targeted by SpeA as unresponsive. Thus, superantigen-responsive T cell subsets are required for nasopharyngeal infection by S. pyogenes. These results support the use of superantigens as vaccine candidates against S. pyogenes and further support an important role for superantigens in acute infection.

Workshop Sessions

MEM-WK300.01 - BAComics: one-step expression of heterologous secondary metabolism clusters in Aspergillus nidulans

JinWoo Bok[1], Jessica C Albright[2], Rosa Ye[3], David Mead[4], Megan Wagner[4], Amanda Krerowicz[4], Anthony Goering[5], Neil L Kelleher[2,5], Cheng-Cang Wu[3], Nancy Keller[1]

[1]Department of Medical Microbiology and Immunology and Bacteriology, University of Wisconsin at Madison, Madison, USA, [2]Department of Chemistry, Northwestern University, Evanston, USA, [3]Intact Genomics, Inc. St Louis, USA, [4]Lucigen Corporation, Middleton, USA, [5]Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, USA

Fungi are prolific producers of anti-microbial secondary metabolites (SMs) and since the turn of the century have provided 45% of bioactive molecules from all microbial sources. However, fungal SM pathways remain largely untapped due to difficulties in efficiently expressing these SM pathways. Here we describe key technological breakthroughs that together result in the next generation functional metagenomic library. This library combined 1) an improved methodology for the isolation and purification of high molecular weight genomic DNA from fungi; 2) a new E. coli-Aspergillus shuttle vector and an A. nidulans host for enhanced autonomous expression of cloned DNAs; 3) a random shear BAC (bacterial artificial chromosome) cloning method to produce unbiased very large insert sizes (>100 kb) for covering the entire set of intact SM pathways of a fungal genome (one BAC clone = one intact SM pathway); and 4) a rapid and improved small molecule identification method using mass spectrometry-based quantitative metabolomics to identify unique compounds. We constructed a BAC library resulting in ~20x genome coverage of the A. terreus genome, or a total of 7,680 BAC clones with an average insert size of 100 kb. End-sequencing of BAC clones allowed identification of all 56 SM gene clusters of A. terreus. We describe the results of characterization of two such SM BACs expressed in A. nidulans; one yielding cryptic spiroquinazoline alkaloids and one astechrome-related molecules. Together, these technologies represent an important advancement for the science of natural product discovery in general and antibiotic discovery in particular.

Workshop Sessions

MEM-WK300.02 - Secondary metabolites inside and outside Aspergillus, Penicillium and Talaromyces

Jens Frisvad[1], Thomas Larsen[1]
[1]Technical University of Denmark, Kongens Lyngby, Denmark

The chemodiversity in Aspergillus, Penicillium and Talaromyces is extremely high, but many of the secondary metabolites in these genera are also found in phylogenetically distantly related fungi. In Penicillium, Aspergillus and Talaromyces natural clades are also phenotypically similar concerning morphology, exoenzymes and physiology. Biosynthetic families of secondary metabolite are common to several species in a natural clade, but seem to form polythetic classes, i.e. no individual secondary metabolite family is common for all species in the clade, but anyway all species in the clade can produce a high number of the characteristic secondary metabolite families. Profiles of secondary metabolites are specific for a species in the trichocomaceous genera. A chemotaxonomic overview, and how many extrolites that has been found outside those chemically prolific genera, is given. Other ascomycetous genera such as Alternaria, Trichoderma, Fusarium, Phoma, Phomopsis, Chaetomium and Aschersonia share some, but not many, extrolites with the trichocomaceous species, while basidiomycetous fungi have very few extrolites in common with the Ascomycetes. Yeasts, ruderal selected filamentous fungi and stress selected filamentous fungi are often poor producers of extrolites, while competition selected fungi often produce up to 40 different secondary metabolite families according to genome sequencing projects. Ways of manipulating fungi to produce as many extrolites as possible are reviewed, especially concerning growth media. The problem of misidentifications of fungi, ITS identifications, and mixed cultures are also discussed.

Workshop Sessions

MEM-WK300.03 - Biodiversity, ecology and secondary metabolite production in the Xylariaceae

Marc Stadler[1]
[1]*Dept. Microbial Drugs, Helmholtz Centre for Infection Research, Braunschweig, Germany*

The Xylariaceae is one of the largest families of Ascomycota and many of its species have an endophytic stage in their life cycle. In the past decade, we studied them intensively, using morphological, chemotaxonomic and molecular phylogenetic methods [1]. Numerous bioactive secondary metabolites were identified, and their production during the life cycle of their producer organisms was followed by HPLC profiling and biolassays. Several new genera and species were recognised in the course of this work, e.g., a comprehensive world monograph of the genus Daldinia and a study on neotropical Hypoxylon have recently been published [2],[3]. Moreover, it was possible to elucidate the life cycle of certain drug producing endophytes [4] and explore highly interesting correlations between endophytes, their host plants and insect vectors [5]. The Xylariaceae seem to represent an ideal “model family” for interdisciplinary work on basic as well as applied aspects of mycology. References 1. Stadler M (2011) Curr Res Environ Appl Mycol 1: 75-133. 2. Stadler M et al. (2014) Stud Mycol, 77:1-143. 3. Kuhnert E et al. (2014) Fungal Divers. 64: 181-203. 4. Bills GF et al. (2012) PLoS ONE 7(10): e46687. doi:10.1371/journal.pone.0046687. 5. Pažoutová S et al. (2013) Fungal Divers 60, 60:107–123.

Workshop Sessions

MEM-WK300.04 – WITHDRAWN - **Secondary metabolites from four marine-derived fungal strains: structure, antifouling, cytotoxic and enzyme inhibiting activity**

Shu-Hua Qi[1], Xiao-Yong Zhang[1], Jie Bao[1], Xin-Ya Xu[1], Xu-Hua Nong[1]
[1]South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China

It is now well known that diverse fungal community is abundant in marine environments, and marine fungi have received considerable attentions as important sources for new drug discovery. In order to obtain new bioactive compounds from marine microorganisms, we isolated culturable fungi from different deep-sea sediment cores and coral samples from the South China Sea. Combined with chemical analysis by HPLC and LC-MS, and various bioassay tests, such as antibacterial, antifungal, antifouling and cytotoxic activity, some bioactive fungal strains were chosen for further study of their bioactive secondary metabolites. Recently, we have obtained more than 30 new compounds with obvious antifouling, cytotoxic or enzyme inhibiting activity from four marine-derived fungal strains. Their antifouling activities were tested by laboratory antilarval settlement bioassay and field test, and two of them were proved to be potential antifouling agents. A dihydrothiophene-condensed chromone oxalicumone A from Penicillium sp. showed significant cytotoxicity against several carcinoma cell lines, and its structure-activity relationship was discussed by semi-synthesis. And in order to enhance the production of oxalicumone A, the wild Penicillium sp. strain was mutated by microwave and ultraviolet light irradiation, and the culture medium was further optimized, which led to the 20–fold increase in oxalicumone A production. In addition, three compounds with strong inhibiting activity towards acetylcholinesterase were isolated from a Aspergillus sp. strain.

Workshop Sessions

MEM-WK300.04 - Curation and characterization of secondary metabolism gene clusters in Aspergillus niger

Sylvester Palys[1]
[1]*Concordia University, Montreal, Canada*

Scientific inquiries into fungal secondary metabolism have a history of yielding highly useful and marketable compounds. These inquires continue to present a high potential for further discovery of novel compounds like anti-cancer drugs and antibiotics as well as the potential for increasing the production of those compounds which are already known. Multiple classes of enzymes, a great many with their own clustered modifying enzymes, are involved in the production of numerous and potentially useful compounds but locating them in a deluge of DNA sequence data can prove challenging. With the advent of the genomics era and advancements in sequencing technologies, the ability to search for and increase or decrease the expression of these compounds has become easier and more targeted. As the amount of data from our sequencing endeavors increases, the need for systematic or standardized methods of annotation and functional prediction becomes ever more pressing. This talk presents a method of annotating secondary metabolite gene classes and defining their relevant gene clusters to provide insight into possible methods of regulation. In addition, a method for predicting the potential molecules the clusters may produce using freely available bioinformatics tools and data from the fully sequenced and manually curated genome of *Aspergillus niger* NRRL3 is also demonstrated. Examples from key enzymes classes are discussed and a more detailed account of the production of tensyuic acids is presented.

Workshop Sessions

MEM-WK300.05 - Multi-microbial metabolite in fish feeds from South-Western Nigeria

Momodu Olorunfemi[1], Adegboyega Odebode[1], Chibundu Ezekiel[2], Michael Sulyok[3], Benedikt Warth[3], Rudolf Krska[3], Patrick Njobeh[4]

[1]Department of Botany, University of Ibadan, Ibadan, Nigeria, [2]Department of Biosciences and Biotechnology, Babcock University, Ilishan Remo, Nigeria, [3]Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna, Tulln, Austria, [4]Department of Food, Environmental and Health Research Group (FEHRG), Faculty of Health Science, Doornfontein Campus, University of Johannesburg, South Africa

A total of 94 fish feed samples were collected from fish farms in six States within South-western Nigeria (Lagos, Ogun, Oyo, Osun, Ondo and Ekiti). The spectrum of microbial metabolites including mycotoxins in the feeds was determined using a simple, rapid Liquid Chromatographic-Tandem Mass Spectrometric method (LC-MS/MS). Altogether, 90 metabolites from diverse fungi and bacteria were found in the feedstuffs. Metabolites from Aspergillus were the most frequent followed by metabolites from Fusarium, Claviceps, Penicillium, Alternaria, other fungal as well as bacterial species. Aflatoxins were detected in 97.9% of the samples while fumonisins were quantified in 88.3% and deoxynivalenol in 87.2%. Aflatoxin B1 concentrations were in the range of 0.7–551 µg/kg while the levels of fumonisin B1 were up to 6100 µg/kg. Considering the high degree of co-occurrence (including those with known toxicities e.g. citrinin, 3-nitropropionicc acid or citreoviridin) of different toxins and the levels of microbial metabolites found in the feeds, the fish industry in South-western Nigeria may be at risk of economic losses due to mycotoxicoses in the fish species. Key words: Aflatoxins; Bacterial metabolites; Fish feeds; Fumonisins; Mycotoxins.

Workshop Sessions

MEM-WK301.01 - Fungal Spores for Dispersion in Space and Time

Jan Dijksterhuis[1]
[1]*Department of Applied and Industrial Mycology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands*

Spores are an integral part of the life cycle of the gross majority of fungi. Their morphology and the mode of formation are both highly variable among the fungi, as is their resistance to stressors. The main aim for spores is to be dispersed, both in space, by various mechanisms or in time, by an extended period of dormancy. Some fungal ascospores belong to the most stress resistant eukaryotic cells described to date. Stabilisation is a process in which biomolecules and complexes thereof are protected by different types of molecules against heat, drought or other molecules. This contribution deals with the most important compounds that are known to protect fungal spores and also addresses the biophysics of cell protection. It further covers the phenomena of dormancy, breaking of dormancy and early germination. Germination is the transition from a dormant cell towards a vegetative cell and includes a number of specific changes. Finally, the applied aspects of spore biology are discussed.

Workshop Sessions

MEM-WK301.02 - Yeasts and their glycan components can have a beneficial or adverse effect on intestinal inflammation

Samir Jawhara[1], Laura Choteau[1], Thierry Fontaine[3], Yann Guerardel[4], Boualem Sendid[1,2], Pascal Vandekerckove[5], Daniel Poulain[1,2]

[1]Université Lille2, INSERM U995, Lille, France, [2]Centre de Biologie-Pathologie-Génétique. Centre Hospitalier et Universitaire, Lille, France, [3]Unité des Aspergillus, Institut Pasteur, Paris, France, [4]CNRS, UMR 8576, Villeneuve d'Ascq, France, [5]Lesaffre International, Division R&D, Marcq-en-Baroeul, France

Saccharomyces cerevisiae (Sc) is widely used in industrial food/beverage production and biotechnology.
S. cerevisiae var. *boulardii* (Sb) is described as biotherapeuthic agent and clinical studies have reported its role in prevention of *Candida albicans* colonization and antibiotic-associated diarrhea and colitis. Previous research has shown that the administration of Sb reduces both *C. albicans* colonization and intestinal inflammation in mice. The aim of this study was to identify dietary yeasts, which have comparable effects to Sb and to assess the capabilities of yeast cell wall components to modulate intestinal inflammation and Candida colonization. Mice received a single oral challenge of either *C. albicans* or *C. glabrata* and were then given 1.5% dextran-sulphate sodium (DSS) for 2 weeks followed by a 3-day restitution period. *S. cerevisiae* strains (Sb, Sc1 to Sc4), as well as mannoprotein (MP) and β-glucan crude fractions prepared from Sc2 and highly purified β-glucans prepared from *C. albicans* were used. Strain Sc1-1 gave the same level of protection against *C. albicans* and *C. glabrata* than Sb when assessed by mortality, clinical scores and colonization levels. When Sc1-1 was compared with the other *S. cerevisiae* strains, the preparation process had a strong influence on biological activity. Interestingly, some *S. cerevisiae* strains dramatically increased mortality and clinical scores. Strain Sc4 and MP fraction favoured *C. albicans* colonization and inflammation, whereas β-glucan fraction was protective against both. Surprisingly, purified β-glucans from *C. albicans* had the same protective effect. Thus, some yeasts appear to be strong modulators of intestinal inflammation and contribute in elimination of both *C. albicans* and *C. glabrata* in the gut. These effects are dependent on the strain, species, preparation process and cell wall fraction. It was striking that β-glucan fractions or pure β-glucans from *C. albicans* displayed the most potent anti-inflammatory effect in the DSS model. Studies to explore the mechanism of β-glucans mediated anti-inflammatory effects are ongoing.

Workshop Sessions

MEM-WK301.03 - Genetic and phenotypic diversity of cryotolerant Saccharomyces species in the Tibetan Plateau: evidence for Far East Asian origin of lager beer yeast

Feng-Yan Bai[1], Jian Bing[1], Pei-Jie Han[1], Wan-Qiu Liu[1], Qi-Ming Wang[1]
[1]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

Lager-brewing arising in 15th century Bavaria is the most popular technique for alcoholic beverage production in the world nowadays. The lager yeast Saccharomyces pastorianus is a domesticated microbe through the hybridization between a S. cerevisiae ale yeast and a cryotolerant Saccharomyces yeast. The latter dubbed S. eubayanus exhibiting 99.56% genome sequence identity with the non-ale subgenome of S. pastorianus has only been discovered in Patagonia, Argentina recently. Consequently, a Patagonian hypothesis for the origin of lager yeast has been proposed. Here we show that S. eubayanus and its close relative S. uvarum commonly occur in various substrates from the Tibetan Plateau and adjacent high altitude regions in west China and exhibit surprisingly high genetic and phenotypic diversity. Three distinct lineages with over 6% inter-lineage sequence divergence were identified from the S. eubayanus strains from China based on analyses of the ribosomal internal transcribed spacer (ITS) region and other 12 loci (10,657 bp) including nine protein genes and three intergenic spacers. Molecular phylogenetic analysis showed that a Tibetan population of S. eubayanus was more closely related with S. pastorianus than the Patagonian type strain. Sequence comparisons of the 12 loci from 10 strains and the whole genome from one representative strain showed that a Tibetan population of S. eubayanus is 99.8% identical to the non-S. cerevisiae subgenome of S. pastorianus. Our results suggest that S. eubayanus is native to Far East Asia, which harbors all the recognized natural species of Saccharomyces, and that the Tibetan population of S. eubayanus is the progenitor of lager yeast. This Far East Asian origin hypothesis has evidently stronger geographic and historic support.

Workshop Sessions

MEM-WK301.04 - Functional analyses of uncharacterized ORFs in wine yeast

Hennie van Vuuren[1], Zongli Luo[1], Christopher Walkey[1]
[1]*Wine Research Centre, University of British Columbia, Vancouver, Canada*

Saccharomyces cerevisiae has been studied extensively in laboratory media, and functions for 5000 of the ca. 6000 ORFs have now been determined. During wine fermentations, however, yeast cells are exposed to a challenging environment; low pH, hypoxia, high osmotic pressure, and a rising ethanol concentration. To investigate how yeast adapts to the harsh conditions during the fermentation of grape must, genome-wide mRNA levels were assayed at five time points during fermentation of grape must. The yeast cells responded to these stresses by activating a gene expression program called the Fermentation Stress Response (FSR). We identified 62 uncharacterized FSR ORFs whose expression was highly induced during fermentation. We used a systems biology approach (transcriptomics, proteomics and metabolomics) to discover functions for these ORFs. A deletion mutant of the YML081W ORF produced less acetic acid than its wild-type counterpart. A yeast strain overexpressing YML081W produced wine with elevated acetic acid levels. We named this gene *AAF1*, Acetic Acid Factor 1. We have now identified two more ORFs encoding putative transcription factors that affect acetic acid production in wine. Moreover, we found that the FSR protein Yfr017p interacts specifically with the glycogen debranching enzyme (Gdb1p). YFR017C null cells displayed a significant reduction in their ability to accumulate glycogen during aerobic growth and fermentation. Moreover, Yfr017p inhibits Gdb1p activity in vitro. These results suggest that Yfr017p functions as an inhibitor of Gdb1p, enhancing the ability of yeast cells to store glucose as glycogen. We therefore renamed the YFR017C ORF as *IGD1* (Inhibitor of Glycogen Debranching). We are currently analysing functions for an additional 150 ORFs that are constitutively expressed during wine fermentations.

Workshop Sessions

MEM-WK301.05 - Adaptive role of horizontal gene transfers in wine yeasts: how to improve resource utilization to survive better

Souhir Marsit[1], Frédéric Bigey[1], Jean-Luc Legras[1], Virginie Galeote[1], Sylvie Dequin[1]
[1]INRA, UMR1083 SPO, Montpellier, France

Saccharomyces cerevisiae wine yeasts display outstanding fermentative capacities during wine fermentation, where they are challenged with stressing and changing environmental conditions. We previously showed that horizontal gene transfers (HGT) from distant yeasts have shaped the genome of wine yeasts and may contribute to their adaptation to the wine environment. To assess the adaptive value of these HGT, we carried out a functional analysis of a mutant deleted for a large, 65kb introgression (region C). We found that this region confers a better cell viability and capacity to complete the fermentation in a Chardonnay grape must. This phenotype is associated to a better assimilation of the oligopeptide fraction of grape must by the horizontally acquired Fot1/2 oligopeptide transporters. We also showed that Fot-mediated peptides uptake affects the glutamate node and the NADPH/NADP+ balance, resulting in altered by-product profile. By carry out competition assays, we demonstrated that Fot deletion mutants were completely outcompeted by the wild-type strain after three successive co-cultures. Thus, the presence of FOT genes improves yeast fitness on grape must. Finally, a population analysis showed a strong conservation of FOT genes in the genome of yeast strains possessing the region C, even when this region is incomplete. These findings demonstrate the adaptive value of FOT genes which improve nutrient resource utilization in a nitrogen-limited environment, and highlight the role of HGT in genome evolution and adaptation of yeasts to their ecological niche.

Workshop Sessions

MEM-WK302.01 - Comparative genomics of Cochliobolus and Setosphaeria genes for secondary metabolism reveals virulence-determining metabolites for both necrotrophic and hemibiotrophic interactions with hosts

B. Gillian Turgeon[1]
[1] *Cornell Univ, Ithaca, USA*

Filamentous fungi produce chemically diverse secondary metabolites (SMs) that have positive and negative effects on other organisms and are implicated in virulence to hosts and development of structures associated with reproduction. SMs also combat nutritional and environmental stresses in niche situations, making them central to fungal survival and proliferation. SMs are at the very heart of the information networks that play out within a single organism, and between communities of interacting organisms. Sequencing of the genomes of highly aggressive plant pathogens in the genus Cochliobolus and in the closely related genus Setosphaeria has provided a clearer picture of the plethora of SM gene clusters encoding unknown SMs. Our lab employs a combination of genetic and comparative phylogenomic methods to explore function of SMs to make initial functional predictions (guilt by association). Comparative analyses have revealed that the suites of SM encoding genes from all Cochliobolus species are astoundingly diverse among species but remarkably conserved among isolates of the same species, except for lifestyle-defining examples that generally map to unique genomic regions. This pattern is also found when comparisons are made between the closely related Cochliobolus and Setosphaeria genera. Functional analysis of several of these strain-unique SMs reveals a strong correlation with a role in virulence for necrotrophs and, surprisingly, also for hemibiotrophs.

Workshop Sessions

MEM-WK302.02 - The bioherbicidal metabolites of the fungus Phoma macrostoma inhibits lycopene β-cyclase in the carotenoid biosynthetic pathway in susceptible hosts

Michelle Hubbard[1], Daniel Hupka[1], Russell Hynes[1], Karen Bailey[1]
[1]*Agriculture and Agri-Food Canada, Saskatoon, Canada*

Phoma macrostoma is a bioherbicidal fungus capable of inducing bleaching in susceptible dicot weeds by means of its metabolites, macrocidins. It is thought that macrocidins interfere with carotenoid biosynthesis. However, the point(s) in the biosynthetic pathway at which macrocidins act are not known. Based on the symptoms produced, we hypothesized that macrocidins interfere with the carotenoid biosynthetic enzyme phytoene desaturase. In order to test the above hypothesis, we treated both susceptible and resistant plants with two doses of macrocidins and the synthetic herbicide diflufenican (a known inhibitor of phytoene desaturase). We measured plant survival, growth, symptom severity, photosynthetic gas exchange and chlorophyll content. After harvesting the plants, we also used HPLC techniques to characterize plant carotenoid profiles. Results collected indicate that macrocidin-treated susceptible plants undergo a macrocidin dose-dependent increase in the ratio of lutein to β-carotene, suggesting impaired functioning of the enzyme lycopene β-cyclase. Enhanced understanding of the interactions between P. macrostoma metabolites and host plants could facilitate more effective use of P. macrostoma and its metabolites as a bioherbicide.

Workshop Sessions

MEM-WK302.03 - Septin-mediated plant tissue invasion by the rice blast fungus Magnaporthe oryzae

Yogesh Gupta[1], Yasin Dagdas[1], Miriam Oses-Ruiz[1], Lauren Ryder[1], Michael Kershaw[1], Wasin Sakulkoo[1], George Littlejohn[1], Nicholas Talbot[1]
[1]*School of Biosciences, University of Exeter, Exeter, UK*

Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, M. oryzae forms a specialised infection structure called an appressorium. The infection cell generates enormous turgor, which is focused as mechanical force to breach the rice cuticle and facilitate entry of the fungus into plant tissue. A hetero-oligomeric septin GTPase complex is necessary for re-organisation of a toroidal F-actin network at the base of the appressorium which allows re-establishment of polarised fungal growth. Re-modeling of F-actin at the appressorium pore is necessary for cortical rigidification and localisation of proteins associated with membrane curvature to the point of plant infection. Septin-mediated cytoskeletal re-modeling is required for development of a penetration peg that ruptures the host cuticle and leads to invasion of epidermal cells by biotrophic invasive hyphae of M. oryzae. Septin-mediated plant infection is controlled by NADPH oxidase activity and a regulated burst of reactive oxygen species occurs within the appressorium. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. The appressorium pore is the site of polarised exocytosis during plant infection and the octameric exocyst complex localises to the pore in a septin-dependent manner and is essential for cytoskeletal regulation. We will also describe the potential operation of both cell cycle and pressure-mediated checkpoints, that are necessary for initiation of septin activation and the re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion.

Workshop Sessions

MEM-WK302.04 - **Determining functional roles of LysM proteins in the ascomycete plant pathogen Cryphonectria parasitica**

Karyn Willyerd[1], Megan McClean[1], Angus Dawe[1]
[1]New Mexico State University, Las Cruces, USA

Proteins containing the lysin motif (LysM) have been shown to act as effectors in fungal pathogens. Secretion of effector proteins enables plant defenses to be overcome, allowing for fungal penetration into the host tissue. Studies have implicated the suppression of chitin recognition and PAMP-triggered defense responses of the plant host by fungal LysM effectors, which likely compete for binding with the pattern recognition receptors of the plant host. Five putative LysM proteins have been identified in the genome of the ascomycete plant pathogen and causative agent of chestnut blight, Cryphonectria parasitica. Using RT-PCR, we have detected transcript for all five of these genes indicating that they are expressed. To assess the potential role of these proteins in the virulence of C. parasitica, we have initially created three gene knockouts by homologous recombination. LM47 is a large predicted protein of 847 amino acids and containing a transmembrane Arv1 domain adjacent to a single LysM motif. The knockout of LM47 resulted in a severely debilitated phenotype that was avirulent on dormant chestnut stems. LM12 is a smaller predicted protein of 229 amino acids. Although the knockout showed no difference in phenotype or virulence on American chestnut when compared to wild-type, use of an epitope tagging strategy has revealed that it is extensively glycosylated. Repeated attempts to create a knockout of LM83 (172 predicted amino acids containing two LysM motifs) resulted in no viable fungal transformants, suggesting that this may be an essential protein. Epitope-tagged contructs for all five predicted proteins have been created in order to test for their secretion and ability to bind chitin or other similar molecules. This work will allow us to further characterize these members of the LysM protein family to act as effectors that allow for the establishment fungal infection.

Workshop Sessions

MEM-WK303.01 - The Met receptor tyrosine kinase interacts with the epidermal growth factor receptor and E-cadherin to induce epithelial cell endocytosis of Candida albicans

Quynh Phan[1], Norma Solis[1], Scott Filler[1,2]
[1]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, USA, [2]David Geffen School of Medicine at UCLA, Los Angeles, USA

Invasion of oral epithelial cells is a key step in the pathogenesis of oropharyngeal candidiasis (OPC). Previously, we found that *Candida albicans* invades oral epithelial cells by interacting with E-cadherin and a complex composed of the epidermal growth factor receptor (EGFR) and HER2. This interaction causes epithelial cells to endocytose *C. albicans* hyphae. Here we investigated the role of the Met receptor tyrosine kinase in triggering the endocytosis of *C. albicans*. The accumulation of EGFR, Met, and E-cadherin around *C. albicans* in the OKF6/TERT-2 oral epithelial cell line was determined by indirect immunofluorescence. The tyrosine phosphorylation of Met and EGFR was assessed by immunoblotting with phospho-specific antibodies. Epithelial cell invasion of *C. albicans* was quantified using a differential fluorescence assay. The therapeutic efficacy of the EGFR kinase inhibitor, gefitinib, and the Met kinase inhibitor, SGX523, was determined using the corticosteroid-treated mouse model of OPC. In epithelial cells infected with *C. albicans*, Met accumulated with both EGFR and E-cadherin around hyphae. Few hyphae were observed to be surrounded by Met without concomitant EGFR or E-cadherin. *C. albicans* infection stimulated the tyrosine phosphorylation of Met and EGFR. SGX523 inhibited phosphorylation of Met, but not EGFR. Similarly, gefitinib inhibited phosphorylation of EGFR, but not Met. Also, both SGX523 and Met siRNA decreased *C. albicans* invasion by approximately 50%. Dual inhibition of Met and EGFR resulted in greater reduction of epithelial cell invasion compared to inhibition of either receptor alone, whereas the combined inhibition of Met and E-cadherin was not additive. Finally, mice treated with SGX523 plus gefitinib had >10-fold lower oral fungal burden after 5 days of infection compared to control mice or mice treated with SGX523 or gefitinib alone. Therefore, *C. albicans* activates both Met and EGFR signaling pathways, which independently induce invasion of epithelial cells, both in vitro and in vivo.

Workshop Sessions

MEM-WK303.02 - Investigation of the role unknown function genes of Candida albicans in biofilm formation

Anna Carolina Borges Pereira Costa[1], Duncan Wilson[2], Bernhard Hube[2], Antonio Olavo Cardoso Jorge[1]
[1]ICT, UNESP – Univ Estadual Paulista, São José dos Campos, Brazil, [2]MPM; Hans- Knöll- Institute, Jena, Germany

Biofilm formation is one of the most important attributes for the virulence of Candida albicans, contributing to resistance to antifungal drugs and defence against attack by host immunity. However, the molecular mechanisms governing biofilm formation and development are not fully understood. In the current study we investigated 34 mutant strains lacking unknown function genes for biofilm formation. Biofilm formation and development of these mutants were evaluated by XTT (metabolic) assay, biomass, visual appearance and determination of disperser cells. Nine mutant strains showed significantly altered phenotypes in one or more of these analyses compared to an isogenic wild type. Deletion of orf19.6847, orf19.3908 and orf19.5417 reduced biofilm biomass. Metabolic activity was negatively altered for the mutant strains lacking orf19.6656, orf19.7504, orf19.7170, orf19.6501, orf19.823 and orf19.6784. Deletion of orf19.6656, orf19.7504 and orf19.6501 significantly reduced the dispersion of cells at early stages. Finally, patchy and irregular biofilms were observed for seven mutant strains. We have now investigated the functions of these previously uncharacterised genes to further our understanding of the biological processes involved in biofilm formation and development. For one gene, orf19.3908, we have identified additional roles in antifungal drug resistance and interactions with human epithelial cells.

Workshop Sessions

MEM-WK303.03 - Elucidating the evolutionary potential of Candida albicans with artificially activated transcription factors

Joachim Morschhäuser[1]
[1]University of Würzburg, Würzburg, Germany

During colonization and infection of its human host, the pathogenic yeast Candida albicans adapts to changes in the environment by reversibly altering its morphology and adjusting its gene expression pattern. C. albicans can also generate genetic variants that are better adapted to new conditions, as exemplified by the evolution of drug-resistant strains during antimycotic therapy. Resistance against the widely used antifungal drug fluconazole is frequently caused by gain-of-function mutations in the zinc cluster transcription factors Mrr1, Tac1, and Upc2. These mutations result in constitutive activity of the transcription factors and overexpression of their target genes, including the multidrug efflux pumps MDR1 and CDR1/CDR2. We found that Mrr1, Tac1, and Upc2 can also be rendered constitutively active by C-terminal fusion with the heterologous Gal4 activation domain, suggesting that this may represent a general strategy for the artificial activation of zinc cluster transcription factors. We therefore created a complete library of all 82 C. albicans zinc cluster transcription factors in a potentially hyperactive form, which were expressed in the wild-type reference strain SC5314. Screening of this library resulted in the discovery of novel regulators of morphogenesis and resistance to drugs and oxidative stress. Among these were Mrr2, which controls the expression of the major multidrug efflux pump CDR1, as well as several transcription factors that induced the expression of FLU1, an efflux pump that is required for tolerance of the antifungal peptide histatin 5. Artificial activation therefore is a highly useful method to elucidate the role of zinc cluster transcription factors of unknown function. As C. albicans frequently acquires natural gain-of-function mutations in these transcriptional regulators under selective pressure, the comprehensive library of strains containing hyperactive forms of all its zinc cluster transcription factors is a valuable resource to probe the evolutionary potential of this fungus when it faces novel environmental challenges.

Workshop Sessions

MEM-WK303.04 - Adhesin-dependent gene expression in Candida albicans biofilm formation

Saranna Fanning[1], Jigar Desai[1], Carol Woolford[1], Wenjie Xu[1], Heather Taff[2], Rachel Ehrlich[1], Tatyana Aleynikov[1], David Andes[2], Frederick Lanni[1], Aaron Mitchell[1]

[1]Department of Biological Sciences, Pittsburgh, USA, [2]Department of Medicine, University of Wisconsin, Madison, USA

Biofilm formation depends upon surface adherence, a property often mediated by adhesive proteins called adhesins. We report here a set of studies that involve genetic manipulation, nanoString profiling, and biofilm formation assays to elucidate the roles of a major Candida albicans adhesin, Als1, in biofilm maturation. Previous studies from several groups demonstrate that Als1 is required for the adherence of C. albicans cells to diverse substrates and to other C. albicans cells. Our studies indicate that Als1 is also required for expression of many biofilm- and hyphal-related genes. We call this response to Als1 levels the Adhesin-dependent Gene Expression (AGE) response. It is mediated by transcription factor Brg1, a known regulator of biofilm formation. Our phenotypic analysis indicates that the AGE response is required for biofilm maturation. Thus the adhesin Als1 functions in biofilm formation in two ways: to promote cell/surface adherence, and to stimulate biofilm maturation through the AGE response.

Workshop Sessions

VIR-WK200.01 - Paramyxovirus entry features with impact on human infection

Ilaria DeVito[1], Samantha Palmer[1], Stephen Jenkins[1], Stefan Niewiesk[2], Laura Marta Palermo[1], Matteo Porotto[1], Anne Moscona[1]

[1]Weill Medical College of Cornell University, NY, USA, [2]College of Veterinary Medicine, The Ohio State University, USA

Human parainfluenza viruses (HPIV) cause widespread respiratory infections, with no vaccines or effective treatments. HPIV3 infection starts when the viral receptor-binding protein engages sialic acid receptors in the lung and the viral envelope fuses with the target cell membrane. Fusion/entry requires interaction between two viral surface glycoproteins: a tetrameric hemagglutinin-neuraminidase (HN), and a fusion protein (F). We have defined structural correlates of HN's features that permit infection in vivo. Specific structural features of the HN dimer interface modulate HN-F interaction and fusion triggering and directly impact infection. The molecular determinants for HPIV3 growth in vitro are fundamentally different from those required in vivo, and these differences also impact inhibitor susceptibility. Fusion/entry molecular machinery that is advantageous for growth in human airway epithelia and in vivo confers susceptibility to peptide fusion inhibitors in the host lung tissue or animal. The same fusion inhibitors have no effect on infections with viruses whose fusion glycoproteins are suited for growth in vitro. We propose that in order to provide information relevant to clinical efficacy, antivirals should be evaluated using clinical isolates or lung adapted strains of virus in natural host tissue, rather than lab strains of virus in cultured cells. The unique susceptibility of clinical strains in human tissues reflects viral inhibition in vivo.

Workshop Sessions

VIR-WK200.02 - Measles virus infection of the central nervous system

Ilaria DeVito[1], Eric Jurgens[1], Jean-Louis Palgen[1], Laura Marta Palermo[1], Anne Moscona[1], Matteo Porotto[1]
[1]*Weill Cornell Medical College, NY, USA*

Measles virus (MV) is a leading cause of child mortality in developing countries despite the availability of a live attenuated vaccine for over 40 years. In the U.S., we are in the midst of a nation-wide outbreak. In a recent MV outbreak in South Africa several HIV-infected people died of MV CNS infection. We analyzed the viruses from these patients and found that specific intra-host evolution of the MV fusion machinery -- receptor binding protein (H) + fusion protein (F) -- had occurred. A mutation in F of the “CNS-adapted” viruses allows it to promote fusion with less dependence on interaction of H with the two known MV cellular receptors; this F is activated independently of H or receptor. We hypothesize that in the absence of effective cellular immunity, MV variants bearing fusion machinery that enabled efficient spread in the CNS underwent positive selection. The measles inclusion body encephalitis (MIBE) F isolated from the HIV-infected patients possesses an inherent ability to mediate fusion in the absence of H; we propose that this feature is ideal for CNS infection but may be detrimental for acute infection.

Workshop Sessions

VIR-WK200.03 - Role of the cytoskeleton in assembly and transmission of human metapneumovirus

Farah El Najjar[1], Rebecca Dutch[1]
[1]*University of Kentucky, Lexington, USA*

The paramyxovirus human metapneumovirus (HMPV) is a leading cause of respiratory tract infections worldwide across all age groups, and is particularly devastating in the pediatric, elderly and immunocompromised populations. Despite its high prevalence and burden on human health, there is currently no treatment against HMPV infections. The process of forming new virus particles requires complex interactions between viral and cellular components and the requirements for particle production differ substantially among paramyxoviruses. The mechanisms by which HMPV assembles and buds are very poorly understood, so we sought to characterize the viral and host factors involved in this late stage of the viral life cycle. We performed spatio-temporal analysis of the main HMPV structural proteins including nucleoprotein N, phosphoprotein P, matrix protein M and fusion glycoprotein F in normal human bronchial epithelial cells, which constitute a relevant model for studying HMPV infection. Our results show that N and P are localized in perinuclear cytoplasmic inclusions during early infection that subsequently relocate to the plasma membrane and incorporate M protein. At late infection, all viral proteins translocate to the plasma membrane and form cell-associated viral filaments. Interestingly, long filamentous extensions containing HMPV proteins extend between cells suggesting a role in direct cell-to-cell transmission. Cytoskeletal inhibitors studies showed that microtubules and actin are critical for the production of released HMPV particles. In addition, these studies suggested a role of actin in HMPV direct cell-to-cell spread. These results are very interesting since paramyxoviruses are generally thought to transmit from cell to cell in the form of cell-free particles. Overall, our data reveal a novel role of the cytoskeleton in HMPV replication and provide new insights into mechanisms of HMPV transmission.

Workshop Sessions

VIR-WK200.04 - The messenger RNA synthesis machinery of vesicular stomatitis virus

Sean P.J. Whelan[1]
[1]*Department of Microbiology & Immunobiology, Harvard Medical School, Boston, USA*

The RNA synthesis machinery of vesicular stomatitis virus comprises the negative-sense genomic RNA encased within a nucleocapsid protein (N) sheath and associated with the viral transcriptase. The transcriptase itself comprises a multifunctional 250 kDa large protein (L) and this requires association with a 29kDa phosphoprotein (P) to bind the N-RNA template. All of the catalytic activities required for the synthesis of mRNA reside within the L protein including an RNA dependent RNA polymerase (RdRP), a GDP:polyribonucleotidyltransferase (PRNTase), and a dual specificity mRNA cap methyltransferase (MTase). We have integrated genetic, biochemical, structural, functional and inhibitor approaches to understand the mechanism by which this minimal mRNA synthesis machine functions during mRNA synthesis. We show that although the L protein alone can copy naked RNA, it requires complex formation with P to induce a structural rearrangement that enhances initiation and processivity of the RdRP. We also demonstrate that the template associated N protein directly influences the enzymatic properties of L, resulting in full processivity of the RdRP, and also in structurally ordering the RdRP to alter its sensitivity to modified nucleotides that are candidate polymerase inhibitors. Structural studies also reveal the architecture of the polymerase itself and the corresponding locations of the RdRP, the PRNTase and the MTase domains. During virus assembly, transcriptase activity is downregulated and the polymerase is packaged into the virion. We now show that this is accomplished by the viral matrix protein directly binding the L protein and inhibiting its activity. Parallels between the VSV RNA synthesis machinery and that of other RNA viruses will be discussed.

Workshop Sessions

VIR-WK200.05 - Immunopathogenesis of Henipavirus infection

Cyrille Mathieu[1], Kevin Dhondt[1], Romain Vives[2], Branka Horvat[1]
[1]International Center for Infectiology Research (CIRI), Lyon, France, [2]Institut de Biologie Structurale Jean-Pierre Ebel, Grenoble, France

Nipah virus (NiV) and Hendra virus (HeV) are closely related, recently-emerged paramyxoviruses, belonging to Henipavirus genus. These zoonotic viruses are capable of causing considerable morbidity and mortality in a number of mammalian species, including humans and have been classified as BSL-4 agents. Both viruses infect cells via ephrinB2 and B3 entry receptors, highly conserved among species. We have characterized a new animal model of henipavirus infection in mice deleted in Interferon-Type I Receptor and extended the study to different transgenic lines lacking particular Pathogen Recognition Receptors, essential for the initiation of immune response. These models allowed to study the immunopathogenesis of the henipavirus infection and to characterize critical control points regulating viral expansion within the host and generation of immunity. Furthermore, we found that in contrast to closely related, highly lymphotropic Morbillivirus, murine and human lymphocytes were not permissive for NiV infection. Interestingly, despite the absence of infection, human lymphocytes could efficiently bind both NiV and HeV and transfer the virus to permissive cells. The transinfection is mediated by an attachment receptor, which we have identified here as heparan sulphate. NiV and HeV infection in hamsters closely reproduces symptoms seen in humans and allowed us to analyze the importance of viral transinfeciton in vivo. We show that circulating leukocytes captured and carried NiV after intraperitoneal infection, without themselves being productively infected and were able to transmit virus to permissive cells ex vivo. Use of heparin efficiently blocked NiV transinfection and significantly improved survival of NiV-infected hamsters. Altogether, these results reveal a remarkable capacity of NiV to hijack lymphocytes in order to transinfect host cells, representing a rapid and potent way for Henipavirus dissemination throughout the organism, which may contribute to its high pathogenicity. They open also novel perspectives for the development of new therapeutic approaches against these emergent infections.

Workshop Sessions

VIR-WK200.06 - Real-time bioluminescence monitoring of henipavirus infection in a small rodent animal model

Tatyana Yun[1], Terence Hill[1], Arnold Park[2], Terry Juelich[1], Jennifer Smith[1], Lihong Zhang[1], Benhur Lee[2,3], Alexander Freiberg[1]

[1]Department of Pathology, University of Texas Medical Branch, Galveston, USA, [2]Department of Microbiology, Immunology and Molecular Genetics, University of California Los Angeles, Los Angeles, USA, [3]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA

Nipah (NiV) and Hendra (HeV) viruses are the deadliest human pathogens within the Paramyxoviridae family (genus henipavirus, HNV), causing acute fatal encephalitis or severe pneumonia with high case fatality rates in humans in Southeast Asia and Australia. Due to their extreme pathogenicity, potential for human-to-human transmission, (agro-) bioterrorism, and the lack of licensed human vaccines, HNVs are the only paramyxoviruses classified as biosafety level 4 (BSL-4) pathogens. HNVs are transmitted through the oronasal-oropharyngeal routes, which leads to systemic infection resulting in end-organ vasculitis, respiratory distress, and acute encephalitis. To characterize the pathogenic symptomology in a small animal model after infection with HNVs, we established a highly efficient reverse genetics system that enabled generation of recombinant NiV (rNiV) and HeV (rHeV) expressing Firefly luciferase (Fluc). Infection of interferon-α/β receptor knockout mice with rNiV-Fluc allowed monitoring incipient and ongoing viral spread in vivo via bioluminescence, a first for BSL-4 conditions. Intraperitoneal inoculation resulted in initial viral replication in the spleen, followed by spread of NiV to the lungs, respiratory tract and nasal turbinates, prior to infection of the olfactory bulb and viral invasion of the brain. These results provide evidence that invasion of the brain may result at least in part from respiratory transmission to the olfactory neurons. In contrast, intranasal inoculation resulted in viral spread that was limited to the upper respiratory tract, and CNS invasion again appeared to follow replication in the olfactory bulb, likely from direct infection of olfactory neurons. Overall, these data demonstrate the applicability of Fluc-expressing HNVs to allow for sensitive detection and identification of early replication sites, spread and development of disease, with the ultimate goal of identifying the molecular determinants of HNV-induced neuropathobiology.

Workshop Sessions

VIR-WK201.01 - Biosecurity implications of new technology and discovery in plant virus research

Robin MacDiarmid[1], Brendan Rodoni[2], Francisco Ochoa-Corona[3], Marilyn Roossinck[4], Ulrich Melcher[5], Fiona Constable[2]

[1]The New Zealand Institute for Plant & Food Research Limited and The University of Auckland, Auckland, New Zealand, [2]Department of Primary Industries, Victoria, Australia, [3]Department of Entomology & Plant Pathology, Oklahoma State University, Stillwater, USA, [4]Plant Pathology and Biology, Center for Infectious Disease Dynamics, Pennsylvania State University, University Park, USA, [5]Department of Biochemistry & Molecular Biology, Oklahoma State University, Stillwater, USA

Human activity is causing new encounters between viruses and plants. Anthropogenic interventions include changing land use, decreasing biodiversity, trade, the introduction of new plant and vector species to native landscapes, and changing atmospheric and climatic conditions. The discovery of thousands of new viruses, especially those associated with healthy appearing native plants, is shifting the paradigm for their role within the ecosystem from foe to friend. The cost of new plant virus incursions can be high and result in the loss of trade and/or production for short or extended periods. We present and justify three recommendations for plant biosecurity to improve communication about plant viruses, assist with the identification of viruses and their impacts, and protect the high economic, social, environmental and cultural value of our respective nations' unique flora: 1) As part of the burden of proof, countries and jurisdictions should identify what pests already exist in, and which pests pose a risk to, their native flora, 2) Plant virus sequences not associated with a recognized virus infection are designated as "uncultured virus" and tentatively named using the host plant species of greatest known prevalence, the word 'virus', a general location identifier, and a serial number, 3) Invest in basic research to determine the ecology of known and new viruses with existing and potential new plant hosts and vectors and develop host-virus pathogenicity prediction tools. These recommendations have implications for researchers, risk analysts, biosecurity authorities, and policy makers at both a national and an international level.

Workshop Sessions

VIR-WK201.02 - Genetic diversity of alphacoronaviruses and betacoronavirus in two bat communities

Xingyi Ge[1], Ben Hu[1], Xinglou Yang[1], Ning Wang[1], Zhengli Shi[1]
[1]Center for Emerging Infectious Diseases, State Key Laboratory of Virology, Wuhan Institute of Virology of the Chinese Academy of Sciences, Wuhan, China

Bats have been identified as natural reservoirs of severe acute respiratory syndrome coronavirus (SARS-CoV) and possibly for Middle East respiratory syndrome coronavirus (MERS-CoV). Diverse bat coronaviruses (CoVs) have been found around the world. To better understand ecological transmission of bat CoVs in the same location, we conducted a CoV surveillance in two bat communities located in Yunnan Province, China. RT-PCR was conducted by amplifying viral partial RNA-dependent RNA polymerase (RdRp), nucleocapsid (N), and spike (S) genes, followed by sequencing confirmation. In bat community one that was dominated with Rhinolophus Subrufus, Rhinolophus affinis, Hipposideros pomona, Hipposideros armiger, and Miniopterus schreibersi, alphacoronaviruses homologous to HKU2, HKU8, HKU7, HKU10, 1A, 1B, BtCoV/A1196/2005, BtCoV/860/2005, and betacoronavirus homologous to Hipposideros/KT/Thailand/2007 were detected, with an infection rate of 56.9% (103/181). In bat community two that was dominated with Rhinolophus sinicus, 7 different SARS-like CoVs were detected, with an infection rate of 16.4% (51/318). Frequent co-infection of different CoVs in same individual bat were detected in bat community one, with an coinfection rate of 15.5% (28/181). Our study revealed high genetic diversity, infection rate, and co-infection rate of CoVs in bat communities with high density. It is could be suspected that the high population density of bats in these caves facilitated CoV persistent circulation in different colonies and promoted CoV transmission. The coinfection of different CoVs in one individual increase the possibility of recombination to produce more novel CoVs.

Workshop Sessions

VIR-WK201.03 - Emerging insights into hantavirus evolution from a newly recognized hantavirus in the long-tailed mole (Scaptonyx fusicaudus)

Hae Ji Kang[1], Se Hun Gu[1], Joseph A. Cook[2], Richard Yanagihara[1]
[1]University of Hawaii at Manoa, Honolulu, USA, [2]University of New Mexico, Albuquerque, USA

Genetically distinct hantaviruses in multiple species of shrews (order Soricomorpha, family Soricidae), belonging to the Soricinae, Crocidurinae and Myosoricinae subfamilies, have spurred the discovery of novel hantaviruses in moles (family Talpidae), in the Talpinae and Scalopinae subfamilies, including the Japanese shrew mole (Urotrichus talpoides), American shrew mole (Neurotrichus gibbsii), European mole (Talpa europaea) and eastern mole (Scalopus aquaticus). We now report the detection of another novel hantavirus, named Dahonggou Creek virus (DHCV), in archival heart and kidney tissues from long-tailed moles (Scaptonyx fusicaudus) captured 17 km south-southeast of Shimian along Dahonggou Creek in Sichuan province, China, in August 1989. Pair-wise alignment and comparison of a 1,058-nucleotide region of the L segment indicated that DHCV was genetically distinct from other rodent- and soricomorph-borne hantaviruses. Phylogenetic analyses, using maximum-likelihood and Bayesian methods, showed that DHCV was basal to a highly divergent lineage comprising Thottapalayam and Imjin viruses (in Asian crocidurine shrews) and Uluguru and Kilimanjaro viruses (in African myosoricine shrews). Direct comparison between DHCV (GenBank HQ616595) and hantavirus YN05-47 (GenBank JF915719), detected in a long-tailed mole from northwestern Yunnan province, was not possible, because they were of different regions of the L segment. However, YN05-47, which was closely related to Jeju virus, a hantavirus harbored by the Asian lesser white-toothed shrew (Crocidura shantungensis), may represent a host-switching event. The fossorial long-tailed mole, which is restricted to montane coniferous forests in central and southern China, extending to northern Myanmar and northern Vietnam, is sympatric with Uropsilus moles, which might also serve as reservoirs of hantaviruses. As evidenced by their phylogenetic positions in each of the four hantavirus clades, mole-borne hantaviruses may be somewhat more catholic in their host proclivity than present-day rodent-borne hantaviruses, suggesting that ancestral moles may have served as the early hosts of primordial hantaviruses.

Workshop Sessions

VIR-WK201.04 - Bringing viral dark matter into the light

Cheryl-Emiliane T. Chow[1], Curtis A. Suttle[1]
[1]*Univeristy of British Columbia, Vancouver, Canada*

"No abstract available at time of publication"

Workshop Sessions

VIR-WK201.05 - Discovery of a divergent hantavirus lineage in soricine shrews in Siberia

Hae Ji Kang[1], Se Hun Gu[1], Shannon Bennett[2], Satoru Arai[3], Liudmila N. Yashina[4], Jin-Won Song[5], Joseph A. Cook[6], Richard Yanagihara[1]

[1]University of Hawaii at Manoa, Honolulu, USA, [2]California Academy of Sciences, San Francisco, USA, [3]National Institute of Infectious Diseases, Tokyo, Japan, [4]State Research Center of Virology and Biotechnology "Vector", Koltsovo, Russia, [5]Korea University College of Medicine, Seoul, Republic of Korea, [6]University of New Mexico, Albuquerque, USA

With the recent discovery of genetically diverse hantaviruses (family Bunyaviridae, genus Hantavirus) in shrews and moles (order Soricomorpha) and insectivorous bats (order Chiroptera), rodents (order Rodentia) are increasingly unlikely to have been the ancestral reservoir hosts of hantaviruses. Most soricomorph-borne hantaviruses fall into older and well-resolved lineages organized largely by host taxa and geographic origin. However, in 2008, we first began detecting hantavirus sequences that did not conform to their reservoir host species or geographic location. That is, within syntopic populations of Sorex araneus, Sorex caecutiens and Sorex roboratus captured at the same time and in the same location in Siberia, we found co-circulation of highly divergent hantaviruses. Moreover, within different Sorex species, such as Sorex minutissimus, captured at the same time in the same location, we found similarly divergent hantavirus sequences. During the past five years, we have accumulated many more examples of these highly distinctive hantavirus sequences from Sorex species in Siberia, as well as from soricine shrew species in Europe and North America. Pair-wise alignment and comparison of partial and full-length S-, M- and L-segment sequences indicated that distinct hantaviruses were being maintained simultaneously in multiple soricine shrew species. Phylogenetic analyses, using maximum-likelihood and Bayesian methods, indicated a unique clade comprising hantaviruses from multiple soricine species that formed a distinct lineage from that harbored by the European mole and insectivorous bats. Our data suggest that this hantavirus lineage may have diverged early in hantavirus evolution. Together with its disjunctive distribution across the Soricidae, this may represent an ancestral hantavirus lineage that subsequently diversified independently within the Soricini tribe in Eurasia and Blarini tribe in North America.

Workshop Sessions

VIR-WK201.06 - Co-circulation of two distinct divergent hantaviruses in Sorex species in Mongolia

Yu Ikeyama[1], Satoru Arai[1], Bazartseren Boldgiv[2], Bazartseren Boldbaatar[2], Kazuko Araki[1], Hiroshi Satoh[1], Keiko Tanaka-Taya[1], Shigeru Morikawa[1], Richard Yanagihara[3], Kazunori Oishi[1]

[1]National Institute of Infectious Diseases, Tokyo, Japan, [2]National University of Mongolia, Ulan Bator, Mongolia, [3]University of Hawai'i at Manoa, Honolulu, USA

Recently, genetically distinct hantaviruses have been detected in multiple species of shrews (order Soricomorpha, family Soricidae) in Europe, Asia, Africa and North America. Seewis virus (SWSV), originally found in the Eurasian common shrew (Sorex araneus) in Switzerland, is now known to be widespread across the geographic range of its reservoir host. In Siberia, Russia, SWSV has been reported in the Eurasian common shrew, tundra shrew (S. tundrensis) and Siberian large-toothed shrew (S. daphaenodon), suggesting host sharing. To ascertain if SWSV is also hosted by Sorex shrews in Mongolia, we extracted total RNA from lung tissues of 21 Laxmann's shrews (S. caecutiens), six Eurasian least shrews (S. minutissimus), one flat-skulled shrew (S. roboratus) and 40 tundra shrews, captured near Khovsgol Lake in Mongolia, during 2010 and 2011. Hantavirus RNAs were detected by RT-PCR in five tundra shrews, two Laxmann's shrews and one Eurasian least shrew. Pair-wise alignment and comparison of partial S-, M- and L-segment sequences indicated SWSV in four tundra shrews. By contrast, two Laxmann's shrews, one Eurasian least shrew and one tundra shrew, all captured at the same time and in the same location, harbored a highly divergent hantavirus, tentatively named Khovsgol Lake virus (KHLV). KHLV was genetically distinct from SWSV and other Sorex-borne hantaviruses in Eurasia, including Yakeshi virus in the taiga shrew (S. isodon), Qian Hu Shan virus in the greater striped-backed shrew (S. cylindricauda), Asikkala virus in the Eurasian pygmy shrew (S. minutus), Kenkeme virus in the flat-skulled shrew and Artybash virus/Amga virus in the Laxmann's shrew. Phylogenetic analyses, using maximum-likelihood and Bayesian methods, indicated that KHLV was most closely related to Altai virus (ALTV), previously reported in a Eurasian common shrew captured in Siberia. The co-circulation of two highly divergent lineages of hantaviruses among syntopic soricine shrew species in Mongolia warrants further intensive investigation.

Workshop Sessions

VIR-WK201.07 - Characterization of a new Chlorovirus type that has permissive and non-permissive features on phylogenetically related algae strains

Cristian Quispe[1], Olivia Sonderman[1], James Van Etten[1]
[1]Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, USA

Chloroviruses are large, icosahedral dsDNA viruses that are ubiquitous in fresh water reaching titers as high as 100 000 plaque forming units (pfu) per mL of native water. About 30 years ago, Paramecium bursaria chlorella virus 1 (PBCV-1) was described as the Chlorovirus prototype that replicates in two distinctive symbiotic algae strains, Chlorella varabilis NC64A and Chlorella sp. Syngen 2-3; both strains are ex-symbionts originally isolated from the protozoan Paramecium bursaria. As part of a three-year longitudinal study to monitor chloroviruses in natural aquatic environments in Nebraska, both strains were used for plaque assays. Surprisingly, pfu numbers on Syngen 2-3 lawns were significantly greater than pfu numbers on NC64A from the same samples. This unexpected result led us to conduct comparative studies on viruses that infected Syngen 2-3 with viruses that infect both hosts. As a result, a novel virus named Only Syngen Nebraska virus 1 (OSyNE-1) that infects exclusively the ex-symbiotic Chlorella sp. Syngen 2-3 but not the NC64A strain was isolated from native water. Importantly, we discovered that Syngen 2-3 is a chlorella strain that can be infected by two distinct virus populations. As part of the virus characterization, we are investigating how OSyNE viruses differ from PBCV-1.

Workshop Sessions

VIR-WK202.01 - Cell Biology of Viroid Infection

Biao Ding[1]
[1]*The Ohio State University, Columbus, USA*

Viroids are circular and noncoding RNAs that infect plants. They replicate in the nucleus or chloroplast and then traffic from cell to cell and from organ to organ to establish systemic infection. Viroids encode all functions within the RNA itself, including stability, intracellular and intercellular trafficking, replication, and pathogenesis. Our recent studies have identified some 3D motif structures of Potato spindle tuber viroid (PSTVd) that are important for replication and trafficking between specific cells. We have also identified a cellular factor that appears to be important for PSTVd replication. I will present our findings and discuss their biological implications.

Workshop Sessions

VIR-WK202.02 - Coilin, the signature protein of Cajal Bodies, differentially modulates the interactions of plants with viruses in widely different taxa

Michael Taliansky[1], Jane Shaw[1], Andrew Love[1], Svetlana Makarova[2], Natalia Kalinina[2], Bryan Harrison[1]
[1]James Hutton Institute, Dundee, UK, [2]MV Lomonosov Moscow State University, Moscow, Russia

Cajal bodies (CBs) are distinct nuclear bodies physically and functionally associated with the nucleolus. In addition to their traditional function in coordinating maturation of certain nuclear RNAs, CBs participate in cell cycle regulation, development, and regulation of stress responses. A key “signature” component of CBs is coilin, the scaffolding protein essential for CB formation and function. Using an RNA silencing (loss-of-function) approach, we describe here new phenomena whereby coilin also affects directly or indirectly, a variety of interactions between host plants and viruses that have RNA or DNA genomes. Moreover, the effects of coilin on these interactions are manifested differently: coilin contributes to plant defence against tobacco rattle virus (tobravirus), tomato black ring virus (nepovirus), barley stripe mosaic virus (hordeivirus) and tomato golden mosaic virus (begomovirus). In contrast, with potato virus Y (potyvirus) and turnip vein clearing virus (tobamovirus), coilin serves to increase virus pathogenicity. These findings show that interactions with coilin (or CBs) may involve diverse mechanisms with different viruses, and that these mechanisms act at different phases of virus infection. Thus coilin (CBs) has novel, unexpected natural functions that may be recruited or subverted by plant viruses for their own needs or, in contrast, are involved in plant defence mechanisms that suppress host susceptibility to the viruses

Workshop Sessions

VIR-WK202.03 - **Intracellular plant virus transport: mapping the highways within cells**

Xiaohua Yang[1], Andres Rodriguez[2], Chengke Liu[1], Carlos Angel[2], James Schoelz[2], Richard Nelson[1]
[1]Division of Plant Biology, The Samuel Roberts Noble Foundation, Ardmore, USA, [2]Division of Plant Sciences, University of Missouri, Columbia, USA

Plant viruses and their components must move within cells to systemically infect their host and cause disease. An understanding of intracellular virus movement will provide information necessary to design and test new methods to prevent systemic virus infections. We have studied the intracellular movement of two viruses,
Tobacco mosaic virus (TMV), and *Cauliflower mosaic virus* (CaMV). These viruses have divergent genomes, however, they both form inclusion bodies (IBs) within cells that are considered the sites for replication of the viral genome. TMV IBs contain the 126 kDa protein whereas the primary component of CaMV IBs is its p6 protein. Both proteins form inclusion bodies during ectopic expression that associate with and traffic along microfilaments. The TMV 126 kDa protein is also required for normal virus intercellular movement and intracellular accumulation. We have identified over 100 host proteins that interact with the 126 kDa and p6 proteins in yeast. We functionally characterized several of these proteins, showing through transient and stable knockout/knockdown studies that they contribute to normal virus spread in plants. Some of these proteins are involved in vesicle trafficking or intra-organelle (vacuole) processing. For one protein known to associate with membranes (Ca-dependent membrane targeting protein), its intracellular localization suggests its involvement in virus transport between cells. From these findings we will present updated models of TMV and CaMV trafficking within cells.

Workshop Sessions

VIR-WK202.04 - Cucumber necrosis virus infection results in de novo generation of peroxisomes from the endoplasmic reticulum and oxidative stress

D'Ann Rochon[1], Bhavana Singh[2], Jane Theilmann[1], Ron Reade[1], Kankana Ghoshal[2], Syed Benazir Alam[2], Ajay Maghodia[2]

[1]Agriculture and Agri-Food Canada, Pacific Agr-Food Research Centre, Summerland, Canada, [2]University of British Columbia, Faculty of Land and Food Systems, Vancouver, Canada

Tombusviruses replicate on pre-existing organelles such as peroxisomes or mitochondria, the membranes of which become extensively reorganized into multivesicular bodies (MVBs) during the infection process. Cucumber necrosis virus (CNV), a positive-sense ssRNA virus and member of the Tombusvirus genus, has previously been shown to replicate in association with peroxisomes in yeast. We show that CNV induces MVBs from peroxisomes in infected plants and that GFP-tagged CNV p33 auxiliary replicase protein colocalizes with YFPSKL, a peroxisomal marker. Most remarkably, the endoplasmic reticulum (ER) of CNV infected N. benthamiana 16C plants undergoes a dramatic reorganization producing numerous new peroxisome-like structures that associate with CNV p33-RFP, thus likely serving as a new site for viral RNA replication. We also show that leaves of plants agroinfiltrated with p33 develop CNV-like necrotic symptoms and contain increased levels of peroxide. Since peroxisomes are a site for peroxide catabolism, and peroxide is known to induce plant defense responses, we suggest that dysfunctional peroxisomes contribute to CNV induced necrosis. Consistent with this we have found that p33 infiltrated plants (but not control vector infiltrated plants) show decreased levels of glycolate oxidase and increased levels of UDP-glucosyl transferase which are features of plants under oxidative stress. p33 agroinfiltrated plants also show increased levels of Hin1 and NPR1 which are markers for the hypersensitive response (HR). Additional experiments are being conducted to assess the role of p33 in oxidative stress and HR.

Workshop Sessions

VIR-WK202.05 - The role of different Argonaute proteins in the defense against a plant RNA virus

Chantal Brosseau[1], Peter Moffett[1]
[1]*Département de biologie, Université de Sherbrooke, Sherbrooke, Canada*

RNA silencing is a major mechanism of constitutive antiviral immunity in plants. *Arabidopsis* encodes four Dicer-like (DCL) proteins and ten different Argonaute (AGO) proteins that are specialized to function in different RNA silencing-related mechanisms. To date, AGO1, AGO2 and AGO7 are known to possess antiviral activity against different viruses. We have previously shown that AGO2 plays an important role in protecting plants against viruses, including resistance to Potato virus X (PVX) in *Arabidopsis*.Despite strong antiviral activity of AGO2, *Arabidopsis* appear to require additional Argonaute for full defense against PVX. To investigate which Argonautes are required for resistance against PVX in *Arabidopsis*, we have used transient expression of AtAGO proteins in the susceptible host *Nicotiana benthamiana*. Surprisingly, using this system we found that all AtAGO proteins possess intrinsic antiviral activity against a PVX mutant that is unable to form a normal replication complex suggesting that viral replication may play a major role in protecting the viral genome. However, only AtAGO2 and AtAGO5 affected wild-type PVX. Consistent with this result, we find that AGO5 is induced during PVX infection and that *Arabidopsis ago2/ago5* double mutants are highly susceptible to PVX, compared to single mutants. We have also used an RNA pull-down technique to show that AGO2 is able to directly bind viral RNA. Using different viral RNA suppressor that sequester small RNAs, we demonstrate that antiviral activity of AGO requires the presence of small RNAs. Together, our results indicate that anti-viral defenses in plants uses a combination of different AGO proteins and these defenses are affected by viral replication strategies and viral suppressors of RNA silencing.

Workshop Sessions

VIR-WK202.06 - A simple protocol for screening pepper genes that confer a hypersensitive response to pepper viruses

Phu-Tri Tran[1], Hoseong Choi[1], Saet-Byul Kim[1], Hyun-Ah Lee[1], Doil Choi[1], Kook-Hyung Kim[1]
[1]Seoul National University, Seoul, Korea

Plant NBS-LRR genes are abundant and have been increasingly cloned from plant genomes. In this study, a method based on agroinfiltration and virus inoculation was developed for the simple and inexpensive screening of candidate R genes that confer a hypersensitive response (HR) to plant viruses. The well-characterized resistance genes Rx and N, which confer resistance to Potato virus X (PVX) and tobamovirus, respectively, were used to optimize a transient expression assay for detection of HR in Nicotiana benthamiana. Infectious sap of PVX and Tobacco mosaic virus were used to induce HR in Rx- and N-infiltrated leaves, respectively. The transient expression of the N gene induced local HR upon infection of another tobamovirus, Pepper mild mottle virus, through both sap and transcript inoculation. When this protocol was used to screen 99 candidate R genes from pepper, an R gene that confers HR to the potyvirus Pepper mottle virus was identified. The protocol will be useful for the identification of plant R genes that confer resistance to viruses.

Workshop Sessions

VIR-WK202.07 - Autonomous long-distance trafficking of a subviral satellite RNA in plants

Chih-Hao Chang[1], Fu-Chen Hsu[1], Yih-Shan Lo[1], Jane Shaw[2], Michael Taliansky[2], Ban-Yang Chang[3], Yau-Heiu Hsu[4], Lin Na-Sheng[1]

[1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, [2]The James Hutton Institute, Invergowrie, Dundee, UK, [3]Department of Biochemistry, National Chung Hsing University, Taichung, Taiwan, [4]Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan

RNA trafficking plays pivotal roles in regulating plant development, gene silencing and adapting to environmental stresses. Satellite RNAs (satRNAs), parasites of viruses, are exclusively associated with plant viruses and depend on their helper viruses (HVs) for replication, encapsidation and efficient spread. However, how satRNAs interact with viruses and the cellular machinery to achieve trafficking remains mostly unknown. In this study, we demonstrated that, unlike other satRNAs, Bamboo mosaic potexvirus satRNA (satBaMV)-encoded P20 protein can functionally complement in trans the systemic trafficking of P20-defective satBaMV in infected plants. Moreover, transgene-derived satBaMV, uncoupled from HV replication, could move autonomously across the graft union. Notably, silencing nucleolar fibrillarin suppressed satBaMV but not HV phloem-mediated movement by grafting or by co-inoculation with the HV. This is the first host factor known to be differentially required for HV and satRNA ribonucleoprotein-complex trafficking mediated by phloem. These findings will shape our view of trafficking of subviral satRNAs and should lead to identifying the host proteins facilitating the cellular RNA transport in plants.

Workshop Sessions

VIR-WK203.01 - The host protease TMPRSS2 plays a major role for influenza virus replication in vivo

Kouji Sakai[1], Yasushi Ami[1], Maino Tahara[1], Toru Kubota[1], Noriko Nakajima[1], Makoto Kuroda[1], Hideki Hasegawa[1], Yoshihiro Kawaoka[2], Masato Tashiro[1], Makoto Takeda[1]

[1]National Institute of Infectious Diseases, Tokyo, Japan, [2]University of Tokyo, Tokyo, Japan

Cleavage of the hemagglutinin (HA) protein of influenza A virus (IAV) is mediated by a host cell protease(s) in vivo and essential for virus infectivity. Although various proteases have been shown to cleave HA in vitro and in cultured cells, it remains unclear whether these proteases redundantly contribute or a specific protease mainly contributes to HA cleavage in vivo. We generated TMPRSS2 knockout (KO) mice, which showed normal reproduction, development, and growth phenotypes. TMPRSS2 is a type II transmembrane serine protease expressed in the respiratory tract. Analysis in vitro and in cultured cells showed that this protease activates a variety of respiratory viruses, including low-pathogenic (LP) IAV possessing a single arginine residue at the cleavage site. Our data showed that, in TMPRSS2 KO mice, the majority of LP IAV particles failed to gain infectivity due to insufficient HA cleavage. Accordingly, unlike WT mice, TMPRSS2 KO mice were highly tolerant against challenge infection by LP IAVs, including seasonal H1N1 and H3N2 and recently emerging H7N9 subtypes. On the other hand, infection with a high-pathogenic (HP) H5N1 subtype IAV possessing a multi-basic cleavage site was lethal for TMPRSS2 KO mice, as observed for WT mice. Our results demonstrate that H1N1, H3N2, and H7N9 subtype LP IAVs mainly use the specific protease TMPRSS2 for HA cleavage in vivo, and that TMPRSS2 is essential for IAV pathogenesis.

Workshop Sessions

VIR-WK203.02 - Functional regulation of the influenza virus nuclear export protein (NEP)

Duncan Paterson[1], Jolyon Claridge[2], Jason Schnell[2], Ervin Fodor[1]
[1]Sir William Dunn School of Pathology, University of Oxford, UK, [2]Department of Biochemistry, University of Oxford, UK

Influenza viruses replicate and transcribe their genomes within the nucleus of infected cells. Consequently, incorporation of newly synthesised viral genomic RNA into progeny virions is dependent on the nuclear export of genomic RNA, in the form of viral ribonucleoprotein complexes (vRNPs). The viral nuclear export protein (NEP) is a multi-functional protein that stimulates vRNP replication, as well as mediating vRNP nuclear export. The mechanism by which these functions are regulated remains unclear. NEP has recently been shown to undergo phosphorylation at a highly conserved patch of serine residues (NEP 23-25) located between two nuclear export signals. We demonstrate that substitution of NEP 24S with a phospho-mimetic amino acid specifically up-regulates nuclear export of NEP. Enhanced nuclear export of NEP is dependent on the cellular nuclear export protein Crm1, consistent with the role of NEP as an adapter between Crm1 and the vRNP. In agreement with these findings, recombinant viruses with mutations to the NEP phosphorylation site are severely attenuated in replication. Further biophysical studies conducted on purified NEP suggest that phosphorylation of 24S may alter the tertiary conformation of NEP, potentially facilitating interaction with Crm1. Accordingly, we propose that phosphorylation of NEP 24S modulates the function of NEP, favouring interaction with the host nuclear export machinery and resulting in enhanced nuclear export of vRNPs.

Workshop Sessions

VIR-WK203.03 - Comparison of antiviral activity between IgA and IgG specific to influenza virus hemagglutinin: increased potential of IgA for heterosubtypic immunity

Mieko Muramatsu[1], Reiko Yoshida[1], Ayato Takada[1]
[1]Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan

Influenza A viruses of 16 hemagglutinin (HA; H1-H16) subtypes are maintained in the waterfowl reservoir. Viruses of H1, H2, and H3 subtypes are known to have caused pandemics in humans, and the emergence of new pandemic viruses of other HA subtypes has been a public health concern. Although both IgA and IgG antibodies are known to play important roles in protection against influenza virus, the contribution of these antibodies to the cross-protective heterosubtypic immunity is not fully understood. In this study, we compared in vitro antiviral activities of monoclonal IgA and IgG that assumed to recognize the same epitope on the HA molecule and found remarkable differences in their cross-reactivities against multiple HA subtypes. Polymeric IgA-producing hybridoma cells were successfully subcloned from those originally producing S139/1, an HA-specific monoclonal IgG that was generated against a virus strain of the H3 subtype but had cross-neutralizing activities against the H1, H2, H13, and H16 subtypes, and S139/1-IgA and S139/1-IgG were used to directly compare antiviral activities between the isotypes. We found that both S139/1-IgA and S139/1-IgG strongly bound to the homologous H3 virus in an enzyme-linked immunosorbent assay, and there were no significant differences in their hemagglutination-inhibiting and neutralizing activities against the H3 virus. In contrast, S139/1-IgA showed remarkably higher cross-binding and antiviral activities against H1, H2, and H13 viruses than S139/1-IgG. It was also noted that S139/1-IgA, but not S139/1-IgG, drastically suppressed the extracellular release of the viruses from infected cells. Electron microscopy revealed that S139/1-IgA deposited newly produced viral particles on the cell surface, most likely by tethering the virus particles. These results suggest that anti-HA IgA has greater potential to prevent influenza A virus infection than IgG, likely due to its multivalency and increased avidity, and that this advantage may be particularly important for heterosubtypic immunity.

Workshop Sessions

VIR-WK203.04 - Two genetically similar mouse strains exhibit differential disease phenotypes after infection with different influenza A viruses

Amie Eisfeld[1], Zachary Najacht[1], Yoshihiro Kawaoka[1,2,3]

[1]Influenza Research Institute, University of Wisconsin-Madison, School of Veterinary Medicine, Madison, Wisconsin, USA,
[2]ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama, Japan,
[3]International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan

The C57BL/6 mouse strain has been used extensively to characterize disease induced by influenza A viruses (IAVs). However, many knockout mice are created using embryos derived from C57BL/6N mice, a closely related strain harboring >50 validated polymorphisms and structural gene differences relative to C57BL/6. To determine whether IAV disease differs between these strains, we performed parallel mouse lethal dose 50 (MLD50) analysis in C57BL/6 and C57BL/6N mice – obtained from The Jackson Laboratory (referred to as '6J' and '6NJ,' respectively) – and compared disease phenotypes after infection with three unique IAVs: a 2009 pandemic H1N1 virus, A/California/04/2009 ('CA04'); a highly pathogenic H5N1 avian influenza virus, A/Vietnam/1203/2004 ('VN1203'); and the recently emerged human H7N9 virus, A/Anhui/1/2013 ('AH1'). 6NJ mice infected with sub-lethal dosages of CA04 exhibited significantly reduced body weight loss relative to 6J mice, suggesting increased resistance to IAV disease. Consistent with this observation, the CA04 MLD50 was 10-fold higher in 6NJ mice. A similar 10-fold increase in the MLD50 was observed in 6NJ mice infected with AH1, although no differences in body weight loss were observed between 6J and 6NJ mice at low dosages. In contrast, both 6J and 6NJ mice exhibited rapid body weight loss, uniform lethality and similar MLD50 values after infection with VN1203. These data suggest that strain-specific genetic differences in 6NJ mice impart resistance to CA04- and AH1-mediated disease, and further imply that the host genetic background differentially affects the disease phenotype caused by distinct IAVs. The mechanisms of 6NJ resistance to IAV disease and the causative genetic factors are currently under investigation.

Workshop Sessions

VIR-WK203.05 - Bat influenza virus harboring the entry machinery of an influenza A virus

Mindaugas Juozapaitis[1], Etori Aguiar Moreira[1], Nacho Mena[2], David Riegger[1], Anne Pohlmann[3], Dirk Höper[3], Gert Zimmer[4], Martin Beer[3], Adolfo García-Sastre[2], Martin Schwemmle[1]
[1]Institute for Virology, University Clinic Freiburg, Freiburg, Germany, [2]Department of Microbiology, Icahn Mount Sinai School of Medicine, New York, USA, [3]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany, [4]Institute of Virology and Immunology (IVI), Mittelhäusern, Switzerland

In 2012 the first complete sequences of a distantly-related influenza virus (H17N10) was discovered in bats from Central America, however due to the lack of infectious virus isolates, further characterization has been impeded. Using polymerase reconstitution and virus-like particle formation assays, we now show partial functional compatibility between bat and influenza A virus components. Guided by these observations, we succeeded to generate an infectious virus containing six out of the eight bat virus genes and having the remaining two genes encoding the HA and NA proteins of a prototypic influenza A virus. This virus replicated to varying degrees in a broad range of mammalian cell cultures, human primary airway epithelial cells, and mice. In sharp contrast, viral growth was poor in avian cells or in ovo. Nevertheless, this species barrier was rapidly overcome by the acquisition of specific adaptive mutations in gene products of the internal segments of the virus. Thus, zoonotic transmissions of bat influenza viruses to other susceptible species, including humans, cannot be excluded. Nevertheless, the inability to reassort with influenza A viruses might further reduce this risk.

Workshop Sessions

VIR-WK203.06 - Development of novel cell-based Influenza H5N1 vaccines

Po-Ling Chen[1], Alan Yung-Chih Hu[1], Pei-Yu Huang[1], Wei-Zhou Yeh[1], Yu-Fen Tseng[1], Min-Shi Lee[1]
[1]*National Health Research Institutes, Miaoli, Taiwan*

Current egg-based influenza vaccine production technology is labor-intensive and lack of flexibility. Moreover, its capacity would not be able to meet the demand during influenza pandemics. This was illustrated in the 2009 H1N1 pandemic where only 22% of expected doses were supplied within the first 6 months after the pandemic was declared. Therefore, cell-based technology is becoming attractive for production of pandemic influenza vaccines. Two cell lines, MDCK and Vero cells are currently used for manufacturing human influenza vaccines. MDCK cells can only be used for manufacturing influenza vaccines. In contrast, Vero cells have been widely used for the production of human vaccines. The current WHO-recommended influenza H5N1 clade-1 vaccine strain (NIBRG-14), a reassortant virus between A/Vietnam/1194/2004 (H5N1) virus and egg-adapted high-growth A/PR/8/1934 virus, could grow efficiently in eggs and MDCK cells but not Vero cells. From 2005 to 2011, NHRI has established MDCK cell culture platforms to manufacture influenza H5N1 clade-1 vaccines and licensed the technology to a local company for further development. In addition, we also adapted the egg-derived NIBRG-14 in Vero cells to become a Vero cell-adapted high-growth H5N1 vaccine virus (Vero-15), which could reach high virus titer (>108 TCID50/ml) in Vero cells in multiple culture systems including T flasks, mirocarriers and TideCell cultures. Recently, H5N1 clade-2 viruses, which are antigenically different from H5N1 clade-1 virus, are circulating widely. Therefore, we have further used the Vero-15 virus as a master donor virus to establish reverse genetics platform and generate high-growth reassortant H5N1 clade-2 vaccine viruses within 2 months. In conclusion, the Vero-15 H5N1 vaccine virus has the commercial potential to become a seed virus for manufacturing H5N1 vaccines. In addition, the Vero-15 H5N1 vaccine virus could become a mater donor virus to generate high-growth vaccine viruses for other influenza A subtypes.

Workshop Sessions

VIR-WK203.07 - Leukocyte trafficking in response to influenza infection in the ferret

Adrian Reber[1], Nedzad Music[1], Jacqueline Katz[1], Ian York[1]
[1]*Influenza Division, Centers for Disease Control and Prevention, Atlanta, USA*

Ferrets are a useful animal model for influenza virus infection due to similarities with the symptoms and disease progression observed in human influenza, but the scarcity of ferret-specific reagents has made assessment of pathogenesis and immunity difficult. We used a panel of cross-reactive antibodies that identify ferret T cells, B cells, granulocytes, and antigen presenting cells (APC) to assess the trafficking of leukocytes of unvaccinated ferrets in response to influenza A/Perth/16/2009(H3N2) virus infection. The proportion of APCs in peripheral blood was significantly reduced on day 1 (d1) post-infection compared to mock-infected ferrets. Bronchoalveolar lavage (BAL) showed a 50% reduction in the proportion of APCs and a threefold increase in granulocytes in lungs of infected ferrets compared to mock-infected animals. The proportion of APCs in the medial retropharyngeal lymph nodes (MRLN) increased on d2 post-infection, and the absolute number of APCs increased in MRLN on d2 and d5. On d2 post-infection, T cells and B cells in peripheral blood dropped dramatically. There was a significant increase in the proportion of CD4+ T cells in the lungs on d2; however, there was no indication of CD8+ T cells and B cells trafficking to MRLN or lungs at this time point. By d5 post-infection peripheral blood lymphocyte counts had partially, but not completely, returned to pre-infection levels. In the MRLN at d5 there was an increase in the proportion of CD8+ T cells, as well as the absolute numbers of CD4+ and CD8+ T cells and B cells . The proportion of CD4+ T cells in the lungs dropped to baseline levels by d5, while the proportion of CD8+ T cells increased. We conclude that much, but not all, of the peripheral blood leukocyte changes observed upon influenza virus infection of ferrets can be accounted for by leukocytes trafficking into the tissues.

Workshop Sessions

VIR-WK203.08 - Identification of a novel viral protein expressed from the PB2 segment of Influenza A Virus

Seiya Yamayoshi[1], Mariko Watanabe[1], Ryuta Uraki[1], Hideo Goto[2], Yoshihiro Kawaoka[1,3,4,5]
[1]*Division of Virology, Institute of Medical Science, University of Tokyo, Tokyo, Japan,* [2]*Department of Microbiology, Wakayama Medical University, Wakayama, Japan,* [3]*ERATO Infection-Induced Host Responses Project, Saitama, Japan,* [4]*International Research Center for Infectious Diseases, IMSUT, Tokyo, Japan,* [5]*School of Veterinary Medicine, University of Wisconsin-Madison, Madison, USA*

Influenza A virus expresses various viral proteins in infected cells. Recently, some novel viral proteins, such as PA-X, which represses host protein expression, have been identified. To date, 16 viral proteins have been identified. However, it remains unclear whether other as yet unidentified viral proteins may be expressed by influenza virus and may play a role in viral replication and/or pathogenicity. Here, we described a novel viral protein derived from the PB2 segment. We infected human 293 cells with A/WSN/33 (WSN) at a multiplicity of infection (moi) of 5. At 6 hours post-infection, total RNA was extracted from the infected cells and cDNA was prepared by reverse-transcription using an oligo-dT primer. Two species of PCR product were produced by PCR using the cDNA and specific primers to the mRNA of PB2 protein. The major PCR product was the mRNA encoding PB2 protein and the minor PCR product was a novel mRNA derived from the PB2 segment. To evaluate whether a viral protein is translated from this novel mRNA, we performed western blot analysis using an anti-PB2 monoclonal antibody, a specific antibody to the novel viral protein, and MDCK cells infected with WSN at an moi of 10. The anti-PB2 monoclonal antibody detected the novel viral protein as well as the PB2 protein. The novel viral protein was also detected by the specific antibody. These results indicate that the PB2 segment encodes a novel viral protein as well as the PB2 protein. The properties and functions of this novel viral protein are now being characterized.

Workshop Sessions

VIR-WK204.01 - Contributions of the Epstein-Bar Virus EBNA1 protein to EBV-associated cancers

Sheila Mansouri[1], Jennifer Yinuo Cao[1], Nirojini Sivachandran[1], Lori Frappier[1]
[1]*University of Toronto, Toronto, Canada*

Epstein-Barr virus (EBV) is a common ɣ-herpesvirus that contributes to the development of several types of lymphomas as well as to nasopharyngeal and gastric carcinomas. The only viral protein that is expressed in all types of EBV-associated cancers is EBNA1. In addition to its role in maintaining EBV episomes, evidence suggests that EBNA1 may alter cells in ways that promote cell survival and transformation. Proteomics profiling has revealed strong interactions between EBNA1 and two host proteins; ubiquitin specific protease 7 (USP7) and CK2 kinase. EBNA1 interactions with USP7 interfere with p53 stabilization by blocking the USP7-p53 interaction. In addition, EBNA1 induces the loss of PML nuclear bodies in EBV-associated carcinomas through degradation of PML proteins, thereby interfering with PML-associated functions including apoptosis and DNA repair. This was found to require EBNA1s interaction with both USP7 and CK2. CK2 phosphorylates PML, priming PML for ubiquitylation and degradation. EBNA1 increases the association of CK2 with PML proteins and their subsequent phosphorylation by CK2. We identified a novel binding pocket in the CK2 regulatory subunit that is bound by EBNA1. Our proteomic studies indicate that some cellular proteins also bind CK2 through this pocket, suggesting that EBNA1 could interfere with the functions of these proteins by competing for CK2. In addition, we recently examined the effect of EBNA1 expression on cellular microRNAs by deep sequencing. The most striking effect was that EBNA1 upregulated multiple let7 family miRNAs. Additional studies on let7a miRNA indicated that EBNA1 increased the levels of let7a primary transcripts and downregulated the let7a target protein, Dicer. These changes in let7a and Dicer inhibited EBV reactivation, suggesting that EBNA1 promotes EBV latency by inducing let7a. As a whole, the data suggest that EBNA1 promotes EBV persistence and cell survival and contributes to tumourigenesis in multiple ways.

Workshop Sessions

VIR-WK204.02 - Function of Akt isoforms in transformation by the Jaagsiekte sheep retrovirus envelope protein

Lisa Santry[1], Wing Ka Amanda Au Yeung[1], Byram Bridle[1], Sarah Wootton[1]
[1]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada*

Jaagsiekte sheep retrovirus (JSRV) is an acutely oncogenic betaretrovirus and the causative agent of ovine pulmonary adenocarcinoma, a contagious lung tumor of sheep. Unlike other oncogenic retroviruses, the envelope (Env) glycoprotein of JSRV is itself an oncogene and sufficient to transform differentiated lung epithelium in vivo and a wide variety of cells in vitro. One of the key pathways activated by JSRV Env is the PI3K/Akt pathway, whose manipulation is a prominent feature of many viruses. In mammals, there are three distinct isoforms of Akt; Akt1, Akt2 and Akt3, each encoded by a separate gene. Originally, it was thought that Akt isoforms were functionally redundant, however studies in knockout mice have revealed that Akt isoforms play distinct roles in a variety of cellular processes, including tumorigenesis and viral pathogenesis. To investigate the role of Akt isoforms in transformation by JSRV Env, the JSRV-Env induced mouse lung tumor cell line, RJenvC1, was transduced with VSV-G pseudotyped lentiviral vectors expressing shRNAs against Akt1-/-, Akt2-/- or Akt 3-/-. Knockdown of the various Akt isoforms was confirmed by western blot and these cell lines were evaluated for their ability to grow in the absence of serum, form colonies in soft agar, invade matrigel, and evade apoptosis. Results show that Akt 1 knockdown cell lines led to smaller colony formation in soft agar, were more invasive and had a lower metabolic rate. Akt 2 knockdown cells had a higher metabolic rate and increased ability to evade apoptosis and finally Akt 3 knockdowns formed larger colonies in soft agar and had an increased ability to evade apoptosis. Finally, we tested whether Akt isoform status influenced susceptibility to oncolytic viruses (OV). Akt isoform ablation had no effect on the susceptibility of RJenvC1 cells to viral oncolysis, suggesting that OV mediated lysis occurred independent of the Akt pathway.

Workshop Sessions

VIR-WK204.03 - A measles virus blind to signaling lymphocytic activation molecule is an oncolytic agent for lung cancer treatment

Tomoko Fujiyuki[1], Misako Yoneda[1], Kunie Obayashi[1], Yosuke Amagai[1], Fusako Ikeda[1], Koichiro Shoji[1], Hiroki Sato[1], Yoshinori Murakami[2], Chieko Kai[1]

[1]Laboratory Animal Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]Division of Molecular Pathology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Wild type measles viruses most efficiently enter into immune cells using signaling lymphocyte activation molecule (SLAM). We previously generated a recombinant measles virus selectively unable to use SLAM based on a wild MV-HL strain (rMV-SLAMblind). The rMV-SLAMblind uses PVRL4 as a receptor to infect breast cancer cells efficiently and showed antitumor activity against human breast cancer xenografts in immunodeficient mice. The oncolytic activity of rMV-SLAMblind was significantly greater than that of rMV-Edmonston. (Sugiyama et al, 2013, Gene Therapy). Thus, it is considered to be useful for breast cancer treatment. In this study, we examined whether rMV-HL-SLAMblind is applicable to lung cancer. Lung cancer is the commonest cancers and is responsible for the most cancer-related death. We screened PVRL4 expressing cell lines, including small cell lung cancers (SCLC) and non-small cell lung cancers (NSCLC). Several cell lines of NSCLC were successfully infected with the virus and a half of them decreased viability after the infection of rMV-SLAMblind. Inoculation of rMV-SLAMblind into lung cancer xenotransplanted immunodeficient mice efficiently suppressed the tumor growth. Thus, rMV-SLAMblind has high oncolytic ability also for other types of cancers in addition to breast cancer, and could be a good candidate as an oncolytic virus for lung cancer treatment, particularly NSCLC.

Workshop Sessions

VIR-WK204.04 - When and where to unwind: host factors regulating the human papillomavirus E1 helicase

David Gagnon[1], Jennifer Alvarez[1], Jacques Archambault[1]
[1]*Institut de recherches cliniques de Montreal (IRCM), Montreal, Canada*

Human papillomaviruses (HPVs) are major pathogens of the skin and mucosa, being the cause of recurrent benign warts, laryngeal papillomatosis and of several cancers of the anogenital tract and head-and-neck region. E1, the only enzyme encoded by HPVs, is an ATP-dependent DNA helicase required for replication of the viral double-stranded DNA genome in the nucleus of infected cells. The N-terminal part of E1 has emerged as an important regulatory region that controls the intracellular localization and activity of the protein through interactions with host factors. One example is the interaction with the cell cycle regulatory kinases cyclin A/E-Cdk2, which serves to regulate the nucleo-cytoplasmic shuttling of E1. By phosphorylating and inactivating the nuclear export signal of E1, these Cdk2 complexes allow the protein to accumulate in the nucleus specifically during S-phase, when viral DNA replication takes place. Nuclear accumulation of E1 is also accompanied by the induction of a cellular DNA damage response that blocks host DNA synthesis and cell cycle progression, thus providing an environment conducive to multiple rounds of viral DNA replication. Another host factor that was recently found to interact with E1 and to regulate viral DNA replication is UAF1, a WD40-repeat protein which forms separate complexes with the deubiquitinating enzymes USP1, 12 and 46. The findings that E1 recruits these UAF1/USP complexes to the viral genome and that their deubiquitinase activity stimulates viral DNA replication suggests that one or more component(s) of the viral replisome, perhaps E1 itself, is a substrate of these enzymes. More generally, the discovery that E1 interacts with cell cycle regulatory kinases and deubiquitinases underscores the importance of post-translational modifications in orchestrating replication of the HPV genome. As such, these findings also provide the necessary impetus for evaluating these interactions and modifying enzymes as candidate antiviral targets.

Workshop Sessions

VIR-WK204.05 - Herpesviral G protein-coupled receptors shortcut NFAT activation by targeting the sarco/endoplasmic calcium ATPase

Junjie Zhang[1], Pinghui Feng[1]

[1]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, USA

G protein-coupled receptors (GPCRs) constitute a large family of receptors that relay extracellular stimulations to regulate an array of fundamental intracellular biological processes. Herpesviruses encode multiple GPCR homologues in their genomes that are implicated in viral pathogenesis. In contrast to cellular GPCRs that are primarily regulated by their cognate ligands, herpesviral GPCRs are constitutively active to instigate downstream signaling pathways, including the nuclear factor of activated T cells (NFAT). However, the roles of NFAT activation and mechanism thereof remain unknown in viral GPCR tumorigenesis. Here we report that viral GPCRs of human Kaposi's sarcoma-associated herpesvirus (kGPCR) and cytomegalovirus (US28) activate NFAT by targeting the ER calcium ATPase (SERCA). Viral GPCR interacted with and inhibited SERCA, leading to increased cytosolic calcium concentration. As such, NFAT activation induced by viral GPCRs was sensitive to cyclosporine A (calcineurin inhibitor) and calcium chelators, while was resistant to inhibitors of PLC, an activator of calcium release. Ectopic expression of SERCA2 diminished NFAT activation by kGPCR in a dose dependent manner. In contrast to kGPCR and US28, Epstein-Barr virus GPCR (BILF1) failed to activate NFAT. A BILF1 chimera protein carrying the cyotoplasmic loops and tail of kGPCR interacted with SERCA2 and activated NFAT. Gene expression profiling of endothelial cells identified a signature of NFAT activation by kGPCR, including chemokines and cytokines that further promoted NFAT activation in an autocrine- or paracrine-mechanism. Furthermore, the expression of NFAT-dependent genes was up-regulated in tumors derived from tva-kGPCR mouse and human KS lesions. Conversely, cyclosporine A treatment diminished NFAT-dependent gene expression in endothelial cells and inhibited tumor formation in a xenograft mouse model induced by kGPCR and US28. These findings unravel the mechanism of "constitutive" action of viral GPCRs in promoting NFAT activation and essential roles of NFAT activation in viral GPCR tumorigenesis.

Workshop Sessions

VIR-WK205.01 - Cutting edge: HCV vaccine and the last frontier

Rowena Bull[1], Andrew Lloyd[1], Fabio Luciani[1]
[1]*Inflammation and Infection Research Centre, Faculty of Medicine, University of New South Wales, Sydney, Australia*

HCV infection is a global pandemic. Following primary infection, ~75% become persistently infected, resulting in severe liver disease. Although clearance occurs, reinfection is well documented. Despite the imminent arrival of new anti-HCV direct-acting antiviral therapies, complex social barriers combined with high cost suggest that treatment-based approaches alone will be insufficient for global control of HCV. By contrast, a multi-pronged approach, likely consisting of transmission control measures, antiviral therapy and vaccine will be required. HCVs ability to not only escape but also subvert the developing immune response, has hindered the progress in vaccine development. HCV vaccine-induced immunity will need to both model and enhance the immunity observed in individuals who naturally clear the infection. However, the virus-host complexities in the critical initial phase of HCV infection remain poorly understood, as early infection is typically asymptomatic. In addition, the labour intensive nature of traditional techniques required to study virus-host interactions has limited inter-disciplinary immunovirology research. However, advances, such as next generation sequencing, now enable high-throughput analysis of biological systems at unprecedented depth. My research objective has been to combine cutting edge technologies to investigate the virus-host interaction during transmission and disease progression in early incident HCV cases from the Australian HITS cohort. We have identified two potential Achille's heels for HCV: new infection is established from only 1-2 'founder' viruses – these viruses dominant infection for ~100 days before they are extinguished. At this point, infection is either cleared or a new variant population emerges and establishes persistent infection. Immunological assays indicate this is driven by both CD4+ and CTL HCV-specific responses. Interestingly, the magnitude of the CTL response is strongly associated with the rapid development of immune escape variants. Better understanding of how these viruses interact with their host(s) innate and adaptive responses will guide the development of a successful vaccine.

Workshop Sessions

VIR-WK205.02 - Dynamics of the virus-specific CD8 T-cell repertoire during HCV reinfection

Mohamed Abdel-Hakeem[1,2], Julie Bruneau[1,2], Hugo Soudeyns[2,3], Naglaa Shoukry[1,2]
[1]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal, Canada, [2]Université de Montréal, Montréal, Canada, [3]Centre Hospitalier de Universitaire Sainte-Justine, Montréal, Canada

We have previously demonstrated that protection upon HCV reinfection correlates with expansion of virus-specific T cells. Protection was also associated with increased breadth of the immune response and shifting epitope dominance, suggesting the generation of de novo T-cell responses. In contrast, viral persistence was associated with limited expansion of T cells and reinfection with variant viral strains. The objectives for this study are; first, to distinguish the role of memory versus de novo T-cell responses in protection and, second, to determine the dynamics of the T-cell receptor (TCR) repertoire relative to changes in viral sequences. We performed longitudinal analysis of the HCV-specific CD8 T-cell repertoire during HCV reinfection in two groups of patients; patients who spontaneously resolved two consecutive infections (SR/SR group) and patients who resolved primary infection but became persistently infected upon reinfection (SR/CI group). Functional avidity was tested, by measuring dose-dependent production of IFNγ in response to stimulation by the different epitope variants. Our results demonstrate that the effector T-cell clonotypes at the peak of the immune response during reinfection were recruited from the pre-existing memory population, with no detection of new clonotypes. Nevertheless, we observed a change in dominance of the clonotypes and more focusing of the TCR repertoire upon reinfection in the SR/SR group. HCV-specific T cells in the SR/SR group showed higher functional avidity in response to reference epitope sequences and higher flexibility in response to epitope variants than T cells in the SR/CI group. These results suggest that effector HCV-specific CD8 T-cell clonotypes associated with protection upon reinfection are recruited from the memory population, with focusing of the repertoire on clonotypes that exhibit superior functional avidity.

Workshop Sessions

VIR-WK205.03 - HCV hijacks the autophagy elongation complex (ATG5-12/16) and blocks maturation of the autophagosome

Ahmed Fahmy[1], Christian Duguay[1], Matthieu Blanchet[1], Patrick Labonté[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada

Hepatitis C virus (HCV) infection is known to induce autophagosome accumulation as observed by the typical punctate cytoplasmic distribution of LC3-II in infected cells. Recently, we showed that viral RNA-dependent RNA polymerase (NS5B) interacts with ATG5, a major component of autophagy initiation. In this study, we evaluate the involvement of the autophagy elongation complex (ATG5-12/16) in HCV replication. We demonstrate that the elongation complex is recruited at the site of viral replication and acts as a proviral factor. Indeed, ATG5-12 as well as ATG16L1 colocalizes with the viral replicase as well as the replicative intermediate dsRNA in infected cells. Furthermore, we show that induction of autophagy by NS4B results in LC3-II colocalization in transfected cells but not in infected cells. Interestingly, LC3-I is not recruited to the elongation complex at the site of viral replication and no sign of colocalization of LC3-II with viral proteins was observed. Using dominant negative forms of ATG5, ATG12 and ATG4B, we demonstrate that ATG5-12 conjugate is important for viral replication but not LC3-II formation. Finally, by monitoring the autophagic process using different techniques we show that HCV induces incomplete autophagic flux. Together, these findings indicate that HCV uses the autophagy elongation complex as a proviral factor for its own replication but blocks the formation of a genuine autophagosome at the site of viral replication.

Workshop Sessions

VIR-WK205.04 - Modulation of Hepatitis C virus genome replication by the glycosphingolipid biosynthetic machinery

Kouacou Konan[1], Irfan Khan[1], Divya Katikaneni[1], Qingxia Han[1], Lorena Sanchez Felipe[1]
[1]Albany Medical College, Albany, USA

Hepatitis C virus (HCV) is a positive sense RNA virus which replicates its genome on cytosolic membranes termed the membranous web (MW) vesicles. HCV infection causes PI4KIIIα activation via interaction with HCV NS5A protein, increased production and retargeting of phosphatidylinositol 4-phosphate (PI4P) lipid to the HCV replication complex. However, the role of PI4P in HCV lifecycle is not completely understood. We hypothesized that PI4P recruits host effectors to modulate the transition from HCV genome replication to virus particles production. To test this hypothesis, we have generated stable cells with doxycycline-inducible expression of shRNAs targeting PI4P adaptor and glycosphingolipid-binding protein, FAPP2. Indeed, FAPP2 knockdown attenuates HCV infectivity and virus RNA synthesis. FAPP2 has two functional domains; the PH domain binds to PI4P lipid, whereas the GLTP domain binds to glycosphingolipids. While expression of the PH domain mutant protein was expected to impede HCV RNA synthesis, a significant decrease in replication was also observed with the glycosphingolipid-binding mutant protein. These findings imply that both domains are crucial for the role of FAPP2 in HCV genome replication. Interestingly, HCV markedly increased the level of some glycosphingolipids during infection, whereas adding glycosphiningolipids to FAPP2-depleted cells partially rescued replication, further arguing for the importance of glycosphingolipids in HCV RNA synthesis. Further study indicates that FAPP2 is relocalized to the HCV replication complex (RC) characterized by virus proteins NS4B and NS5A or dsRNA foci. Additionally, FAPP2 knockdown disrupts the RC and alters the co-localization of HCV replicase proteins. Altogether, our findings suggest that HCV hijacks FAPP2 for its direct role on virus genome replication via PI4P-binding, and its indirect role via glycosphingolipid binding and transport to the HCV RC.

Workshop Sessions

VIR-WK205.05 - Hepatitis B and C in Morocco: epidemiological and molecular aspects

Warda Baha[1], Roxana-Delia Trimbitas[1], Abderrahim Foullous[1], Fatiha Lazaar[1], Abdelouahed El Malki[1], Abdelouaheb Benni[1]

[1]*Pasteur Institute of Morocco, Casablanca, Morocco*

Viral hepatitis is a serious public health problem affecting billions of people globally. Together, hepatitis B virus (HBV) and hepatitis C (HCV) are the leading cause of liver cancer in the world. The objective of this exhaustive study was to draw an epidemiological and molecular portrait of HBV and HCV infections in Morocco. A seroprevalence survey was conducted in the general population and blood donors. We confirmed the intermediate endemicity for HCV infection and noted a downward trend in the incidence of HBV, which might reclassify Morocco in low HBV endemicity area. The HCV infection was primarily associated with nosocomial exposures. Additionally, sexual risk behaviors were associated with higher prevalence of HBV among adults. The study of the HBV genetic diversity showed a predominance of genotype D and apparition for the first time of genotype E and mixed infections A/D and D/F. We also reported that precore variants could be found in more than three quarters of Moroccan patients with HBV infection. Their prevalence was related to HBV genotypes. In addition, we have characterized the first cases of antiviral resistance in patients under treatment and no mutation was detected in naive subjects. Concerning hepatitis C, its diversity was analyzed over a period of fifteen years. Five genotypes and eight subtypes have been identified. We have shown a predominance of genotype 1 and a global codominance of subtypes 1b and 2a/2c. Drug addiction was the primary mode of spread of 3a subtype. Subtype 1b was prevalent in patients with cirrhosis confirming thus, its association with severe liver disease. Pilgrims follow comparably the national trend with an increased prevalence in older people and the presence of genotypes 1, 2 and 4. Among multi-transfused patients, despite the unique presence of genotype 1, the contribution of transfusion in HCV transmission has not been demonstrated.

Workshop Sessions

VIR-WK205.06 - Human genetic variation and hepatitis C virus infection in Moroccan patients

Sayeh Ezzikouri[1], Rhimou Alaoui[2], Naima Elmdaghri[1], Soumaya Benjelloun[1]
[1]Virology Unit, Viral Hepatitis Laboratory, Institut Pasteur du Maroc, Casablanca, Morocco, [2]Service de Médecine B, CHU Ibn Rochd, Casablanca, Morocco

Background: Morocco is a medium endemic country for chronic Hepatitis C virus (HCV) infection. The aim of the present study was to investigate the role of polymorphisms at several locus (IL28B, L-SIGN and PNPLA3 genes) with progression and outcome of HCV infection in a Moroccan population. Methods: We analyzed a cohort of 232 patients with persistent HCV infection, 68 individuals who had naturally cleared HCV and 109 healthy controls. The IL28B SNPs rs12979860 and rs8099917, PNPLA3 SNP rs738409 and the L-SIGN repeat region in exon 4 were genotyped, using a TaqMan 5' allelic discrimination assay or PCR respectively. Results: The protective rs12979860-C and rs8099917-T alleles (IL28B) were more common in subjects with spontaneous clearance (p = 0.00001 and 0.0025, respectively). Patients with advanced liver disease carried the rs12979860- T/T genotype more frequently than patients with mild chronic hepatitis C. The frequency of the 7/4 genotype was higher in spontaneous resolvers (14.3%) as compared with the persistent group (4%) (p=0.022). In addition, we found that 4-L-SIGN was associated with spontaneous resolution of HCV infection (p=0.005). Interestingly, patients with 4-L-SIGN had lower viral loads when compared with carriers of the 5 (p 0.001), 6 (p 0.021) and 7-alleles (p 0.048). Variation at rs738409 (PNPLA3) was not associated with significant changes in resolution rate of hepatitis C, by contrast, M/M genotype was associated with a 3-fold increase of liver cancer risk. Conclusions: In the Moroccan population, polymorphisms near the IL28B gene play a role both in spontaneous clearance and progression of HCV infection. Neck region polymorphism of L-SIGN can influence the outcome of HCV infection and the four-tandem repeat is associated with clearance of HCV infection while he PNPLA3 I148M variant apparently stimulates liver cancer development without interfering on the HCV clearance process.

Workshop Sessions

VIR-WK205.07 - Comprehensive analysis of B-lymphoma cells spontaneously developed in transgenic mice that express the full hepatitis C virus genome in B cells

Kyoko Tsukiyama-Kohara[1], Yuri Kasama[1], Takuo Mizukami[2], Hideki Kusunoki[2], Jan Peveling-Oberhag[4], Toshiaki Mizuochi[3], Michinori Kohara[5]

[1]Kagoshima University, Kagoshima, Japan, [2]Kurume University, Kurume, Japan, [3]National Institute of Infectious Diseases, Tokyo, Japan, [4]Goethe-University Hospital, Frankfurt, Germany, [5]Tokyo Metropolitan Institute, Tokyo, Japan

Hepatitis C virus (HCV) infection leads to the development of hepatic diseases but also extrahepatic disorders such as B-cell non-Hodgkin's lymphoma (B-NHL). To reveal the molecular signalling pathways responsible for HCV-associated B-NHL development, we utilised transgenic (Tg) mice that express the full-length HCV genome specifically in B cells and develop non-Hodgkin type B-cell lymphomas (BCLs). The gene expression profiles in B cells from BCL-developing HCV-Tg mice, from BCL-non-developing HCV-Tg mice, and from BCL-non-developing HCV-negative mice were analysed by genome-wide microarray. In BCLs from HCV-Tg mice, the expression of various genes was modified; however, the modified genes in male and female mice were essentially the same. Markedly modified genes such as Fos, C3, LTβR, A20, NF-κB, and miR-26b in BCLs were further characterised using specific assays. We propose that activation of both canonical and alternative NF-κB signalling pathways and down-regulation of miR-26b contribute to the development of HCV-associated B-NHL.

Workshop Sessions

VIR-WK206.01 - Insect Viruses - Mutualistic bracoviruses exhibit both novel and conserved features with pathogenic ancestors

Michael Strand[1]
[1]*University of Georgia, Athens, USA*

Microorganisms form symbiotic associations with animals and plants that range from parasitic (pathogens) to beneficial (mutualists). Although numerous examples of obligate, mutualistic bacteria, fungi, and protozoans exist, viruses are generally thought to form parasitic associations. A notable exception is the family Polydnaviridae, which consists of large DNA viruses that are obligate mutualists of insects called parasitoid wasps. Polydnaviruses fully rely on wasps for vertical transmission while wasps rely on polydnaviruses for successful development of their offspring. Prior studies show that polydnaviruses in the genus Bracovirus evolved approximately 100 million years ago from nudiviruses, which are closely related to a large family of insect pathogens called baculoviruses. Polydnaviruses are thus of fundamental interest for understanding the processes by which viruses can evolve into mutualists. In this presentation, I will discuss polydnavirus evolution, key traits polydnaviruses still share with nudiviruses and baculoviruses, and some of the functional novelties polydnaviruses and wasps that are essential for maintenance of their mutualistic association.

Workshop Sessions

VIR-WK206.02 - Mapping dual nuclear localizers essential for nuclear translocation of the insect baculovirus AcMNPV ME53

Yang Liu[1], Eva Nagy[1], Peter Krell[1]
[1]University of Guelph, Guelph, Ontario

Me53 of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) (family Baculoviridae, genus Alphabaculovirus) is one of five immediate early genes and is highly conserved in all sequenced lepidopteran baculoviruses. ME53 contains a C-terminal C4 zinc finger whose function is not yet clear. Late in infection ME53 colocalizes in membrane foci in a GP64 dependent manner with the envelope glycoprotein GP64, but also translocates to the nucleus. However, the nuclear transport mechanism for ME53 and whether ME53 interacts with other viral/host proteins to facilitate this translocation are still unknown. In order to map ME53 determinants that facilitate nuclear translocation recombinant AcMNPV bacmids containing a series of ME53 truncations, internal deletions and peptides tagged with either HA or GFP (for detection by immunofluorescence/fluorescence microscopy) were constructed. Intracellular localization in bacmid-transfected Sf21 cells was monitored at early (18 hrs) and late (48 hrs) times post transfection. Of several peptides tested, peptide AA (83-152)-HA was translocated into the nucleus in the late phase, while ME53 with AA (83-152) deleted failed to localize in the nucleus, suggesting that residues within AA (83-152) are required for the nuclear transport of ME53. Bacmids with carboxy GFP-fused ME53 truncations were used to more finely map residues essential for nuclear translocation. When ME53 AA (2-106) was deleted, nuclear transport of the remaining peptide ME53 AA (107-449)-GFP was observed. However, with a larger truncation comprising AA 2 to 121, the remaining peptide ME53 AA (122-449)-GFP localized mostly in the cytoplasm, and its nuclear transport was greatly abolished, suggesting that amino acids 107 to 121 are essential for its nuclear translocation. However, when AA (107-130) was internally deleted, compared to the deletion of AA (2-121), it still translocated to the nucleus, indicating a second nuclear localizer upstream of AA (107-121), and that ME53 has dual localizers for its nuclear translocation.

Workshop Sessions

VIR-WK206.03 - Development of baculovirus gene therapy vectors targeting AAVS1

Jondavid de Jong[1], Sarah Wootton[1]
[1]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph ON*

Baculovirus vectors have garnered attention as gene delivery vectors because of their ability to transduce human cells and large transgene coding capacity. Additionally, baculoviruses are non-replicative in mammalian cells, do not integrate into mammalian chromosomes, and humans lack pre-existing baculovirus antibodies and T-cells, making them a safe choice for gene therapy. Despite these advantages, use of baculovirus for gene therapy has been hampered by their sensitivity to serum complement and transient nature of transgene expression. To address these short-comings we have generated DAF-displaying baculoviruses that target transgene insertion into the AAVS1 "safe harbour" within human chromosome 19. These vectors display DAF within the virion envelope, thus providing increased resistance to serum-complement. To target transgene insertion into the AAVS1 locus we have adopted two strategies. The first is the generation of DAF-Bac/AAV hybrids which encode AAV-2 rep68 and a transgene cassette flanked by the AAV2 ITRs. This provides all necessary elements to catalyze the integration of any ITR flanked cassette into the AAVS1 locus. The second strategy utilizes an AAVS1 specific TALEN pair to introduce a double-stranded break within AAVS1 to mediate site-specific homologous recombination (HR). DAF-Bac/AAVS1TAL, encodes an AAVS1 TALEN pair and transgene cassette flanked by AAVS1 homologous arms which provides template DNA for HR directed repair. Using a GFP/Hygromycin cassette DAF-BAC/AAV and DAF-BAC/AAVS1TAL will be evaluated in terms of both frequency of transgene integration as well as fidelity of integration within the AAVS1 locus. Additionally, we will discuss the incorporation of vaccinia virus immune modulating genes into the baculovirus backbone in order to counter host innate and inflammatory immune responses. Transduction of cells with these vectors not only provides short-term transient expression, but will also lead to the integration of a transgene cassette into a known safe genomic location, leading to permanent expression for the life of that cell.

Workshop Sessions

VIR-WK206.04 - Investigation of protein-protein interactions between Baculovirus RNA polymerase subunits using fluorescent in vivo Protein Complementation Assays

Jessica Breznik[1], Nicola Johnson[1], Mustapha El-Ayoubi[1], Eric Carstens[1]
[1]Queen's University, Kingston, Canada

Baculovirus transcription is directed by two different DNA-directed RNA polymerases (RNAPs): early genes are transcribed by the insect host RNA polymerase while late and very late genes are transcribed by a virus-coded RNA polymerase consisting of four proteins: P47, LEF-4, LEF-8 and LEF-9. The presence of conserved catalytic motifs in LEF-8 and LEF-9 suggests that the interface of these subunits forms the catalytic RNA polymerase activity while LEF-4 has enzymatic activities associated with RNA capping. No function has yet been demonstrated for P47. We investigated the in vivo intracellular localization and potential interacting partners of these subunits by fusing two individually non-fluorescent fragments (V1 and V2) of the Venus yellow fluorescent protein with the N-termini of each of the RNAP subunits. Since there is a strong correlation between the switch from early to late transcription and the initiation of virus DNA replication, we also produced similar fusions with two components of the virus replisome complex, LEF-3 and P143 in plasmid expression vectors. Bacmids expressing each of these fusion proteins were also constructed to generate recombinant viruses expressing each of the V1- or V2-tagged protein subunits. Protein-protein interaction potentials of these subunits were investigated using bimolecular fluorescence complementation (BiFC) assays. Reciprocal co-transfections were performed to investigate the potential self-interaction of these proteins to form homo-oligomers, as well as their ability to interact with heterologous partners in the absence of any other viral proteins. Co-infections were used to investigate the interactions of these subunits in the presence of a full complement of all the other virus proteins. We also prepared similar V1- and V2-tagged fusions with the Spodoptera frugiperda (Sf) TATA binding protein (TBP) and investigated the ability of this cellular protein to interact with the viral genes. The results of co-transfection and co-infection will be presented.

Workshop Sessions

VIR-WK206.05 - Analysis of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) AC141 (EXON0) interaction with the lepidopteran *Trichoplusia ni* (*T. ni*) kinesin-1

Siddhartha Biswas[1], Gary Blissard[2], David Theilmann[1,3]
[1]Plant Science, Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada, [2]Boyce Thompson Institute at Cornell University, Ithaca, USA, [3]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, Canada

The baculovirus AcMNPV produces two forms of virions, occlusion derived virus (ODV) and budded virus (BV). Nucleocapsids (NCs) for both ODV and BV are synthesized in the nucleus of infected cells. ODV NCs are retained in the nucleus, whereas BV NCs are transported from the nucleus, through the cytoplasm and bud from the plasma membrane. Previous studies from this laboratory showed that the viral protein AC141 (EXON0) is required for efficient BV production and it interacts directly or indirectly with beta-tubulin. Studies by others using FRET-FLIM have also shown that AC141 associates with drosophila kinesin-1 TPR domain. The objective of this study was to confirm that AC141 can associate with the lepidopteran kinesin-1 during NC egress. To enable these studies the, cDNAs of *T. ni* kinesin-1 heavy (KHC) and light (KLC) chains were identified from a transcriptome analysis of *T. ni* Tnms42 cells then isolated by RT-PCR, cloned and sequenced. In addition, KHC and KLC were cloned into plasmid expression vectors, tagged at the 5' and 3' ends with GFP or the Myc and HA epitope tags and used to generate High Five (BTI-Tn5B14) stably transformed cell lines. High Five or the stable KHC and KLC expressing cell lines were used to determine if AC141 associates with kinesin-1. Initial experiments showed that in both N- and C-terminal HA-tagged KLC stable cell lines infected with WT virus, AC141 co-immunoprecipitates with tagged KLC. In addition, HA-tagged AC141 was found to co-immunoprecipitate WT KLC. In Myc-tagged stable cell lines, KLC co-localizes with HA-AC141 in regions adjacent to the plasma membrane at 20, 24 and 48 hpi. Confocal microscopy was also used to examine co-localization of AC141, microtubules and tagged kinesin-1 molecules. These studies continue to support the association of AC141 with microtubules suggesting that baculovirus NCs utilize them for egress.

Workshop Sessions

VIR-WK206.06 - Structure of entomopoxvirus spindles reveals in vivo crystals of a chitin oxidase

Kaheina Aizel[1], Marcel Hijnen[1], Marion Boudes[1], Vincent Olieric[2], Clemens Schulze-Briese[2], Max Bergoin[3], Fasseli Coulibaly[1]

[1]Monash University, Melbourne, Australia, [2]Swiss Light Source at the Paul Scherrer Institut, Villigen, Switzerland, [3]Universite de Montpellier 2, Montpellier, France

Entomopoxviruses produce two types of microcrystals in infected cells: virus-containing spheroids representing their main infectious form; and spindles, bipyramidal crystals of the viral fusolin protein, that contribute to the oral virulence of these viruses. In co-feeding experiments, spindles also enhance the insecticidal activity of baculovirus and Bacillus thuringiensis, which suggested their use as bioinsecticide additives. To understand how fusolin contributes to virulence and assembles in vivo, we determined the structures of entomopoxvirus spindles by X-ray micro-crystallography using functional crystals isolated from infected common cockchafers. This structure reveals that fusolin is composed of a globular domain followed by an extended C-terminal molecular arm (CT). The globular domain is structurally homologous to CBP21, a protein that is secreted by Gram-negative bacteria to degrade chitin as a source of energy. Like CBP21, fusolin has all the hallmarks of a lytic polysaccharide monooxygenase enzyme (LPMOs) with two conserved histidine residues forming a copper binding site and a conserved di-tryptophan motif positioned to bind the planar surface of crystalline chitin. The LPMO domain assembles in vivo into ultra-stable crystals crosslinked by CT. This molecular arm mediates the formation of domain-swapped dimers and their assembly into a crystalline lattice stabilized by a 3-D network of inter-dimer disulfide bonds. Overall, the molecular organization of spindles indicates a mode of action where controlled released of the LPMO domain of fusolin by proteolytic removal of the CT extension leads to disruption of the chitin-rich peritrophic matrix of larvae to facilitate the initial steps of viral invasion of the host.

Workshop Sessions

VIR-WK206.07 - C-terminus features of Autographa californica nucleopolyhedrovirus DNA polymerase important for its nuclear localization, viral DNA synthesis and virus replication

Guozhong Feng[1,2], Peter Krell[2]
[1]China National Rice Institute, Hangzhou, China, [2]University of Guelph, Guelph, Canada

The 984 aa DNA polymerase (DNApol) of the baculovirus *Autographa californica* nucleopolyhedrovirus (AcMNPV), with exonuclease and polymerase domains between aas 109 to 750, is essential for viral DNA synthesis but the role of the carboxy end is unknown. To this end we generated bacmids expressing different DNApol C-terminal truncations starting from aa 800 to 949 either on their own, or as EGFP:DNApol fusions. The full length EGFP:DNApol showed clear nuclear localization in an infection-independent manner and virus production was rescued by an EGFP:DNApol bacmid. In contrast, bacmids with C terminal DNApol truncations (independent or as EGFP:DNApol fusions) were compromised for viral DNA synthesis and virus production; i.e. the exonuclease and polymerase domains alone were insufficient for DNApol function. We identified one monopartite and one bipartite putative nuclear localization sequences (NLSs) within the C terminus along with a highly conserved 10 aa motif at the extreme C terminus (Cter consensus). Each NLS (fused to EGFP) was independently functional in nuclear localization, but an EGFP:DNApol fusion with a deletion of either NLS remained cytoplasmic. No virus production was observed from bacmids expressing DNApol lacking either one of these NLSs or with an alanine-substituted Cter consensus. A bacmid expressing a DNApol chimera with aa 1 to 800 from the AcMNPV DNApol followed by the C-terminal aa 798 to 990 from the DNApol of another alphabaculovirus *Choristoneura fumiferana* MNPV (CfMNPV) rescued virus spread and replication. The CfMNPV DNApol C terminus, despite its low sequence similarity to that of AcMNPV, also encoded two, but different, NLSs plus the Cter consensus suggesting that these three features were responsible for DNApol nuclear localization and function and not the C-terminal sequence per se. Our data indicated that both NLSs and the DNApol Cter consensus features were critical to nuclear localization of AcMNPV DNApol, viral DNA synthesis and virus production.

Workshop Sessions

VIR-WK207.01 - Three-Dimensional Cell Models for the Study of Enterovirus Entry and Infection

Coyne Drummond[1], Katharine Harris[1], Carolyn Coyne[1]
[1]University of Pittsburgh, Pittsburgh, USA

Despite serving as the primary cell portal for coxsackievirus B (CVB) entry, very little is known regarding the specific molecular events that regulate CVB entry and replication in, and egress from, the intestinal epithelium. Although the use of cultured intestinal cells grown in two-dimensions (2-D) has provided the foundation for many of the studies regarding CVB-intestinal cell interactions, an inherent limitation with this approach is the inability to recapitulate the three-dimensional architecture and multicellular complexity associated with the human gastrointestinal tract. To overcome this limitation, we have applied a previously characterized three-dimensional (3-D) organotypic cell culture model, which recapitulates many of the properties of the human gastrointestinal tract, to study CVB-intestinal cell interactions. Intestinal cells cultured in the rotating wall vessel (RWV) bioreactor culture system exhibit many characteristics normally associated with fully differentiated functional intestinal epithelia in vivo, including distinct apical and basolateral polarity, increased expression and better organization of tight junctions, extracellular matrix, brush border proteins, and highly localized expression of mucins, and also exhibit multicellular complexity (including the presence of M/M-like cells, goblet cells, Paneth cells, and enterocytes) which is not possible using standard 2-D culture systems. This model will allow us to provide unprecedented insights into the complex dialogue that likely exists between CVB and the intestinal epithelium, which may have a profound impact on CVB pathogenesis.

Workshop Sessions

VIR-WK207.02 - Cytoplasmic viral RNA-dependent RNA polymerase disrupts the intracellular splicing machinery by entering the nucleus and interfering with Prp8

Yen-Chin Liu[1,2], Rei-Lin Kuo[1,2], Jing-Yi Lin[5], Peng-Nien Huang[1], Yi Huang[3], Hsuan Liu[3], Jamine J. Arnold[6], Robert Yung-Liang Wang[1,4], Craig E. Cameron[6], Shin-Ru Shih[1,2]

[1]Research Center for Emerging Viral Infections, Chang Gung University, Taoyuan, Taiwan, [2]Department of Medical Biotechnology, Chang Gung University, Taoyuan, Taiwan, [3]Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan, [4]Institute of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan, [5]School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan, [6]Biochemistry and Molecular Biology, Pennsylvania State University, State College, USA

The primary role of cytoplasmic viral RNA-dependent RNA polymerase (RdRp) is viral genome replication in the cellular cytoplasm. However, picornaviral RdRp denoted 3D polymerase (3Dpol) also enters the host nucleus, where its function remains unclear. In this study, we describe a novel mechanism of viral attack in which 3Dpol enters the nucleus through the nuclear localization signal (NLS) and targets the pre-mRNA processing factor 8 (Prp8) to block pre-mRNA splicing and mRNA synthesis. The fingers domain of 3Dpol associates with the C-terminal region of Prp8, which contains the Jab1/MPN domain, and interferes in the second catalytic step, resulting in the accumulation of the lariat form of the splicing intermediate. Endogenous pre-mRNAs trapped by the Prp8-3Dpol complex in enterovirus-infected cells were identified and classed into groups associated with cell growth, proliferation, and differentiation. Our results suggest that picornaviral RdRp disrupts pre-mRNA splicing processes, that differs from viral protease shutting off cellular transcription and translation which contributes to the pathogenesis of viral infection.

Workshop Sessions

VIR-WK207.03 - Role of human SRp20 protein in poliovirus translation initiation

Ilya Belalov[1], Bert Semler[1]
[1]Department of Microbiology and Molecular Genetics, School of Medicine, University of California, Irvine, USA

Translational regulation of gene expression in eukaryotic cells predominantly depends on the step of initiation. Internal ribosome entry directed translation of poliovirus RNA is no exception to this tenet. The highly structured 5' noncoding region of poliovirus genomic RNA contains six structural domains. Five of these domains comprise the internal ribosome entry site (IRES), a composite structure required for binding the uncapped viral RNA to the small ribosome subunit. Stem-loop IV, the major domain of the poliovirus IRES, binds cellular poly(rC) binding protein 2 (PCBP2), which in turn binds to cellular splicing related protein 20 (SRp20; also known as SRSF3). These interactions are required for efficient initiation of viral protein synthesis. SRp20 contains two functional domains that contribute to its activities in the cell. In uninfected cells, the C-terminal arginine/serine rich RS domain of SRp20 plays a role in spliceosome recruitment via protein-protein interactions. For poliovirus translation initiation, the RS domain appears to anchor SRp20 to the IRES by a PCBP2-stem loop IV bridge. In uninfected cells, the N-terminal RNA binding domain (RRM) provides substrate specificity by binding short pre-mRNA splicing enhancer sequences. We hypothesize that the role of the RRM domain in poliovirus IRES ribosome recruitment is the direct binding of 18S rRNA in the 43S preinitiation complex. In our preliminary analysis of the structure of the poliovirus IRES by small-angle X-ray scattering, we have detected large-scale flexibility of this RNA element. As such, this structure may require stabilization in an active conformation by SRp20 and other IRES trans-acting factors. Ongoing experiments are aimed at investigating the precise role of the SRp20 RRM domain during poliovirus ribosome recruitment.

Workshop Sessions

VIR-WK208.01 - Bluetongue virus replication: understanding a complex molecular machine

Polly Roy[1]

[1]*London School of Hygiene and Tropical Medicine, London, UK*

My lecture will be centred on the molecular dissection of orbiviruses, particularly Bluetongue virus, a model complex RNA virus with a view to understanding the role of each protein in the virus replication cycle. BTV is an insect-vectored emerging pathogen of wild ruminants and livestock (with mortality reaching 70% in sheep) in many parts of the world. The virion particle is an architecturally complex structure of 4 consecutive layers of protein surrounding a genome of ten double-stranded (ds) RNA segments. We have used a multi-disciplinary approach to understanding BTV replication and assembly, including atomic and 3D structure reconstructions, protein engineering, synthetic biology and reverse genetics. Specifically, we have probed the complexity of the virion, defined the virus encoded enzymes required for RNA replication, provided an order for the assembly of the capsid shell and the protein sequestration required for it, and interrogated the role of host proteins in the virus replication cycle. A reconstituted in vitro transcription/replication complex has defined the individual steps involved in the replication and packaging of the viral dsRNA genome. These findings illuminate BTV replication and lead directly to the design of novel assemblies and attenuated strains. Equally, they indicate the pathways that related viruses might use, including viruses that are pathogenic to man and animals, to provide an informed starting point for intervention or prevention.

Workshop Sessions

VIR-WK208.02 - The essential roles of template RNA and replication proteins in the formation alphavirus replication spherules

Tero Ahola[1], Kirsi Hellström[1], Katri Kallio[1]
[1]University of Helsinki, Helsinki, Finland

All known positive-strand RNA viruses modify the intracellular membranes of the host cells to create specific membrane structures or mini-organelles for virus replication. The replication sites of alphaviruses and many other animal and plant viruses consist of numerous bulb-shaped membrane invaginations, also known as spherules, which contain the viral double-stranded replication intermediates. The spherule interior is connected to the cytoplasm by a narrow neck structure. We have devised an efficient trans-replication system for alphaviruses, which allows us to dissect the requirements for spherule formation. In this system, replication proteins and templates are expressed in mammalian cells from separate plasmids. Mutants lethal in the context of virus infection can easily be analysed, since template and protein production is maintained. To detect spherules and study their properties in transfected cells, we use correlative light and electron microscopy (CLEM). We have shown that the presence of a replication template is absolutely essential for spherule formation for alphaviruses. Furthermore, template length determines the size of the spherule: shorter templates generate much smaller spherules than those observed during the replication of full-length viral templates. A combination of different template lengths also yields different size classes of spherules in the same cell. We have also shown that the polymerase and helicase activities of the replication proteins nsP4 and nsP2 are essential for spherule formation. In contrast, mutations disrupting the RNA capping activities of nsP1 permit the synthesis of minus strand RNAs and the concomitant generation of spherule structures. Altogether, these results indicate that RNA synthesis and spherule formation are tightly linked processes for alphaviruses. Comparison with other viruses suggests that although the spherules are similar in appearance, the requirements for spherule formation can be different. This work highlights the fundamental similarities as well as differences in the membrane modification induced by positive-strand RNA viruses.

Workshop Sessions

VIR-WK208.03 - Exogenous Rubella Virus capsid proteins enhance virus genome replication

Min-Hsin Chen[1], Cara C. Burns[1], Emily Abernathy[1], Adaeze A. Ogee-Nwankwo[1], Joseph P. Icenogle[1]
[1]*National Center For Immunization And Respiratory Diseases, Centers For Disease Control And Prevention, Atlanta, USA*

Enhanced replication of rubella virus (RV) and replicons by de novo synthesized viral structural proteins has been described. Such enhancement can occur by viral capsid proteins (CP) alone in trans. In this study, we found that the exogenous addition of RV CP also enhanced viral genome replication, when used to package replicons as well as when mixed with RNA during transfection. We demonstrated that CP does not affect translation efficiency from genomic or subgenomic RNA (sgRNA), the intracellular distribution of nonstructural protein (NSP) or sgRNA synthesis. However, significantly more double stranded RNA was observed in cultures co-transfected with recombinant CP (rCP), resulting primarily from increases in negative-strand RNA genome synthesis. rCP was found to restore replication of a few mutants in NSP but failed to rescue the replicons with defects in positive-strand RNA synthesis. These results suggest that the extracellular RV CP increases efficiency of early viral genome replication by modulating the negative-strand RNA synthesis, possibly through a general mechanism such as RNA stability rather than interaction with specific domain in NSP.

Workshop Sessions

VIR-WK208.04 - Structure based modification of Bluetongue virus VP6 to produce a viable VP6-truncated BTV

Eiko Matsuo[1,2], Esther Leon[3], Steve Matthews[3], Polly Roy[2]
[1]Kobe University, Kobe, Japan, [2]London School of Hygiene and Tropical Medicine, London, UK, [3]Imperial College London, London, UK

Bluetongue virus VP6 protein is an ATP hydrolysis dependent RNA helicase whose precise localization and role in the virus replication cycle remains unclear. Preliminary data suggests that VP6 exists as both a monomer and as higher order oligomers in different experimental conditions, in contrast to helicases in general which act as monomers or dimers. To define further the role of VP6 we have combined a number of approaches, virological, biochemical and structural, to address the requirement for the complete VP6 in BTV replication. NMR structure studies were performed on full-length VP6 and several truncated variants following expression, labelling and purification in E.coli. Our studies demonstrated the sequence specific assignment of the 1H-15N-HSQC spectrum of the 15N, 13C-labelled full length VP6. Approximately half of the protein sequence was unambiguous. The remaining half of the protein is predicted to be formed of two large loops, whose flexibility makes them “invisible” to NMR as the signals are broadened. Further, the sequence specific assignments of the 1H-15N-HSQC spectrum of a loop truncated variant of VP6 suggested that loss of the loop did not affect to the overall folding of the protein. Using a BTV reverse genetics system, we showed that the VP6-truncated BTV was viable in BHK cells in the absence of any helper VP6 protein, suggesting that a part of VP6 was not absolutely necessary for BTV replication. The characterization of these viruses in both mammalian and insect cells and the deduced role of VP6 in the BTV replication cycle will be discussed.

Workshop Sessions

VIR-WK208.05 - Identification and characterization of the role of C-terminal Src Kinase in dengue virus replication

Guruprasad Medigeshi[1]

[1]*Translational Health Science and Technology Institute, Gurgaon,India*

Tyrosine kinases (TK) regulate diverse cellular functions such as cell cycle regulation, protein trafficking, organelle biogenesis, cytoskeletal organization and immune responses. Many viruses hijack TK signaling pathways at various stages of viral life-cycle such as entry, replication, assembly, egress and also to evade host immune response. In order to identify tyrosine kinases involved in flavivirus life-cycle we have performed screening of a siRNA library targeting 88 human tyrosine kinases in Huh-7 cells and have identified TKs that block infectious virus production. One of the identified TKs was CSK (C-terminal Src Kinase) and we show that depletion of CSK by siRNAs blocks DENV infection at the stage of RNA replication and has no effect on viral entry. We are further characterizing the role of CSK and mapping the domains of CSK that is involved in dengue replication. We are also investigating whether this function of CSK is mediated via regulation of SRC kinase signaling pathways to facilitate dengue replication.

Workshop Sessions

VIR-WK208.06 - Molecular determinants of turnip mosaic virus 6K2 protein in viral replication complex cellular biogenesis

Jun Jiang[1], Camilo Patarroyo[2], Juan Wan[1], Maxime Agbeci[1], Daniel Garcia[1], Romain Grangeon[1], Huanquan Zheng[2], Jean-François Laliberté[1]

[1]Institut National de la Recherche Scientifique (INRS), Institut Armand-Frappier, Laval, Canada, [2]Department of Biology, McGill University, Montreal, Canada

Turnip mosaic virus (TuMV) is a positive-sense single-stranded RNA virus. TuMV infection reorganizes the host cell secretory pathway and induces the formation of at least two types of structures: a large perinuclear globular structure and peripheral motile endoplasmic reticulum (ER)-associated vesicles. However, the molecular determinants for the biogenesis of these 6K2 structures are unknown. According to secondary structure predictions, 6K2 is characterized by the presence of a 19-amino acid N-terminal tail, an 11-amino acid C-terminal tail and a trans-membrane domain (TMD) composed of 23 amino acids that are responsible for its membrane association. Cellular membrane fractionation experiments together with different chemical treatments established that 6K2 is an integral membrane protein. When the N-terminal tail was progressively deleted, the truncated 6K2 was retained in the ER and accumulated in the perinuclear globular structure. A conserved tryptophan residue in the N-terminal tail was substituted with alanine and introduced into an infectious clone of TuMV (W15A). The mutated virus was able to replicate but intercellular movement was inhibited. By yeast two-hybrid and Co-IP experiments, we detected the interaction of 6K2 with COPII coatomer Sec24. These results indicate the N-terminal tail of 6K2 contains an ER export motif, which is indispensable for virus movement.

Workshop Sessions

VIR-WK208.07 - Selective HIV-1-mediated recruitment of nucleoporin p62 for viral assembly

Valerie Le Sage[1], Sean Seltzer[1], Andrew Mouland[1]
[1]Lady Davis Institute at the Jewish General Hospital and McGill University, Montréal, Canada

Our earlier work showed that HIV-1 induces dramatic changes in the composition of nuclear pore complexes (NPCs) in human immunodeficiency virus type 1 (HIV-1)-infected T cells (Monette et al., J. Cell Biol. 2011). NPCs are composed of 30 different nucleoporins (Nups) that stud the nuclear envelope to regulate nucleocytoplasmic trafficking of macromolecules. As a direct consequence of the nuclear export of the HIV-1 genomic RNA (vRNA), Nup62 is ejected from NPCs and translocates to the cytoplasm. In this work, we report that Nup62 is found associated to vesicular structures in the cytoplasm in HIV-1-expressing cells, as determined by immunogold electron microscopy. S100/P100 fractionation and membrane flotation assays showed that whereas Nup62 was virtually absent in membrane fractions in mock-expressing cells, a strict partitioning of Nup62 to membrane fractions was found in HIV-1-expressing cells. The Nup62 membrane association was not dependent on the presence of Gag as Nup62 remained exclusively in membrane fractions upon expression of a Gag-less proviral construct. To characterize the cytoplasmic complexes containing Nup62, comparative proteomic analyses of immunoprecipitated FLAG-Nup62 were undertaken in the presence and absence of HIV-1. FLAG-Nup62 interacting partners found solely in HIV-1-expressing cells included numerous ER, Golgi, nuclear, exocyst and mitochondrial factors, suggesting that Nup62 may associate with a wide variety of organellar membranes. Combined, sensitive fluorescence in situ hybridization/immunofluorescence and super-resolution structured illumination microscopy allowed us to visualize vRNA and Nup62 at an ~120nm resolution. A proportion of vRNA and Nup62 colocalized in small discrete punctae in the cytoplasm and at juxtanuclear positions, suggesting that there may be a population of Nup62 associated to the vRNA to make up a HIV-1 ribonucleoprotein. Finally, the overexpression of Nup62 in HeLa cells resulted in a 3-fold increase in virus production. Our results suggest that Nup62, that finds itself in the cytoplasm following HIV-1-induced rearrangements of the NPC, plays a role in virus assembly.

Workshop Sessions

VIR-WK209.01 - A bivalent vaccine based on a replication-incompetent Influenza A virus possessing Pneumococcal Surface Protein A protects mice from secondary Pneumococcal infection

Ryuta Uraki[1], Zhenyu Piao[2,3], Yukihiro Akeda[2], Kiyoko Iwatsuki-Horimoto[1], Maki Kiso[1], Makoto Ozawa[4,5], Kazunori Oishi[2,6], Yoshihiro Kawaoka[1,7,8]

[1]Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, [3]Division of Infection Control and Prevention, Osaka University, Osaka, Japan, [4]Laboratory of Animal Hygiene, Kagoshima University, Kagoshima, Japan, [5]Transboundary Animal Diseases Center, Kagoshima University, Kagoshima, Japan, [6]National Institute of Infectious Diseases, Tokyo, Japan, [7]ERATO Infection-Induced Host Responses Project (JST), Saitama, Japan, [8]Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, USA

Secondary bacterial infections after influenza can be a serious problem, especially in young children and the elderly. Yet, the efficacy of current vaccines is limited. We previously demonstrated that a replication-incompetent PB2-knockout (PB2-KO) influenza virus possessing a foreign gene in the coding region of its PB2 segment can serve as a platform for a bivalent vaccine. Here, we generated the PB2-KO virus expressing pneumococcal surface protein A (PspA), which is a promising antigen against Streptococcus pneumoniae infection. PB2-KO virus replication was restricted to PB2-expressing cells. We then examined the efficacy of this virus as a bivalent vaccine in a mouse model. High levels of influenza virus-specific and PspA-specific antibodies were induced in the serum, bronchoalveolar lavage fluid, and nasal washes of immunized mice, which were protected from lethal doses of influenza virus and Streptococcus pneumoniae. Moreover, the immunized mice were completely protected from secondary pneumococcal infection after the influenza virus infection. Our PB2-KO virus bearing PspA thus provides a promising option to prevent primary and secondary pneumococcal infection, as well as influenza.

Workshop Sessions

VIR-WK209.02 - Codon-optimized filovirus DNA vaccines delivered by intramuscular electroporation protect cynomolgus macaques from lethal Ebola and Marburg virus challenges

Connie Schmaljohn[1], Louis Altamura[1], Catherine Badger[1], Drew Hannaman[2], Lesley Dupuy[1], Rebecca Grant-Klein[1]
[1]USAMRIID, Frederick, USA, [2]Ichor Medical Systems Inc., San Diego, USA

Filoviruses are highly pathogenic negative strand RNA viruses, which cause outbreaks of hemorrhagic fever in humans and nonhuman primates with high case-fatality rates. We evaluated the immunogenicity and protective efficacy of plasmid DNA vaccines expressing codon-optimized filovirus glycoprotein (GP) genes administered to cynomolgus macaques by intramuscular electroporation. The vaccines tested expressed the GP genes from two viruses in the Ebolavirus genus, Zaire (EBOV) and Sudan (SUDV) ebolaviruses, and two viruses in the Marburgvirus genus, Marburg Musoke (MARV) and Ravn (RAVV) viruses. Macaques were vaccinated with the individual DNA vaccines or with a mixture of all four vaccines and challenged with either EBOV or MARV. All macaques that received filovirus DNA vaccines developed GP-specific IgG antibodies, with no significant differences in titer between single and multiagent groups. Most of these macaques also developed virus-neutralizing antibodies. Analysis of peripheral blood mononuclear cells by IFN-gamma ELISpot demonstrated that vaccinated macaques generated filovirus GP-specific cytotoxic T cell responses. Both the MARV and multiagent vaccines were able to protect animals from lethal MARV challenge (5/6 vs. 6/6). In contrast, although 5/6 macaques vaccinated with only the EBOV vaccine survived challenge, only 1/6 given the multiagent vaccine survived. These results suggest possible immunological interference; thus, additional studies are underway to measure differences in the immune response to EBOV by macaques vaccinated with the individual vs the combination DNA vaccines. Our results provide evidence that codon-optimized DNA vaccines against filoviruses can elicit protective immune responses in cynomolgus macaques, but that prechallenge total and neutralizing antibody titers and IFN-gamma T cell responses do not accurately predict protective efficacy of the vaccines.

Workshop Sessions

VIR-WK209.03 - Attenuated recombinant measles virus expressing highly pathogenic avian influenza virus (HPAIV) antigen is protective against HPAIV challenge in cynomolgus monkeys

Tomoko Fujiyuki[1], Ryo Horie[1], Misako Yoneda[1], Takeshi Kuraishi[2], Fumihiko Yasui[3], Keisuke Munekata[3], Shosaku Hattori[2], Hiroshi Kida[4], Michinori Kohara[3], Chieko Kai[1]

[1]Laboratory Animal Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]Amami Laboratory of Injurious Animals, The Institute of Medical Science, The University of Tokyo, Kagoshima, Japan, [3]Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [4]Graduate School of Veterinary Medicine, Hokkaido University, Hokkaido, Japan

Highly pathogenic avian influenza virus (HPAIV) continues to threaten human health, thus there are urgent needs for development of effective vaccines against HPAIV. We generated a recombinant measles virus (MV) expressing haemagglutinin (HA) protein of HPAIV (H5N1, clade 2.3). By using a backbone of wild type MV derived-mutant defective for the expression of V [MV-HL-V(-)] or a MV vaccine strain (MV-Ed), H5N1 HA gene whose multibasic site necessary for its cleavage was deleted was inserted between N and P genes of MV genome. Infection of the rescued recombinant viruses [rMV-Ed-HA and rMV-HL-V(-)-HA] resulted in HA expression in vitro. Vaccination of the recombinant viruses to cynomolgus monkeys induced production of anti-H5N1 HA antibody and anti-MV antibody, while the vaccinated monkeys appeared healthy. Protective effects of the recombinant viruses against HPAIV infection was examined using a monkey model, which that we previously established. The monkeys inoculated with either rMV-HL-V(-)-HA or rMV-Ed-HA, and then challenged with a wild water bird-derived HPAIV (A/Whooper swan/Hokkaido/1/2008, H5N1, clade 2.3.2.1). The vaccinated monkeys were recovered earlier from influenza symptoms than unvaccinated monkeys. The results suggest that both of rMV-Ed-HA and rMV-HL-V(-)-HA are candidates of a safe and effective vaccine against HPAIV infection to protect from HPAI severity.

Workshop Sessions

VIR-WK209.04 - Development of live attenuated H7 (H7N3, H7N7 and H7N9) influenza vaccines

Zhongying Chen[1], Janine Lu[1], Qi Xu[1], Yumiko Matsuoka[2], Kanta Subbarao[2], Hong Jin[1]
[1]MedImmune LLC, Mountain View, USA, [2]NIAID, NIH, Bethesda, USA

To prepare for potential pandemics caused by influenza H7 subtype viruses, we have developed live attenuated influenza vaccines against H7N3 (A/British Columbia/CN-6/2004, BC04 ca), H7N7 (A/Netherland/209/20003, NL03 ca) and the recently emerged H7N9 (A/Anhui/1/2013, AH13 ca) viruses based on cold-adapted influenza virus A/Ann Arbor/6/60 (AA ca). The reassortant H7N9 vaccine virus with the wt A/Anhui/1/2013 HA and NA sequences replicated poorly in eggs; we developed high yield vaccine viruses by in vitro passage followed by re-engineering by reverse genetics. The candidate vaccine contains the N133D and G198E substitutions in the HA head region that improved viral titer by >10-fold, reaching a titer of 8.6 log10 Fluorescent Focus Units/mL without affecting viral antigenicity and immunogenicity. These two amino acid changes also significantly improved yield of an H7N9 PR8 reassortant virus in eggs. The HA receptor binding preference affects viral replication in the upper respiratory tract and immunogenicity in ferrets. The BC04 ca and AH13 ca viruses exhibit α2,3-SA and α2,6-SA dual receptor binding preference while the NL03 ca virus preferentially binds α2,3-SA. Substitution of the Q226 and G228 (Q-G) residues by L226 and S228 (L-S) in the HA of NL03 ca improved its binding to α2,6-SA and immunogenicity as measured by H7-specific antibody and cellular responses in ferrets. These H7 vaccine viruses elicited protective immune responses in ferrets and have been evaluated in Phase I clinical studies. The three live attenuated H7 influenza vaccines represent coverage for viruses from North American, Eurasian and Asian lineages and have some cross-reactivity against each other. The production of these live attenuated H7 vaccines will be valuable in our pandemic preparedness program to protect the public from influenza H7 subtype virus infections.

Workshop Sessions

VIR-WK209.05 - A nonspreading Rift Valley fever virus vector expressing the hemagglutinin of H1N1 virus fully protects mice from influenza infection after a single vaccination

Nadia Oreshkova[1,2], Lisette Cornelissen[1], Alexander de Haan[2], Rob Moormann[1,2], Jeroen Kortekaas[1]
[1]*Department of Virology, Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands,* [2]*Virology Division, Faculty of Veterinary Medicine, Utrecht University,Utrecht, The Netherlands*

Virus replicon particles are capable of infection, genome replication and gene expression, but are unable to produce progeny virions, rendering their use inherently safe. By virtue of this unique combination of features, replicon particles hold great promise for vaccine applications. We have previously developed replicon particles of Rift Valley fever virus (RVFV) and we have demonstrated their high efficacy as a RVFV vaccine in the natural target species. Here we investigate the feasibility of using this nonspreading RVFV (NSR) as a vector vaccine, using a model influenza A virus (IAV) infection in mice. NSR particles were designed to express either the full-length hemagglutinin of IAV H1N1 (NSR-HA) or the respective soluble ectodomain (NSR-sHA). The efficacies of the two NSR vaccines, applied via either the intramuscular or the intranasal route, were tested. A single vaccination with NSR-HA protected all mice from a lethal challenge, while none of the NSR-sHA-vaccinated mice survived the challenge. Interestingly, whereas intramuscular vaccination elicited superior systemic immune responses, intranasal vaccination provided optimal clinical protection.

Workshop Sessions

VIR-WK209.06 - Highly efficacious influenza vaccination using αGal carbohydrate modification

Brian Martin[1], Jinjin Zhang[1], Carol Martin[1], Alex Chen[1], Serguei Kisselev[1], Katie Bauer[1], Emily Dasen[1], Nicholas Vahanian[1], Charles Link[1]

[1]*BioProtection Systems/NewLink Genetics, Ames, USA*

Recent influenza pandemics (such as H7N9) have highlighted the need for flu vaccine improvement. The gal-α1,3-gal-β1,4-N-acetylglucosamine (αGal) epitope is present on glycoproteins and lipids in most mammals, but is not found in Old World primates or humans. Exposure to carbohydrates present on normal intestinal flora induces development of high-titer anti-αGal antibodies in αGal(-) species, which is the primary reason for rapid rejection of transplanted αGal(+)tissues in αGal(-) animals. This natural antibody response is the basis for HyperAcute(R) technology that is in multiple clinical trials as cancer immunotherapy. In the current studies, we hypothesized that modification of influenza vaccines would enhance the immunogenicity of the vaccine. Since most mammals are αGal(+), experiments are done mainly in α-1,3-galactosyltransferase (αGT) knockout mice and we spent some time optimizing this model system. We developed a novel chemical modification system that allowed us to add αGal epitopes to vaccines that were made in an αGal(-) setting and we have demonstrated enhanced vaccine efficacy using multiple vaccine platforms. We examined a mouse-adapted H1N1 virus strain (A/Puerto Rico/8/34) and two pandemic virus strains (H5N1 and H7N9). Our data demonstrate that modification with the αGal epitope in the absence of any additional adjuvant drastically increases the immunogenicity of the vaccine (with greater than 10-fold increase in antibody responses). Our experiments demonstrate enhanced vaccine efficacy in every platform tested. In mice vaccinated against and subsequently challenged with the mouse-adapted virus, survival increased significantly. In summary, our results show that αGal-modification of influenza vaccines can greatly enhance immunogenicity, increasing immunity against viral disease. These data lend strong support for the use of the αGal influenza vaccine platform in human clinical trials and suggest that αGal-modification may be efficacious for other infectious disease vaccines.

Workshop Sessions

VIR-WK209.07 - VaxCelerate: the Use of MTBhsp70-Avidin as an adjuvant to rapidly generate self-assembling vaccines with biotinylated, antigen-specific peptides targeting emerging pathogens

Pierre Leblanc[1], Thimothy Brauns[1], Cybelle Luza[1], Christine Boyle[2], Lenny Moise[2], Anne De Groot[2], Jordan B. Fishman[3], Eric A. Berg[3], Russel Coleman[4], Mark Poznansky[1]

[1]Vaccine & Immunotherapy Center, Massachusetts General Hospital, Charlestown, USA,, [2]EpiVax Incorporated, Providence, USA, [3]21st Century Biochemicals, Marlboro, USA, [4]Pfenex Incorporated, San Diego, USA

Development of effective vaccines against emerging infectious diseases can take years to progress from pathogen isolation/identification to clinical approval. As a result, conventional approaches fail to produce field-ready vaccines before the EID has spread extensively. The VaxCelerate Project's goal is to address the need for more rapid vaccine development by creating a platform capable of generating and pre-clinically testing a new vaccine against specific pathogen targets in less than 120 days. A self-assembling vaccine is at the core of the approach. It consists of a fusion protein composed of the immunostimulatory Mycobacterium tuberculosis heat shock protein 70 (MTBhsp70) and the biotin binding protein, avidin. Mixing the resulting protein (MAV) with biotinylated pathogen specific immunogenic peptides yields a self-assembled vaccine (SAV). To meet the constrained time requirement for this project we used a distributed R&D model involving experts in the fields of protein engineering and expression, bioinformatics, peptide synthesis/design and GMP/GLP manufacturing and testing standards. This approach was first tested in a model system, Ovalbumin in C57Bl/6 mice, and then progressed to testing Flu (H1N1) specific peptides and ultimately a Lassa fever virus specific vaccine in transgenic HLA DR3 mice. Using a GLP validated assay we demonstrated that the Lassa fever vaccine assembled in this way induced significantly increased class II peptide specific interferon-γ CD4+ T cell responses in transgenic mice compared to peptide or MAV alone controls. The VaxCelerate approach may also facilitate accelerated regulatory review by using an identical design for each vaccine reducing review requirements for subsequent vaccines, and by developing safety assessment tools that are more relevant to human vaccine responses than current preclinical models.

Workshop Sessions

VIR-WK209.08 - Encoded natural adjuvants increase the efficacy of DNA vaccines

Eric Gowans[1], Tessa Gargett[1], Tamsin Garrod[1], Stanley Yu[1], Paul Radspinner[2], Branka Grubor-Bauk[1]
[1]Discipline of Surgery, The University of Adelaide, Adelaide, Australia, [2]FluGen Inc, Madison, USA

DNA vaccines are attractive because the immogen is expressed in an endogenous manner, leading to Th1 responses. However, their potential has not been realised due to suboptimal delivery, poor antigen expression and the lack of a localised inflammatory response, essential for antigen presentation and the development of an effective immune response to the vaccine-encoded antigens. To address these problems, we encoded membrane bound and secreted versions of heat shock protein 70 in a DNA vaccine encoding the HIV protein, gag, that act as natural adjuvants. Similarly, as we believed that the non-cytolytic nature of DNA vaccination was a factor contributing to its inefficiency, we developed a DNA vaccine encoding HIV gag and a cytolytic protein (perforin-PRF) that induces necrosis in vaccine-targeted cells in the dermis after intradermal delivery. This leads to the expression and extracellular localization of damage associated molecular patterns which are also effective adjuvants that bind to pathogen recognition receptors in antigen presenting cells. These vaccines generated increased levels of cell mediated immunity resulting in significant increased protection against challenge with EcoHIV, a chimeric HIV that replicates in mice, compared with responses generated after vaccination with a canonical DNA vaccine. We also encoded PRF in a DNA vaccine which encodes the HCV NS3 protein and vaccinated mice and pigs, representing a large animal model, with this vaccine. The results of this study showed that the PRF-encoding vaccine generated statistically significant higher levels of cell mediated immunity in mice and in pigs, as determined by ELIspot, after intradermal vaccination. The intradermal vaccine was delivered to the pig dermis by a microneedle device to ensure effective and reproducible delivery. An effective DNA vaccine may be used in a homologous multi-dose regimen without inducing vector-specific immunity, or as a DNA prime to increase the efficacy of a heterologous regimen.

Workshop Sessions

VIR-WK210.01 - Classic nuclear localization signals and a novel nuclear localization motif are required for nuclear transport of porcine parvovirus capsid proteins

Maude Boisvert[1], Véronique Bouchard-Lévesque[2], Sandra Fernandes[3], Peter Tijssen[4]
[1]Institut National de la Recherche Scientifique, Montreal, Canada

Nuclear targeting of capsid proteins (VPs) is important for genome delivery and precedes assembly in the replication cycle of porcine parvovirus (PPV). Clusters of basic amino acids, corresponding to potential nuclear localization signals (NLS), were found only in the unique region of VP1 (VP1up). Of the five identified basic regions (BR), three were important for nuclear localization of VP1up: BR1 was found to be a classic Pat7 NLS and the combination of BR4 and BR5 was a classic bipartite NLS. These NLS were both essential for viral replication. VP2, the major capsid protein contained no region with more than two basic amino acids in proximity. We identified three regions of basic clusters in the folded protein, assembled into a trimeric structure. Mutagenesis experiments showed that only one of these three regions was involved in VP2 transport to the nucleus. This structural NLS, termed "nuclear localization motif" (NLM) was located inside the assembled capsid, and thus can be used to transport trimers to the nucleus in late steps of infection but not for virions in initial infection steps. The two NLS of VP1up are located in the N-terminal part of the protein, externalized from the capsid during endosomal transit, exposing them for the nuclear targeting during early steps of infection. Globally, the determinants of nuclear transport of structural protein of PPV were different from those of closely-related parvoviruses.

Workshop Sessions

VIR-WK210.02 - Distribution of Parvovirus 4 and KI / WU Polyomaviruses in blood samples identified HIV positive, France.

Mhammed Touinssi[1], Vital Galicher[1], Philippe de Micco[1], Philippe Biagini[1]
[1]Viral Emergence and Co-Evolution Unit, UMR 7268, French Blood Agency, Marseille, France

Parvovirus 4 (PARV4) is a small DNA virus that belongs to family Parvoviridae (Jones et al., 2005). It exhibits a low sequence homology with parvovirus B19 (<30% aa). Epidemiological studies revealed significant prevalence of PARV4 in human blood, i.e. 6%-30%, depending on detection systems adopted and cohorts tested. The virus had been also detected in blood of individuals positive for HCV and HIV (Simmons et al., 2012). Several studies have been conducted already in our laboratory, demonstrating its presence in French blood donors and in some groups of patients (Biagini et al., 2008; Touinssi et al., 2010, 2011). KI and WU polyomaviruses (KIPyV, WUPyV) (family Polyomaviridae) are small non-enveloped DNA viruses harboring a circular double-stranded genome. They have been characterized since 2007. The initial identification of these two viruses in samples from the respiratory tract has been gradually extended to blood and urine samples, with prevalence ranging from 0.5% to 13%. Recent work has allowed us to demonstrate their presence in French blood donors (Touinssi et al., 2013). Until today, PARV4 and KIPyV / WUPyV implications for human health remain unknown. Literature analysis reveals that few studies have been conducted on blood samples positive for HIV, especially in France. We conducted an epidemiological and molecular study of PARV4 and KIPyV / WUPyV viruses on plasma samples positive for HIV RNA (n=128) (French Blood Agency, National Plasma Bank, period 2008-2012). A prevalence of 11.7% for PARV4 and 5.5% for KIPyV / WUPyV was observed for the cohort studied. Molecular approaches and results of this study are exposed here.

Workshop Sessions

VIR-WK210.03 - Experiences with fatal cases of Human Parvovirus B19 infections

Janak Kishore[1]
[1]Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

Parvovirus B19 (B19), family parvoviridae (discovered 1975) was listed as newly emerging virus (1981-1987); now known to cause myriads of clinical diseases still it could not gain much importance due to asymptomatic/self-limiting infections coupled with limited diagnostic will/ facilities and unknown disease burden. B19 has great tropism for erythroid progenitor cells, hepatocytes, vascular endothelium. B19 inflicts direct virus injury due to NS1, VP1u proteins, virus persistence and immune mediated injuries and occasionally uncontrolled macrophage activation syndrome. Review of literature revealed just a few case-reports on fatal B19 infections. Hence to highlight that B19 infections can even be fatal such cases encountered are described here. First, one was a fatal missed case of HLH (hemophagocytic lymphohystiocytosis) (in press) triggered by B19 (anti-B19 IgM, IgG were positive) and Epstein Barr virus in a two months male infant with fever, rash, bicytopenia and hepato-splenomegaly who was thought to have septicaemia and given antimicrobial agents, packed red cell and platelet transfusions. Testing for serum hyperferritinemia/ hypertriglyceridemia could have diagnosed HLH and saved a life. Secondly, a 46 yr old women with pure red cell aplasia and myelodysplasia untreated with I.V.I.G. but given hundreds of packed red cell transfusions over five years when she died (Kishore et al., 2003). Third, was a study on viral hepatitis where 16 children had FHF (fulminanat hepatic failure) of which four died and all four (100%) had B19 co-infection (author's unpublished data). Fourth again was a 5 yr male child with FHF had B19 co-infection with other hepatitis viruses but he died despite ICU treatment (Kishore J et al., 2009). These few fatal cases (tip of the ice-berg) denoted sinister complications of B19 infections. Hence B19 virus be regarded as potentially serious/fatal and be investigated in all cases with fever and rash, sudden pallor/drop of hemoglobin or fulminant hepatitis.

Workshop Sessions

VIR-WK210.04 - The discovery of three new iteraviruses (Densovirinae) and characterization of their expression strategies

Qian Yu[1], Peter Tijssen[1]

[1]*INRS- Institut Armand-Frappier, 531, boulevard des Prairies, Laval, Québec, Canada*

The Iteravirus genus, which belongs to the Densovirinae subfamily of the Parvoviridae family, so far includes three densoviruses, i.e. Casphalia extranea Densovirus (CeDNV), Dendrolimus punctatus Densovirus (DpDNV) and Bombyx mori Densovirus (BmDNV). In this study, we used a Sequence-Independent Single-Primer Amplification (SISPA) method to detect the pathogens of larvae from two additional insect species (Papilio polyxenes , Sibine fusca and Danaus plexippus), killed by some unknown pathogens. Sequencing of the clones that were obtained and BLAST analysis revealed the existence of three previously unknown densoviruses (provisionally named PpDNV , SfDNV and DppIDV). The genome of the new densoviruses were cloned into pCR2.1-topo or pBluescript(SK-) vectors. All of the virus sequences (including ITRs) have high identities with CeDNV and BmDNV. The identical genome organizations indicated that these three new densoviruses should be classified in the Iteravirus genus. Together with the infectious clones of CeDNV and BmDNV, we investigated the expression strategies of five different iteraviruses (PpDNV, SfDNV, CeDNV, BmDNV, DppIDV). Total RNA was obtained both from LD cell line transfected by infectious clones of the iteraviruses and virus infected larvae (Papilio polyxenes). RACE methods were used to identify the 5' and 3' transcription ends. The nonstructural (NS) and structural (VP) genes were located on the same strand of the genome. The NS cassette consists of two genes with NS1 and overlapping NS2. The NS2 transcripts all start at 7 nts downstream of the NS1 start codon. So far the transcription start for NS1 genes are still lacking. NS and VP transcripts do not overlap. The four VPs were similarly generated by leaky scanning translation of unspliced mRNA. The VP transcripts just start 2nts downstream of the poly (A) motif for NS transcripts. Interestingly, poly (A) signals for VP transcripts all overlap with the stop codons of the VP genes.

Plenary Sessions

BAM-PL04.01 - Folding of newly synthesized polypeptides

Bernd Bukau[1]
[1]Center for Molecular Biology, University of Heidelberg, Heidelberg, Germany

Protein homeostasis is established by a complex cellular machinery which assists and regulates regular folding pathways and counteracts protein misfolding and aggregation. A particularly critical process in the life of a protein is the native folding of newly synthesized proteins, which therefore is tightly controlled. Already during ongoing synthesis by the ribosome nascent polypeptides are subject to enzymatic processing, chaperone-assisted folding to the native state or targeting to translocation pores at membranes. The ribosome itself plays a key role in these different tasks by serving as platform for the regulated association of enzymes, targeting factors and chaperones that act upon the nascent polypeptides emerging from the exit tunnel. The molecular mechanisms integrating the different co-translational processes leading to the maturation and native folding of nascent chains will be described.

Plenary Sessions

BAM-PL04.02 - Architecture of the FtsZ ring *in vivo* and *in vitro* suggests a sliding filament mechanism of constriction

Piotr Szwedziak[1], Qing Wang[1], Matthew Tsim[1], Jan Löwe[1]
[1]*MRC Laboratory of Molecular Biology, Structural Studies Division, Cambridge, UK*

Cell membrane constriction is a prerequisite for cell division. The most common membrane constriction system in the prokaryotic kingdoms is based on the tubulin homologue FtsZ, whose filaments are anchored to the membrane by FtsA and enable formation of the Z ring and the divisome. The precise architecture of the FtsZ ring has remained enigmatic. Here, we report three-dimensional arrangements of FtsZ and FtsA filaments in *E. coli* cells and inside constricting liposomes by means of electron cryomicroscopy and cryotomography. In both situations, the Z-ring is composed of a small, single-layered band of filaments parallel to the membrane, creating a continuous ring through filament overlap. Detailed visualisations of the *in vitro* reconstituted constrictions as well as a complete tracing of the helical paths of the filaments with a molecular model favour a mechanism of FtsZ-based cell membrane constriction that is accompanied by filament siding.

Plenary Sessions

BAM-PL05.01 - Mechanisms and regulation of bacterial surface attachment and biofilm formation

Yves Brun[1]
[1]*Indiana University, Bloomington, USA*

The attachment of bacteria to surfaces provides advantages such as increasing nutrient access and resistance to environmental stress. Attachment begins with a reversible phase, often mediated by surface structures such as flagella and pili, followed by a transition to irreversible attachment, typically mediated by polysaccharides. The presentation will focus on stimulatory and inhibitory mechanisms of cell attachment and biofilm formation. I will describe how the interplay between pili and flagellum rotation of *Caulobacter crescents* cells stimulates the rapid transition between reversible and polysaccharide-mediated irreversible attachment by stimulating the biosynthesis of the holdfast adhesive polysaccharide. I will also describe how programmed cell death in a biofilm produces extracellular DNA that inhibits cell settling in the biofilm by directly binding to nascent holdfast, thereby stimulating cell dispersal in response to declining environmental quality.

Plenary Sessions

BAM-PL05.02 - Ecology and physiology of proteorhodopsin containing bacteria in the oceans

Carlos Pedrós-Alió[1]
[1]*Institut de Ciencies del Mar, CSIC, Barcelona, Spain*

About half of the bacteria living in the surface ocean contain a gene coding for proteorhodopsin (PR). PR acts as a light driven proton pump. In this way it provides energy to bacteria in the surface ocean when concentrations of suitable organic matter are low. Some bacteria can only use this extra energy to survive longer under starvation conditions. Flavobacteria, however, grow faster and more efficiently in the light than in the dark. Recent experiments show the intricate regulation of central metabolic pathways necessary for this positive growth effect of light. Finally, recent surveys in deep ocean (4000 m) and in Polar winter waters suggest that PR is present and sometimes active even under such dark conditions.

Plenary Sessions

MEM-PL04.01 - Sexual reproduction and the evolution of eukaryotic microbial pathogens

Joseph Heitman[1]
[1]*Department of Molecular Genetics and Microbiology, Duke University, Durham, USA*

Viral, bacterial, and eukaryotic pathogens evolve via genetic exchange. We focus on sexual reproduction of human fungal pathogens. Many fungal and parasitic pathogens were thought to be asexual, mitotic, and clonal; we now appreciate the majority have extant unusual sexual cycles. We study fungal mating-type identity. Many fungi are bipolar with two mating-types and a biallelic mating-type locus. Many basidiomycete fungi have more complex tetrapolar systems with two unlinked multi-allelic mating-type loci, yielding thousands of mating-types that enhance outcrossing but restrict inbreeding. Our studies reveal transitions from ancestral outcrossing to derived inbreeding systems occurred in pathogenic species aligned with nonpathogenic species. This transition has occurred repeatedly in plant and animal pathogens, possibly for host adaptation. Pathogenic *Cryptococcus* species took this one step further to a unipolar sexual cycle. These global human pathogens have largely unisexual populations that reproduce via an unusual sexual cycle involving only one mating-type. Like a-α opposite sex, α-α unisexual reproduction can admix parental diversity in progeny. However, in other cases solo α-α unisex involves selfing of identical genomes with no genetic diversity to exchange. Why organisms do so challenges conventional models on sex. We find unisexual reproduction provides multiple routes to adaptive benefit. First, unisex generates genetic diversity de novo, preserving well-adapted genomic configurations yet generating limited genetic diversity. Second, unisex promotes a dimorphic yeast to filamentous hyphae transition, enabling nutrient foraging and infectious spore generation. Third, unisex reverses Muller’s Ratchet, avoiding mutation accumulation that dooms asexual species to extinction. Other fungi and eukaryotic parasites also reproduce unisexually, generalizing these findings. Unisex may have evolved to mitigate sex costs associated and afford advantages associated with conventional sexual modes. Studies of fungal sex evolution illustrate general principles with implications for model and pathogenic microbes and multicellular eukaryotes. Roles of sex in *C. gattii* outbreaks will be considered.

Plenary Sessions

MEM-PL04.02 - Exploitation of the host cell membrane fusion machinery by Leishmania

Albert Descoteaux[1]
[1]*INRS-Institut Armand-Frappier, Laval, Canada*

Leishmania is particularly adept at transforming the macrophage into a hospitable host cell, and as such, constitutes a unique tool to study basic mechanisms of macrophage biology. Our laboratory focusses on the subversion of the macrophage membrane fusion machinery during *Leishmania* infection. We previously established that the promastigote forms of this parasite inhibit phagolysosome biogenesis. The surface lipophosphoglycan is responsible for this inhibition through its insertion in the phagosome membrane, causing destabilization of lipid microdomains and exclusion of the membrane fusion regulator Synaptotagmin V from the phagosome. We recently made the striking discovery that the *Leishmania* metalloprotease GP63 cleaves a subset of SNAREs and synaptotagmins that modulate phagosome functions, including antigen crosspresentation and cytokine secretion. Disruption of the host cell membrane fusion machinery thus appears as an efficient strategy to create an intracellular niche favorable to the establishment of infection and to the evasion of the immune system. Here, we will discuss the mechanisms by which *Leishmania* subverts the fusion machinery of host cells and the consequences of this subversion on the autophagic response. These studies will deepen our knowledge of *Leishmania* pathogenesis, and will provide novel insights into the regulation of phagosome functions. Knowledge gained from studying this host-parasite interaction may be extended to other intracellular pathogens. *Supported by the Canadian Institutes of Health Research*

Plenary Sessions

MEM-PL05.01 - Using a Yeast Model of Congenital Sideroblastic Anemia to Determine the Function of the Human Gene that Causes the Disease

Christopher McMaster[1]
[1]*Dalhousie University, Halifax, Canada*

A common type of congenital sideroblastic anemia is due to mutations in human SLC25A38. The function of SLC25A38 is not known. We used Sacharomyces cerevisiae to delineate that SLC25A38 is a mitochondrial glycine transporter required for the initiation of heme synthesis, providing a molecular explanation for how SLC25A38 mutations cause sideroblastic anemia. Within the mitochondria, glycine has several other roles including the synthesis of folate derivatives through the glycine cleavage system. We found that the addition of glycine plus folate ameliorated congenital sideroblastic anemia in the yeast and zebrafish congenital sideroblastic anemia models. We speculate that this dual requirement is due to the role of mitochondrial glycine as both a source of heme and of 5,10 methylenetetrahydrofolate. Given the tolerability of glycine and folate in humans, this study may represent a paradigm shift in the treatment of congenital sideroblastic anemia.

Plenary Sessions

MEM-PL05.02 - Anti-prion systems: Normal levels of Btn2p and Cur1p cure most newly formed [URE3] prion variants

Reed Wickner[1]
[1]*National Institutes of Health, Bethesda, USA*

[URE3] is an amyloid-based prion of the S. cerevisiae Ure2p, a regulator of nitrogen catabolism. We have shown that infectious amyloid of Ure2p has a folded in-register parallel β-sheet architecture that can explain how a protein can template its conformation, thus acting as a gene. The yeast prions [URE3] and [PSI+] are often lethal or severely toxic, and even their mildest forms are detrimental to their hosts. Overproduction of Btn2p, involved in late endosome to Golgi protein transport, or its paralog Cur1p, cures [URE3]. Btn2p, in curing, is co-localized with Ure2p in a single locus suggesting sequestration of Ure2p amyloid filaments. We find that most [URE3] variants generated in a btn2 cur1 double mutant are cured by restoring normal levels of Btn2p and Cur1p, with both proteins needed for efficient curing. The [URE3] variants cured by normal levels of Btn2p and Cur1p all have low seed number, again suggesting a seed sequestration mechanism. Hsp42 overproduction also cures [URE3], and Hsp42p aids Btn2-overproduction curing. Cur1p is needed for Hsp42 overproduction curing of [URE3], but neither Btn2p nor Cur1p is needed for overproduction curing by the other. While hsp42Δ strains stably propagate [URE3-1], hsp26Δ destabilizes this prion. Thus, Btn2p and Cur1p are anti-prion system components at their normal levels, acting with Hsp42. Btn2p is related in sequence to human Hook proteins, involved in aggresome formation and other transport activities.

Plenary Sessions

VIR-PL03.01 - Immuno-biology of re-emerging alphaviruses: from bed to bench and back

Lisa F.P. Ng[1]
[1]*Singapore Immunology Network, A*STAR, Singapore*

Vector-borne infectious diseases have been emerging or resurging due to socio-demographic changes, and to genetic mutations in the pathogens. The changing disease pattern, and adaptation of viruses from different mosquito vectors are important new features that impact public health. Indeed, the spread of the *Aedes* mosquito in temperate countries introduces a new risk of epidemics in countries where the entire population is immunologically naive. Chikungunya fever has re-emerged as an important human arboviral infection. Sporadic infections are still being reported in many parts of the world, causing severe morbidity with extensive incapacitation in naïve populations. Questions remain about the role of possible microevolution on viral virulence and severity of the associated disease. Importantly, the exact nature of the protective immune defense and the pathogenic mechanisms of debilitating arthralgia and arthritis upon Chikungunya virus (CHIKV) infection are still poorly known. With the increasing spread of the virus around the world, integrated approaches would be essential in order to gather fundamental knowledge on the immune responses mounted against CHIKV. Studies have demonstrated how understanding innate and adaptive immunity against CHIKV could be exploited to develop new immune-based preventive and treatment strategies. These findings will be relevant for the rational design of effective therapies against arthralgia-inducing CHIKV and other re-emerging arthrogenic alphaviruses.

Plenary Sessions

VIR-PL03.02 - Influenza Virus Transmission

Yoshihiro Kawaoka[1]
[1]*University of Wisconsin-Madison & University of Tokyo, Tokyo, Japan*

"No abstract available at time of publication"

Plenary Sessions

VIR-PL03.03 - Oncolytic viruses as cancer therapeutics

John Bell[1]
[1]*Ottawa Hospital Research Institute, Ottawa, Canada*

As tumours arise from normal tissues they acquire genetic mutations that provide them with a growth/survival benefit compared to their normal counterparts. We discovered that the same genes that are mutated in cancer cells that allow them to become immortal normally function to also provide cells with the ability to resist virus infection. Thus while cancer cells develop the ability to have unrestricted growth they are at the same time compromised in their ability to resist virus infection. We therefore designed viruses that can specifically infect and kill tumour cells but cannot infect normal tissues. These so-called oncolytic viruses have entered into clinical testing and encouraging new data is emerging. Data will be presented on how these viruses function and where they are in clinical development.

Tuesday, 29 July 2014 **10:35 - 11:00 Room 517 C**

Plenary Sessions

VIR-PL03.04 - Are viruses in the sea the largest reservoir of genetic diversity on Earth?

Curtis Suttle[1]

[1]*University of British Columbia, Vancouver, Canada*

Viruses are by far the most abundant "life" on Earth, with an estimated 10exp30 viruses in the sea alone. Stretched end-to-end they would transverse ~10exp7 light years, or further than the nearest 60 galaxies. Each of these viruses has its own genome, and its own evolutionary trajectory. With an estimated 10exp26 infections per second, events that are rare on an individual basis, can still be happening all of the time. Each one of these infections is an opportunity to transfer genetic material between a virus and its host, or among other viruses, making viruses the powerhouse of evolution on the planet. Yet, the vast majority of this genetic diversity, and hence its biological potential remains unexplored and its functions unknown. Moreover, because of climate change, we are at risk of loosing vast amounts of this biological diversity without ever knowing its function. Should we be worried about loosing viral diversity, the largest reservoir of biological diversity on the planet?

Tuesday, 29 July 2014 **13:05 - 13:30 Room 517 C-D**

Bridging Plenary Sessions

BR-02.01 - A new synthesis for antibody-mediated immunity

Arturo Casadevall[1]

[1]*Albert Einstein College of Medicine, New York, USA*

It is generally acknowledged that most vaccines mediate protection by eliciting protective antibody responses that neutralize the infecting inoculum and protect the host from infection progressing to disease. Consequently, the harnessing of humoral immunity in vaccine development has been a highly successful strategy. However, the continued success of such vaccine approaches requires a healthy understanding of mechanisms of antibody-mediated immunity. The study of antibody-mediated immunity flourished through the early and middle years of the 20th century but then entered the doldrums as immunologists moved to other topics convinced that the major problems involving immunoglobulins had been solved. The late 20th century synthesis posited that antibody-mediated immunity was well understood, that it was important in host defense against extracellular pathogens and that this protection was mediated by one of five well-established mechanisms: opsonization, complement activation, antibody-directed cellular cytotoxicity (ADCC), toxin neutralization and viral neutralization. However, in the closing decades of the 20th century a series of observations revealed that the above view was simplistic with major aspects of antibody function having been ignored or misinterpreted. The notion that humoral immunity had no role against classical intracellular pathogens was undermined by the observation that it was possible to make protective monoclonal antibodies against such diverse organisms as *Mycobacterium tuberculosis, Cryptococcus neoformans* and *Histoplasma capsulatum*. New functions were discovered for antibody-mediated immunity including the ability to mediate direct antimicrobial functions through a variety of mechanisms that were complement and cell-independent. Specific antibody was found to have the capacity of modulating microbial gene expression directly, thus establishing a new connection by which the immune system could affect microbial physiology. From a structural level, the constant region was found to affect antibody specificity and affinity by mechanisms independent of avidity that likely involved connectivity between variable and constant regions. Perhaps most importantly, single mAbs were demonstrated to mediate protective, indifferent or enhancing functions depending on host conditions thus highlighting the connectivity between humoral immunity and other functions of the immune system. These observations have led to a new synthesis which views antibody molecules as structurally connected and capable of many complex roles beyond the classical functions. The new synthesis for antibody-mediated immunity provides a theoretical framework for new approaches to vaccine design.

Bridging Plenary Sessions

BR-02.02 - Next generation antibiotics

Gerry Wright[1]
[1]*McMaster University, Hamilton, Canada*

Resistance to antibiotics is a challenge of global proportion that is undermining the advances of modern medicine and public health agencies across the globe are sounding the alarm. New antimicrobial agents are an essential component of addressing the resistance crisis. The next generation antimicrobials must preserve the best aspects of 1st generation drugs while improving on their limitations. This includes the identification of narrow spectrum and even organism-specific agents, compounds that enhance the activities of 1st generation antibiotics even in the face of resistance (antibiotic adjuvants), agents that do not kill bacteria but only impair their ability to cause disease (anti-virulence agents), and strategies that enhance the host immune system to clear infection. Examples of our efforts to identify such molecules will be presented.

Bridging Plenary Sessions

BR-02.03 - The Philadelphia measles epidemic of 1991: lessons from the past

Paul Offit[1]

[1]Children's Hospital of Philadelphia, Philadelphia, USA

We will discuss the Philadelphia measles epidemic of 1991. During a few month period, more than 1,400 people developed measles and nine children died. The outbreak centered on two fundamentalist churches that refused vaccines and medical care for their children. City officials eventually obtained a court order to immunize children against the will of their parents. This was accomplished despite the fact that the Commonwealth of Pennsylvania had allowed religious exemptions to vaccination for more than 10 years.

Poster Session

BAM-PT1001 - Bacterial communication between Veillonella and Streptococcus in biofilm formation

Izumi Mashima[1], Futoshi Nakazawa[2]
[1]Health Sciences University of Hokkaido Graduate School of Dentistry, Hokkaido, Japan, [2]Health Sciences University of Hokkaido, Hokkaido, Japan

It is suggested that oral Veillonella and Streptococcus have important roles in oral biofilm formation at early stage. Recently, we have established V. tobetsuensis as a novel oral Veillonella species, and demonstrated that S. gordonii formed large amount of biofilm in the presence of V. tobetsuensis. In the present study, the biofilms formed by S. gordonii were studied with culture supernatant or bacterial cells of V. tobetsuensis in the supernatant to determine the bacterial communication. Also, autoinducer (AI) activity in the supernatant from V. tobetsuensis was examined. The wire method established in our laboratory was used for the biofilm formation. The supernatant from V. tobetsuensis was prepared at late growth phase. After biofilms were formed by S. gordonii on the wires under the anaerobic condition for 1 day, these wires were inserted into test tubes containing the supernatant from V. tobetsuensis in a concentration of 20%, 40% and 60%. Then, biofilms on the wires were incubated for 6 days under the same condition. Also, an effect of bacterial cell of V. tobetsuensis in the supernatant was examined on biofilm formation. Finally, genomic DNAs were extracted from these biofilms to quantify the biofilm formation. Also, activity of AI-2 in the supernatant was tested by a previously luminescence assay. As results, the supernatant from V. tobetsuensis showed concentration-dependent inhibition in the biofilm formed by S. gordonii. On the other hand, the bacterial cell of V. tobetsuensis in the supernatant enhanced the biofilm formation. Furthermore, V. tobetsuensis showed high AI-2 like activity. The present study demonstrated that AI-2 like substances produced by V. tobetsuensis inhibit the biofilm formed by S. gordonii, and another cell-to-cell signal molecule between V. tobetsuensis and S. gordonii had important roles in the biofilm formation.

Poster Session

BAM-PT1003 - Actinobacillus pleuropneumoniae can acquire pyridine compounds from other swine pathogens to form multispecies biofilms

Abraham Loera Muro[2], Mario Jacques[1], Francisco Javier Avelar González[2], Josée Labrie[1], Yannick D.T. Tremblay[1], Alma Lilián Guerrero Barrera[2]

[1]Université de Montréal, Quebec, Canada, [2]Universidad Autónoma de Aguascalientes, Aguascalientes, México

Actinobacillus pleuropneumoniae is the etiological agent of porcine contagious pleuropneumonia, which causes important worldwide economic losses in the swine industry. Several respiratory infections are associated with biofilm formation, and A. pleuropneumoniae has the ability to form biofilms in vitro. Multispecies biofilms are probably the dominant form of microbial growth in nature and clinical data suggest that bacterial species form multi-species biofilms in vivo. The goal of this study was to determine the capacity of A. pleuropneumoniae to form multi-species biofilms with other swine pathogens (Streptococcus suis, Bordetella bronchiseptica, Pasteurella multocida, Staphylococcus aureus and Escherichia coli). A serotype 1 field isolate of A. pleuropneumoniae, strain 719, was used in a 96-well microtiter plate biofilm assay. A. pleuropneumoniae was able to form strong two-species biofilms in the presence of S. suis, B. bronchiseptica and S. aureus under growth conditions that are not favorable for A. pleuropneumoniae (BHI media without NAD supplementation) when both species were added at the same time. When inoculated with P. multocida or E. coli, A. pleuropneumoniae biofilm formed a weak biofilm. In all cases, viable bacteria were recovered from the two-species biofilms. The live and dead populations, and the matrix composition of the multi-species biofilms were characterized using fluorescent markers (SYTO-9, propidium iodide, wheat germ agglutinin, Sypro Ruby and BOBO-3) and enzymatic treatments (dispersin B, proteinase K and DNase). The results indicated that poly-N-acetyl-glucosamine remains the primary component responsible for the biofilm structure. A. pleuropneumoniae was also able to incorporate into pre-formed biofilms of S. aureus, S. suis or E. coli, under conditions that favour biofilm formation by S. aureus, S. suis or E. coli but not A. pleuropneumoniae. In conclusion, our data suggest that A. pleuropneumoniae is able to acquire pyridines compounds from other swine pathogens and form or incorporate into biofilms with other swine pathogens.

Poster Session

BAM-PT1005 - Biofilm formation and phosphorus mobilization by mycorrhizosphere bacteria

Salma Taktek[1], Yves Piché[2], Hani Antoun[1]
[1]Département des Sols et de Génie Agroalimentaire, CRH, Université Laval, Québec, Canada, [2]Département des sciences du bois et de la forêt, U. Laval, Québec, Canada

Soluble phosphorus (P) fertilizers added to soil rapidly precipitate, forming sparingly soluble phosphates, not available to plants. The use of phosphate solubilizing bacteria (PSB) proved to be a promising alternative to the use of high rates of fertilizers required to increase P availability. PSB can also improve phosphate rock (PR) efficiency when directly applied to soil. Most published work on PSB overlooked the possible interaction between PSB and arbuscular mycorrhiza (AM), which are ubiquitous in cultivated plants. We hypothesized that PSB closely attached to AM hyphae and able to form biofilms on PR will be more efficient than PSB isolated from the rhizoplan (thin layer of soil covering the mycorrhizal roots). Indeed our results indicated that Rhizobium miluonense Rm3 and Burkholderia anthina Ba8 isolated from the hyphae of the AM fungus Glomus irregulare, produced significantly more biofilm on PR than Rahnella sp. Rs11 and Burkholderia phenazinum Bph12 isolated from isolated from the mycorrhizosphere (soil attached to the roots of a mycorrhizal plant). Moreover, as indicated by the hydrolysis of the fluorescein diacetate assay, the biofilm formed by Ba8 on a PR from Quebec or on hydroxyapatite (HA), contained substantially more viable cells that the biofilms formed by the three other bacteria tested. In fact, the highest P mobilized from PR or HA was observed with Ba8 biofilm. PSB biofilm formation and cells viability was further investigated using confocal laser scanning microscopy (CLSM). CLSM micrograph of Ba8 biofilm showed that viable cells adhere to the whole surface of PR particles as compared to Rs11 and Bph12 viable cells that were scattered. Importance of the biofilm formed by Ba8 was also confirmed by observations made by using scanning electron microscopy (SEM).

Poster Session

BAM-PT1007 - A comparative study of the effect of Malaysian and Brazilian Propolis on biofilm formation and expression of biofilm-associated genes in Enterococcus faecalis

Ebenezer Fabian[1], Sriyutha Murthy[2], Venugopalan VP[2], Stephen Ambu[1], Fabian Davamani[1]
[1]International Medical University, Kuala Lumpur, Malaysia, [2]Baba Atomic Research Center Facilities, Kalpakkam, India

Background: Enterococcus faecalis is a gram-positive bacterium that is a leading cause of nosocomial infections. It forms prolific biofilms, which are particularly resistant to antibiotic treatments. Bacteria growing inside biofilms are reported to express more virulent phenotypes than their planktonic counterparts. Propolis is a resinous substance produced by honey bees, and is composed of resins, essential oils, bioflavonoids, phenols and aromatic compounds. It is known for its bactericidal properties and is being explored for practical applications in replacing antibiotics to control bacterial growth. The aim of this study was to investigate the antibiofilm properties of Propolis from two spatially different habitats. Method: Ethanolic extracts of Brazilian red propolis and Malaysian propolis were prepared. E. faecalis was cultured in vitro and allowed to form biofilms in 96-well and 24-well plates in the presence of different concentrations of propolis (10-200 µg/ml). The concentration at which biofilm formation was inhibited without killing the bacteria was used for bacterial treatment. Total RNA was extracted from treated bacteria and converted to cDNA. The effect of propolis on the expression of bacterial virulence-associated genes was analyzed by quantitative PCR. Results: Brazilian propolis was found to be more effective in controlling biofilm formation by E. faecalis compared to Malaysian propolis. The effect of the former was even comparable to that of triple antibiotic mixture treatment. Continuous presence of propolis in a 24-h culture inhibited biofilm formation at lower concentrations, whereas at higher concentrations, it was bactericidal. Quantitative PCR analysis showed that propolis is capable of modulating the expression of virulence-associated genes in biofilm-forming E. faecalis. Conclusion: Ethanolic extract of propolis seems to be a promising candidate for the development of natural-product-based anti-biofilm therapeutic agents.

Poster Session

BAM-PT1009 - Characterization of a seawater denitrifying biofilm

Geneviève Payette[1], Christine Martineau[1], Florian Mauffrey[1], Richard Villemur[1]
[1]*Institut Armand-Frappier (INRS), Laval, Canada*

Nitrate accumulation often appears in closed-circuit aquarium which causes toxicity to many life forms. Montreal Biodome has used a methanol-fed, moving bed (MB) denitrification reactor to lower the concentration of nitrate in its 3250 m3 seawater mesocosm. The reactor contained many carriers that were colonized by a denitrifying biofilm composed of about fifteen naturally occurring microorganisms. Two methylotrophs, Hyphomicrobium nitrativorans NL23 and Methylophaga nitratireducenticrescens JAM1, were shown to be the most abundant species in the biofilm and responsible for the major part of the denitrification. In the MB reactor, the biofilm was operated in continuous mode and did not provide optimal performance. In previous studies, we suggested to use the batch mode for better performance and easier management operations. The project aims to assess the impact of the batch mode on the composition of the biofilm's bacterial populations, and on the denitrification rate. The biofilm taken from the MB reactor was dispersed and cultivated in synthetic seawater containing carriers under denitrifying conditions and batch mode. The influence of temperature, C/N ratio and nitrate, methanol and salt concentration on the bacterial populations and the denitrification rate were assessed. The carriers were transferred each week in fresh medium. Our results showed important development of biofilm on carriers. The denitrification rates remained constant with an exposure of 300 mg-N/L to 900 mg-N/L (C/N ratio of 1,5), and also under low NaCl concentrations. This rate was however improved by a 30 ^{0}C incubation temperature. We observed that the bacterial populations stabilized from the fourth transfer. Contrary to the continuous mode, we observed a strong decline of strain NL23 in the biofilm in batch mode cultures. This operating mode did not however influence the level of strain JAM1. Our results suggest that other denitrifying bacteria have taken over strain NL23 to perform complete denitrification.

Poster Session

BAM-PT1011 - Acoustic monitoring of *Pseudomonas aeruginosa* PAO1 biofilm growth and viscoelastic properties using QCM-D

Adam Olsson[1], Michael Mitzel[2,3], Nathalie Tufenkji[1]
[1]Department of Chemical Engineering, McGill University, Montreal, Canada, [2]Department of Natural Resource Sciences, McGill University, Montreal, Canada, [3]McGill School of Environment, McGill University, Montreal, Canada

Bacterial adhesion and cohesion are known to be crucial for the development of biofilms, however these behaviours remain poorly understood. Better understanding of the viscoelastic properties at the contact points between bacteria and surfaces during biofilm development will aid efforts towards improving biofilm control strategies on various surfaces. A quartz crystal microbalance with dissipation monitoring (QCM-D) was used to study bacterial attachment and subsequent biofilm growth of *Pseudomonas aeruginosa* PAO1 to a model silica surface. QCM-D provided direct, non-disruptive, in situ measurements offering new insights into the acoustic properties of the biofilm as it developed. Biofilm-surface bond maturation dynamics are identified and biofilm formation and development investigated using confocal laser scanning microscopy (CLSM) and mutant PAO1 strains in combination with QCM-D. Findings are interpreted and discussed in the context of well-known models (i.e., Voigt model, coupled-resonance model) to assess the applicability of the QCM as a 'mass sensor' versus an acoustic monitor in the context of biofilms.

Poster Session

BAM-PT1013 - Snow crab antimicrobial peptides to reduce microbial induced corrosion and biofilms formation on submerged metallic structures

Kim Doiron[1], Lucie Beaulieu[2], Karine Lemarchand[1]
[1]ISMER/UQAR, Rimouski, Canada, [2]UQAR, Rimouski, Canada

In aquatic environments, bacteria exist in sessile communities but are frequently organised as biofilms on all submerged surfaces. In marine environments, this biofilm formation can cause important damages (e.g. corrosion, biofouling) and generate important cleaning costs for operators of metallic submerged structures. As it was previously demonstrated that the modification of microbiological growth on submerged structure can modify the occurrence of corrosion, modification of bacterial species in the core architecture of the biofilm could have a beneficial impact to protect metallic structures from corrosion. The aim of our study was to demonstrate the efficacy of antimicrobial peptides (AMP) from the snow crab Chionoecetes opilio as an organic film on mild steel plates in order to reduce the corrosive marine biofilms over 10 days of immersion in seawater. The use of marine natural coproducts as new natural antimicrobial, appeared to be a new avenue not only in reducing corrosive biofilms but also in marine biomass enhancement. The results of our study showed that, in comparison to the control without AMP, the use of AMP reduced 30 to 40% the viability of the biofilm, the bacterial diversity and the corrosion during the first seven days of the experiment. This loss of diversity could be due to the elimination of AMP-sensitive species and selection of AMP-resistant bacterial strain within the biofilm core leading to an additional protection against corrosion. These preliminary results allowed us to better understand the early stages of multi-species biofilms formation and their interactions with the conditioning film on submerged surfaces.

Poster Session

BAM-PT1015 - Biofilm formation of Staphylococcus epidermidis strains isolated from contaminated platelet units and implications in transfusion medicine

Shruti Mallya[2], Mariam Taha[1,2], Sandra Ramirez-Arcos[1,2]
[1]Canadian Blood Services, Ottawa, Canada, [2]University of Ottawa, Ottawa, Canada

BACKGROUND: Transfusion of bacterially-contaminated platelet concentrates (PCs) poses the major transfusion-associated infectious risk in developed countries. The predominant microorganisms isolated from contaminated PCs are skin flora coagulase negative staphylococci, which are able to form surface-attached communities of cells (biofilms) during platelet storage. AIMS: 1) To determine the prevalence of coagulase negative Staphylococcus epidermidis in contaminated PCs at Canadian Blood Services. 2) To characterize these isolates for their biofilm-forming ability. METHODS: Results of platelet screening for bacterial contamination at Canadian Blood Services from 2008 to 2013 were analyzed. Twenty-four S. epidermidis strains isolated during this period were used in this study. Biofilm formation assays were performed in trypticase soy broth supplemented with glucose (TSBg) with overnight incubation at 37^0 C. A subgroup of nine strains, including eight biofilm-negative and one biofilm-positive isolates, were tested for biofilm formation in PCs with incubation at 22 ± 2^0 C for five days. Biofilms were quantified using a crystal violet method. All assays were done in triplicate with three independent repetitions. RESULTS: S. epidermidis was the predominant platelet contaminant (30%) from 2008 to 2013 at Canadian Blood Services. Out of the 24 strains studied here, five (20.8%) displayed a biofilm-positive phenotype in TSBg while all nine strains tested in PCs (100%) were able to form biofilms in this milieu. CONCLUSIONS: Our studies demonstrated that S. epidermidis is the predominant species isolated from contaminated PCs at Canadian Blood Services. It is also shown that a high proportion of strains isolated from healthy blood donors are able to form biofilms. Notably, the platelet storage environment stimulates biofilm development by biofilm-negative bacteria increasing the potential for missed detection during platelet screening and the clinical risk for platelet recipients.

Poster Session

BAM-PT1017 - Role of alkyl hydroperoxide reductase in the biofilm formation of Campylobacter jejuni

Euna Oh[1], Byeonghwa Jeon[1]
[1]University of Alberta, Edmonton, Canada

Campylobacter jejni is a major cause of human gastroenteritis worldwide. Biofilm formation of C. jejuni contributes to the survival of this pathogenic bacterium in different environmental niches; however, molecular mechanisms for its biofilm formation have not been fully understood yet. In this study, the role of oxidative stress resistance in biofilm formation was investigated using mutants defective in catalase (KatA), superoxide dismutase (SodB), and alkyl hydroperoxide reductase (AhpC). Biofilm formation was substantially increased in an ahpC mutant compared to the wild type, and katA and sodB mutants. In contrast to the augmented biofilm formation of the ahpC mutant, a strain overexpressing ahpC exhibited reduced biofilm formation. A perR mutant and a CosR-overexpression strain, both of which upregulate ahpC, also displayed decreased biofilms. However, the introduction of the ahpC mutation to the perR mutant and the CosR-overexpression strain substantially enhanced biofilm formation. The ahpC mutant accumulated more total reactive oxygen species and lipid hydroperoxides than the wild type, and the treatment of the ahpC mutant with antioxidants reduced biofilm formation to the wild-type level. Confocal microscopy analysis showed more microcolonies were developed in the ahpC mutant than the wild type. Our findings successfully demonstrate that AhpC plays an important role in the biofilm formation of C. jejuni.

Poster Session

BAM-PT1019 - Human tissue proteins determine polymicrobial biofilm composition, with additional population changes induced by antimicrobial treatment

Patricia Alves[1], Kevin Purdy[2], Sarah Maddocks[1]
[1]Cardiff School of Health Science, Cardiff Metropolitan University, Cardiff, UK, [2]School of Biological Sciences, University of Warwick, Warwick, UK

Polymicrobial biofilms are commonly associated with acute and chronic wound infection; microorganisms adhere to exposed tissue proteins such as fibronectin, fibrinogen and collagen, and complex communities that are notoriously difficult to treat, develop. Biofilm organisms, especially those in mixed-culture are afforded intrinsic protection against antimicrobial treatments which do not effectively permeate the extra-cellular polysaccharide layer. Where this occurs, sub-lethal doses can drive changes in the microbial composition, leading to the selection of resistant isolates. Using a three-species static biofilm model consisting of Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes we demonstrate that the species composition of the biofilm is distinct depending on which human ligand is present on the substratum. We also demonstrate that presence of each human ligand in solution impacts upon the aggregation of these bacterial species in co-culture, within discrete species composition profiles observed for each protein. The treatment of polymicrobial biofilms with antimicrobial manuka honey altered the biofilm composition, changed the typical species profiles observed and impeded co-aggregation. Where viable organisms remained following honey treatment, S. pyogenes predominated at the expense of P. aeruginosa; numbers of S. aureus remained relatively stable. A small percentage of the microorganisms that peristed following treatment also exhibited increased resistance to honey treatment. Therefore it is likely that the human tissue proteins within a wound could determine the composition of colonising microbiota and have an impact on the development of infection. Moreover treatment with broad spectrum antimicrobials could exacerbate this situation if bactericidal concentrations are not achieved throughout the microbial community, favouring the emergence of a particular pathogen and as well as resistant variants.

Poster Session

BAM-PT1021 - Investigating the bioperformance of cranberry-modified silicone materials for use in implantable medical devices

Louis Briggs[1], Naser Muja[1], Showan Nazhat[1], Nathalie Tufenkji[1]
[1]McGill University, Montreal, Canada

Catheter associated urinary tract infections are the most common type of hospital acquired infection in North America with over a million cases reported each year. This high incidence rate, together with the dissemination of antibiotic resistant uropathogens, has resulted in much interest in the development of preventative measures within the research community. The consumption of Vaccinium macrocarpon, the North American cranberry, has been linked with the prevention of bacterial infections in the urinary tract for over 100 years. The McGill Biocolloids and Surfaces Laboratory has shown that cranberry derived materials can inhibit bacterial adherence to surfaces, impair bacterial motility, and influence various bacterial virulence functions. It is therefore hypothesized that cranberries and cranberry derived materials could prevent the development of catheter associated urinary tract infections via the disruption of various stages of pathogenesis. Herein, we report on the bioactivity of cranberry impregnated biomedical grade silicone against Proteus mirabilis, a clinically relevant uropathogen. First, the release of bioactive cranberry materials into aqueous environments has been demonstrated over the course of 48 hours in human urine, lysogeny broth and distilled water. The release of dissolved cranberry material is measured spectrophotometrically. Second, swarming motility of P. mirabilis is blocked on the cranberry-modified silicone surfaces during clinically relevant timescales exceeding 24 hours. Finally, the ability of P. mirabilis to form biofilms on the cranberry-modified silicone surface is studied over the course of 96 hours via confocal microscopy and biomass measurements. Biofilm thickness and architecture is examined on control biomedical grade silicone and a cranberry-modified hybrid material. The potential medical applications and implications of this research will be discussed.

Poster Session

BAM-PT1023 - Biofilm formation, compositon and antibiotic resistance of *Escherichia coli* and *Salmonella* spp. environmental isolates

Raluca Tutulan[1], Robin Slawson[1], Joel T. Weadge[1]
[1]*Wilfrid Laurier University, Waterloo, Canada*

Bacterial biofilms are often implicated in more than 65% of hospital acquired infections. The biofilm matrix can provide the bacteria with protection from harsh environmental factors, antimicrobial agents and host defenses, allowing them to survive in the environment and thrive within a host. *Escherichia coli* and *Salmonella* spp. can be found in our soil, water and food and can cause illness if ingested. Many strains of E. coli and Salmonella spp. are known to produce biofilms composed of curli fimbriae and cellulose. These components aid in bacterial processes such as adhesion to surfaces and water retention, as well as providing high tensile strength to the biofilm. The aim of this study is to test the biofilm forming capabilities of environmental isolates of *E. coli* and *Salmonella* spp. and correlate this with antibiotic susceptibility. Isolates were tested for their ability to form biofilms in liquid medium at a variety of temperatures (15°C, 23°C and 37°C) using microtiter assays. The expression of curli fimbriae and cellulose at these temperatures was also tested using plate assays containing Congo Red or Calcofluor as detection agents. The majority of isolates (approx. 97%) could form biofilms under at least one of the experimental conditions and were subsequently categorized as weak, intermediate and hyper biofilm formers based on the amount and physical properties of the biofilm matrix. Correlation between antibiotic resistance and biofilm formation proved to be inconclusive by the disk-diffusion method, so a modified liquid-based microtiter assay has been developed and has yielded promising results. This research will bring us closer to understanding the non-specific nature of biofilm resistance and how this can contribute to the pathogenic potential of environmental bacteria.

Poster Session

BAM-PT1025 - Heterotrophic bacteria growing on biofilms of Malinalco; an archaeological site in Mexico

Nora Beatriz Medina-Jaritz[1], Roberto Antonio Rios-Gonzalez[1], Cesar Hugo Hernandez-Rodriguez[1]
[1]I. P. N. Mexico City, Mexico, [2]I. P. N. Mexico City, Mexico, [3]I. P. N. Mexico City, Mexico

Archaeological areas exposed to weather conditions are severely threatened by biodeterioration caused by the active growth of different microorganisms on the surfaces through several physicochemical and biological processes. These processes include humidity and temperature changes, dissolution or crystallization of salts (carbonates, sulfates and silicates), etc. All of the above result in aesthetic and structural alterations of the areas. The composition of the biofilms on the archaeological site of Malinalco (Mexico) is not known. The aim of this study is the isolation, characterization and identification of heterotrophic bacteria from biofilms in Malinalco, an archaeological zone in Mexico, in order to analyze part of the microbial community diversity of fresh biofilms, growing on the biodeteriorated wall surfaces of two buildings: the so called Monumento III and the monument called Casa de las Aguilas. Samples from the biofilms were obtained and cultivated on LB plates. Bacterial strains isolated from biofilms growing on buildings were tested for siderophore production using LB-Cas Azurol Chrome media; for organic acids production with agar Foster plus bromocresol green (presumptive test) and agar Foster plus calcium carbonate (confirmatory test); finally for potassium salts solubilization with Pikovskaya modified media. The halos obtained were measured with 24 and 48 hour-intervals. Routine microbiological techniques (colonial morphology, Gram, etc.), biochemical tests (stain, fermentation, nitrate reduction, indole and hydrogen sulfide production, motility, etc.) and analysis of 16S gene sequences were performed to determine the strains. To conclude, among the heterotrophic bacteria present on the surface of the archaeological zone are Bacillus, Arthrobacter, Pantoea and Proteus; these strains produce different metabolites such as organic acids and siderophores, which contribute to deterioration of archaeological monuments.

Poster Session

BAM-PT1027 - Natural compounds targeting biofilm formation and other virulence factors related to Staphylococcus aureus pathogenicity

Said Desouky[1], Mohamed Abu Elghait[1], Ally F. Mohamed[2], Mamdouh S. El-Gama[1]
[1]Department of Botany and Microbiology, Faculty of Science, Al-azhar University, Cairo, Egypt, [2]Applied Research Sector, VACSERA, Egypt

Growing problems of antibiotic-resistance and increasing of mortality/morbidity rate caused by Methicillin-Resistant Staphylococcus aureus (MRSA) have necessitated alternative therapeutic strategies such as searching for new promising anti-virulence compounds that able to repress or attenuate pathogenicity rather than affecting bacterial growth. Discovering effective natural compounds open the gate to find a novel compound or new strategy that may serve as a cornerstone in drug development. Staphylococcal virulence factors, e.g., blood hemolysis, biofilm formation, coagulase production and staphyloxanthin pigment can cause infection separately or in synchronization. Controlling these virulence factors act as a disarming of this ruthless microorganism and freezing their harmful activity. With the aim to discover an effective anti-virulence compound(s) targeting one or more of S. aureus virulence factors, a library of plant extracts and essential oils were screened by investigation of previous staphylococcal virulence factors in addition to antibiotic susceptibility test. One of promising compounds coming out from Plant extract of Rhamnus sp that successfully blocked blood hemolysis in ppm concentration and attenuated biofilm formation as well as increase antibiotic susceptibility for assigned antibiotics including methicillin. Identification of active compounds, chemical and structural analyses are ongoing in addition of purification of other effective compounds. The purified and identified compounds could be a lead compound for MRSA treatment.

Poster Session

BAM-PT1029 - Hypochlorite stimulates c-di-GMP synthesis and biofilm formation in Pseudomonas aeruginosa

Nikola Strempel[1], Anke Neidig[1], Michael Nusser[1], Robert Geffers[2], Gerald Brenner-Weiss[1], Joerg Overhage[1]
[1]Karlsruhe Institute of Technology, Karlsruhe, Germany, [2]Helmholtz Center for Infection Research, Braunschweig, Germany

The opportunistic pathogen Pseudomonas aeruginosa is a versatile Gram-negative bacterium which is able to adapt to a variety of often harmful environmental conditions due to different survival strategies including the formation of resistant biofilms. In this study, we investigated the stress response of P. aeruginosa towards hypochlorite (HOCl), a strong oxidant frequently used in human neutrophils to kill invading bacteria. In static biofilm assays, we observed a significant increase of up to 3-fold in initial cell attachment after 2 hours of incubation with sub-lethal concentrations of hypochlorite. Microarray analyses revealed a substantial upregulation of genes involved in oxidative stress response and biofilm formation in the presence of hypochlorite. Among others, we identified a 26-fold upregulation of ORF PA3177 coding for a putative diguanylate-cyclase. These enzymes catalyze the synthesis of the second messenger c-di-GMP which considerably influences motility, biofilm formation and persistence in P. aeruginosa. Subsequent LC-MS/MS analyses of bacterial lysates showed indeed an increase in c-di-GMP levels suggesting a key role of this second messenger in hypochlorite induced biofilm development. Moreover, PA3177 overexpression in P. aeruginosa resulted in increased attachment and biofilms, impaired motility and elevated intracellular c-di-GMP levels. Quantitative real-time PCR analyses demonstrated an increased expression of ORF PA3177 in THP1 human macrophage-like cells. The role of PA3177 in oxidative stress response and biofilm formation in P. aeruginosa was further investigated in more detail by analyzing mutants, environmental and clinical isolates.

Poster Session

BAM-PT1031 - Structural and functional characterization of the role of BcsG in bacterial cellulose biosynthesis

Erum Razvi[1], Laura Kell[1], Joel T. Weadge[1]
[1]*Wilfrid Laurier University, Waterloo, Canada*

Bacteria commonly live in multicellular communities, such as biofilms. The extracellular matrix of a biofilm, which surrounds and supports bacteria, is predominantly composed of exopolysaccharides produced by the encased bacteria. For example, *Escherichia* and *Salmonella* spp. produce the exopolysccharide, cellulose. This exopolysaccharide leads to the formation of a biofilm that helps these bacteria elude detection by the immune system and increases their tolerance to antimicrobial agents and harsh environmental factors. Genetic studies have demonstrated that at least four genes, bcsABZC, are essential for the biosynthesis of cellulose in bacteria. However, enteric pathogens, such as *E. coli* and *Salmonella* spp., possess an additional three genes, bcsEFG proven critical to the proper synthesis of the polymer. BcsG is predicted to play a role in the release of growing cellulose chains from bacterial cells, but biochemical verification of this hypothesis is lacking. The goal of the present research is to overcome this deficit of understanding through the cloning, overexpression, purification and structure-function characterization of BcsG. Two protein constructs of BcsG, the entire polypeptide sequence and a truncated derivative lacking the N-terminal transmembrane region, have been generated. Purification of both constructs has been successful using immobilized-metal ion affinity chromatography, but only the soluble truncated derivative of BcsG can be isolated to high yields. Extensive protein crystallization trials with this truncated construct have led to the identification of promising conditions that are currently being optimized. Biochemical exploration of the predicted enzymatic activity is also being conducted through cellulase assays to confirm and characterize BcsG. Studies probing the potential interaction between BcsG and another periplasmic protein, BcsC, are also underway. Combined, these structural and functional analyses will improve our understanding of the role BcsG has in the cellulose biosynthetic complex and increase our overall knowledge of how biofilms develop in *E. coli* and *Salmonella* spp.

Poster Session

BAM-PT1033 - Field trials of biocontrol fungus Coniothyrium minitans WP against Sclerotinia sclerotiorum

Liu Yuanming[1], Sun Guangzhong[1], Peng Chaomei[1], Xu Lingfeng[1]
[1]*Plant Protection Station of Hubei Province, Wuhan, China*

Oil rape sclertiniose, an important disease caused by Sclerotinia sclerotiorum (Lib.) de Bary, is widely distributing in Hubei Province. Although using chemicals to control the disease has some effects , but because of the pathogenic fungus gradually resistants to commonly-used fungicides and the chemical residues in field have some impacts on environment , so biological control is more and more important in the comprehensive treatment of the disease.Coniothyrium minitans Campbell is a parasitic fungus to Sclerotinia sclerotiorum (Lib.) de Bary ,which can effectively inhibit the formation and germination of sclerotium.In recent years, biocontrol fungus preparation,Coniothyrium minitans wettable powder, has been developing in Hubei province . In 2013,in order to clarify its actual control effect and dose in the field, we carried out field efficacy trials in Huanggang City of Hubei Province.The first application of 4 billion spores / g Coniothyrium minitans WP for the disease was at the beginning of oilseed rape flowering (March 13) , the second was at rape flowering period (March 20).30 days later after 2nd application ,control effect is investigated. The result shows that : with 4 billion spores / g Coniothyrium minitans WP (750 g /hm2) , control effect is 74.36% , which was significantly higher than 40% Dimetachlone WP (1875 g / hm2),50% carbendazim WP (1125 g / hm2) and 500 g / l thiophanate-methyl SC (1500ml / hm2).In the production, optimal application dose is 750 g per hm2, apply it at early flowering and flowering season , on cloudy days or after 15:00, by dissolving the vigor spores in water , and spraying on the lower part of the rapeseed stem, leaves (especially on the main stem) to wet it all,the disease could be effectively controlled and the environment could be friendly protected.

Poster Session

BAM-PT1035 - L(+)-Lactic acid production from Cassava starch by immobilized Rhizopus oryzae TISTR 3523 on polyurethane sponge in 3L airlift fermentor

Srisakul Trakarnpaiboon[1], Kuakoon Piyachomkwan[2], Nantana Srisuk[1], Vichien Kitpreechavanich[1]
[1]Department of Microbiology Faculty of Science, Kasetsart University, Bangkok, Thailand, [2]National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand

L(+)-Lactic acid is an important organic acid that has many applications such as, acidulant, food preservative, curing agent, flavoring agent, and a monomer for biodegradable plastic (PLA) production leading to a rapid growth for L(+)-lactic acid global demand. L(+)-Lactic acid production by fermentation of low cost agricultural products leads to reduction in production cost and the value added of agricultural products. In present work, repeated batch culture of Rhizopus oryzae for L(+)-lactic acid fermentation using cassava starch as a substrate was attempted. Firstly, the effects of inoculum types on the form of fungus which will be able to recycle were investigated in 3L airlift fermentor with the controlled pH of 6 with NaOH and temperature of 35 oC. L(+)-lactic acid production by uniform pellet, previously prepared in shaking flask with 2 x107 spore/ml and shaking speed at 150 rpm for 24 h gave the highest L(+)-lactic acid concentration and productivity as 91 g/l and 0.67 g/l/h, respectively at aeration rate of 0.75 vvm at 72 h cultivation cultivation. However, clamp biomasses of R. oryzae were formed at the bottom of the fermenter which made difficulty to reuse the biomass. Whereas, high L(+)-lactic acid concentration of 83.7 g/L was obtained by immobilized R. oryzae on 0.25 x 0.25 x 0.25 cm3 polyurethane sponge (PUS) as an inoculum and the obtained uniform of PUS immobilized cells made the repeated batch culture feasible. In repeated batch culture experiment, the maximum lactic acid concentrations, 62.2 g/L with Yp/s of 0.49 g /g and 53 g/L with Yp/s of 0.54 g was repeatable for 4 and 6 cycles when α- amylase liquefied and saccharified cassava starch were used as substrate, respectively.

Poster Session

BAM-PT1037 - Formation of extracellular 5-aminolevulinic acid by mutants of strain MB-1

Koesnandar Sastrawijono[1], Diana Nurani[1], Ruzka Radwamina[1], Yoshikatsu Murooka[2]
[1]Agency for the Assessment and Application of Technology, Jakarta, Indonesia, [2]Hiroshima Institute of Technology, Hiroshima, Japan

It was found that 5-aminolevulinic acid (ALA), precursor of tetrapyrrole synthesis in living organism has the ability to improve photosynthesis activity in plant and increase the amount of chlorophyll in plant, therefore increasing plant growth and productivity. It has been reported recently that ALA have photodynamic effect to some deseases. The aim of this study is to develop mutant strain for the production of extracellular 5-aminolevulinic acid. Isolation and selection of bacteria was based on their ability to synthesize 5-aminolevulinic acid. Selected strains was mutated by mean of NTG. The mutants were tested for their formation of extracelluler 5-Aminolevulinic acid. Selected strain MB-1 produced 13,3 μmol ALA. Hundreds of mutants from MB-1 has been isolated and tested for their ALA formation. ALA formation was enhanced by mutant strain. Mutant No.2-45 and parent strain produced 268 μmol ALA and 82 μmol ALA respectively. Supplementation of levulinic acid, an ALA-dehydratase inhibitor did not significantly influnce the ALA formation by mutants strain. Keywords : ALA formation, NTG mutation, ALA-dehydratase.

Poster Session

BAM-PT1039 - Production of endoglucanase by Aspergillus niger IMI 502691 using solid substrate fermentation of Cassava root fibers

Adetunji Adegoke[1], Frederick Odibo[2], Samuel Obi[3]
[1]*Kogi State University, Anyigba, Nigeria,* [2]*Nnamdi Azikiwe University, Awka, Nigeria,* [3]*Kogi State University, Anyigba, Nigeria*

Cell-free culture supernatant of Aspergillus niger from Solid Substrate Fermentation of cassava root fibers showed endoglucanolytic activity. Optimization of cultural conditions for endoglucanase production, using cheap and available raw materials, was carried out at laboratory scale. The extracted enzyme was purified by a combination of 5M sucrose fractionation, ion-exchange chromatography on Carboxy-methyl Sepharose fast flow (FF) and gel filtration on Biogel P4. The kinetic properties were characterized. Optimization studies shows that endoglucanase was optimally produced with 0.3% (w/v) yeast extract, 1.0% (NH4)2SO4, 0.5% (v/v) Tween 60 and 5mM Mn^{2+}. Time course of endoglucanase production showed that it was maximally produced at day 5 with a corresponding culture pH of 4.973. The ion-exchange purification step resolved the crude endoglucanase into two forms, Endoglucanase I and Endoglucanase II. Endoglucanase I was purified 2.94 fold to give 0.93% yield and a specific activity of 112.34U/mg proteins. Endoglucanase II was purified 1.83 fold with a yield of 1.14% and a specific activity of 57.35U/mg proteins. Endoglucanase I was optimally active at 40°C and retained above 65% of its original activity at 70°C for 1h. Endoglucanase II was maximally active at 50°C and retained above 38% of its initial activity at 60°C for 1h. Endoglucanase I shows optimal activity at pH 3.0 and was maximally stable at pH 6.0 for 1h. Endoglucanase II was maximally active and stable for 1h at pH 7.0 and pH 6.0, respectively. Endoglucanase I and Endoglucanase II hydrolysis of carboxy-methyl cellulose showed Km value of 5.69mg/ml and 4.91mg/ml, respectively with Vmax values of 0.55mM/mg/min and 0.40mM/mg/min, respectively. Endoglucanase I and Endoglucanase II were significantly activated by Sr^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+}. Both were inhibited by Ni^{2+} and Cu^{2+} .

Poster Session

BAM-PT1041 - Molecular characterizations of the c-di-GMP pathway from the extremophile bacterium Acidithiobacillus thioooxidans

Mauricio Díaz[1], Silvia Copaja[2], Nicolas Guiliani[1]
[1]Universidad de Chile, Facultad de Ciencias, Dpto. de Biología, Santiago, Chile, [2]Universidad de Chile, Facultad de Ciencias, Dpto. de Química, Santiago, Chile

Gram-negative bacteria from the genus Acidithiobacillus play a relevant role in bioleaching process. As biofilms have an impact on bioleaching performance, the understanding of biofilm formation acquires relevance to design biological strategies to improve the efficiency of this industrial process and to prevent environmental damages caused by acid mine/rock drainages. In Acidithiobacillus sp. the molecular mechanisms involved in biofilm formation are still mainly unknown. In many bacteria, the intracellular levels of c-di-GMP molecules regulate the transition from motile planktonic state to sessile community-based behaviors. These levels depend on the balance between the enzymatic activities of diguanylate cyclases (DGCs) (synthesis) and phosphodiesterases (PDEs) (degradation). A functional a c-di-GMP pathway has been characterized in the iron/sulfur-oxidizing bacterium At. ferrooxidans. However, sulfur-oxidizing species that are capable to form biofilms have to be also characterized. Thus, we started studies of c-di-GMP pathway in At. thioooxidans and At. caldus. Here, we report our work related to At. thiooxidans ATCC 19377. Several putative-ORFs encoding DGCs, PDEs and effector proteins have been identified in the genome sequence of At. thiooxidans ATCC 19377. The c-di-GMP production was analysed in thiosulfate-, tetrathionate- and elemental sulphur-grown cells. Furthermore, in elemental sulfur we analyzed the c-di-GMP production in adhered and planktonic cells. The nucleotide-enriched fraction analysis extracted from At. thiooxidans cells showed that this bacteria is capable of produce c-di-GMP, where adhered cells have a mayor production than planktonic cells. The transcriptional analysis of adhered and planktonic cells grown in elemental sulfur showed differences in transcript levels of putative genes between physiological states. These results indicate that At. thiooxidans has a functional c-di-GMP pathway.

Poster Session

BAM-PT1043 - Antimicrobial efficacy of tea tree oil and its components on the growth of bacteria, yeast and molds

Elisabeth Mantil[1], Grace Daly[1], Tyler Avis[1]
[1]*Carleton University, Ottawa, Canada*

Melaleuca alternifolia is a native Australian evergreen and is the main producer of tea tree oil (TTO) used in industrial applications. TTO produced in the leaves of M. alternifolia is classified as one of the six chemotypes. Because of this variability in oil type, an international standard is in place to ensure consistency amongst commercial TTO products. The aim of this study was to evaluate and compare the efficacy of commercial TTO and their components against different microorganisms. Various concentrations of four commercially available TTO and their chemical components were tested for inhibition of growth of bacteria (Escherichia coli, Bacillus subtilis), yeast (Saccharomyces cerevisiae), and molds (Pythium sulcatum, Rhizopus stolonifer, Botrytis cinerea). Virtually no growth was observed for the bacteria, yeast and R. stolonifer at 0.8% TTO for two of the four commercial TTO. Pythium sulcatum and B. cinerea also exhibited significant decreases in growth at 0.8% TTO with all TTO formulations. Inhibition of microbial growth with 0.8% TTO was not significantly different than the inhibition provided by 0.3% methyl- or propylparaben. Minimal inhibitory concentrations (MIC) for the most inhibitory TTO was evaluated for each of the previously tested microorganisms. Complete inhibition of E. coli, B. subtilis, S. cerevisiae, P. sulcatum, R. stolonifer and B. cinerea was achieved with 0.4%, 0.5%, 0.2%, 0.8%, 0.4% and 0.8% TTO, respectively. A comparison of the antimicrobial activities of major TTO components showed that terpinen-4-ol and γ-terpinene were generally more effective in inhibiting microbial growth than α-terpinene and 1,8-cineole. All inhibitory concentrations of the individual components were higher than that of the inhibitory concentrations found with tea tree oil. Results from this study support that antimicrobial efficacy of TTO can be attributed to varying levels of its major components and suggests a possible synergistic relationship between the components within tea tree oil.

Poster Session

BAM-PT1045 - Molecular characterization of Nostoc and Anabaena microalgae isolates for agricultural purposes

Nóra Makra[1], Gyöngyvér Gell[1], Angéla Juhász[1], Vilmos Soós[1], Zoltán Molnár[2], Vince Ördög[2], Ervin Balázs[1]
[1]Department of Applied Genomics, Agricultural Institute, Centre for Agricultural Research, Martonvásár, H-2462 Brunszvik u 2 Hungary, [2]Plant Biology Institute, Faculty of Agriculture University of West Hungary, Mosonmagyaróvár, H-9200, Kolbai u 8. Hungary

The Mosonmagyaróvár Algal Culture Collection (MACC) is one of the largest in Europe. So far, the studies focused on their hormone production and antimicrobial effects, however due to it's size this unique collection can be utilized for different microbiological, agricultural and industrial purposes. The characterization of the MACC strains was primarily carried out using morphological markers, however, the identification was limited mostly to genera-level due to the small size and slight variety of the cells and the degradation of phenotipical characteristics during the breeding. Therefore, it is important to develop a molecular taxonomy method, which enables a genotype level identificaion and the exploration of the strains' phylogenetic relationships, hereby facilitate the selection of strains for special economic utilisation. 40 deposits of Nostoc and 40 deposits of Anabaena microalgae isolate was analysed in our laboratory using probes specific for the 16S rRNA gene sequences. Complete gene sequences were used to build a phylogenetic tree . The results have highlighted some discrepancies for several strains, based on which the accuracy of previous morphological genera determination became uncertain. Furthermore, some strains appiered to be identical based on their 16S rRNA sequences. The confirm these results some other conserved gene sequences will be involved in the study. This work is supported by TÁMOP-4.2.2.A-11/1/KONV-2012-0003 "MICROALGAL BIOTECHNOLOGY IN SUSTAINABLE AGRICULTURE" grant.

Poster Session

BAM-PT1047 - Activation of secondary metabolite production in Streptomyces spp. by exposure to subinhibitory concentrations of lincomycin

Yu Imai[1], Seizo Sato[2], Yukinori Tanaka[3], Kozo Ochi[3], Takeshi Hosaka[4]
[1]Interdisciplinary Graduate School of Science and Technology, Shinshu University, Nagano, Japan, [2]Nippon Suisan Kaisha, Ltd., Tokyo, Japan, [3]Faculty of Life Sciences, Hiroshima Institute of Technology, Hiroshima, Japan, [4]Faculty of Agriculture, Shinshu University, Nagano, Japan

Antibiotics have been known to have bactericidal or bacteriostatic activity. In contrast, it has been also reported that at subinhibitory concentrations [below the minimum inhibitory concentration (MIC)], the use of antibiotics, such as protein synthesis inhibitors, causes global changes in bacterial gene expression[1−3)]. In this study, we found that lincomycin, the large ribosomal subunit-targeting antibiotic, at subinhibitory concentrations is effective in activating the expression of genes involved in secondary metabolism in *Streptomyces* strains. In *S. coelicolor* A3(2) strain 1147, lincomycin at 0.1 × the MIC caused a marked increase in the expression of pathway-specific regulatory gene *act*II-ORF4 in the blue-pigmented antibiotic actinorhodin biosynthetic gene cluster, leading to the actinorhodin overproduction. Interestingly, *S. lividans* 1326 grown on MR5 agar medium containing lincomycin at subinhibitory concentrations (0.33 × the MIC) produced abundant antibacterial compounds that were not detected in cells grown on lincomycin-free medium. Bioassay and mass spectrometry analysis revealed that some of these compounds are new congeners of known calcium-dependent antibiotic. These results suggest that lincomycin at subinhibitory concentrations activates the ability to produce secondary metabolites in *Streptomyces* strains, and the activation approach utilizing the dose response effects of lincomycin can be applicable to eliciting yield of cryptic secondary metabolites. Our next research goal is to clarify the mechanism by which lincomycin at subinhibitory concentrations activates the potential to produce secondary metabolites in *Streptomyces* strains.

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Poster Session

BAM-PT1049 - Growth profile of different colony phenotypes of Saccharomyces cerevisiae varying sugar concentrations

Isis Gabriela Barbosa Carvalho[1], Vanda Renata Reis[1,2], Amanda Ribeiro Almeida[1], Anna Lívia Paraluppi[1], Ana Paula Guarnieri Bassi[2], Sandra Regina Ceccato-Antonini[1]

[1]DTAiSER, Centro de Ciências Agrárias, Universidade Federal de São Carlos, São Paulo, Brazil, [2]Post-Graduation in Food Science and Tecnology, ESALQ/USP, São Paulo, Brazil

Different colony phenotypes of Saccharomyces cerevisiae can be found in fermentation process for fuel alcohol production. The starter yeast is usually a smooth-colony type with dispersed cells, however, a rough-colony type with cells in pseudohyphae is frequently isolated from the fermentation tanks. Our previous study has demonstrated that the latter is a slow fermenter yeast with high rate of cell sedimentation, which can bring about operational problems to the industry and decrease in the ethanol yield. In this work, we verified the ability of these two colony phenotypes to grow in YNB medium with glucose concentrations ranging from 5 to 25 g/L, in shaken flasks, at 30oC, for 48 hours. Growth was monitored by plating the samples on YPD medium and counting the number of colony forming units after 48 hours of incubation at 30oC. Three strains of rough colonies and one strain of smooth colony of S. cerevisiae (the industrial strain PE-2) were evaluated. Two rough-colony yeasts displayed slow growth and it was higher in the highest glucose concentrations. The smooth-colony yeast grew faster and best in the lower sugar concentration (5 g/L). Among the rough yeasts, one strain (named 36) showed a growth profile similar to the smooth-colony yeast but in a slower rate. The ability to grow in low sugar concentrations (starvation conditions), that can occur at the end of the fermentation, can confer to the yeasts a competitive ability to survive in the environment. Variability among the rough strains was detected, which suggests that a unique profile should not be found. Despite that, the rough-colony strains of S. cerevisiae should be further investigated regarding the ability to grow in sucrose as carbon source, which is the major constituent of sugar cane juice and molasses for ethanol production. Support: FAPESP (2011/17928-0, 2013/18845-7 and 2012/18082-0).

Poster Session

BAM-PT1051 - TNF-binding protein of Variola virus as a possible TNF antagonist

Irina Orlovskaya[2], Sergey Sennikov[2], Sergei Shchelkunov[1], Irina Gileva[1]
[1]State Research Center Virology and Biotechnology "Vector", Koltsovo Novosibirsk reg., Russia, [2]Institute of Clinical Immunology, Novosibirsk, Russia

Genome of Variola virus codes a gene for a TNF-binding protein (VARV-CrmB) as a strategy to neutralize human TNF in order to escape of the host immune response. The recombinant VARV-CrmB protein was produced using molecular cloning approach. That recombinant protein is effective both in vitro and in vivo as anti-TNF agent. It protects murine fibroblast L929 cells against cytotoxic effects of human and murine TNFs and increases significantly the survival rate of mice experiencing endotoxic shock due to LPS exposure. For further exploring of anti-TNF potential of VARV-CrmB as a possible TNF antagonist its influence on hTNF-induced production of IL-1β and IL-6 of donor's mononuclear cells and on MuTNF-induced migration of cells from the skin of BALB/c mice have been studied. Results obtained strongly demonstrate the TNF blocking activity of VARV-CrmB protein and suggest that viral TNF binding protein might be considered as a new TNF antagonist.

Poster Session

BAM-PT1053 - Kinetics of culture growth and ethanol production from Pichia stipitis and Pachysolen tannophilus using different Hemicellulosic sources

Guilherme Peixoto[1], Gustavo Mockaitis[2]
[1]UNESP - São Paulo State University, Araraquara, Brazil, [2]National Research Council of Canada, Montréal, Canada

The advances in ethanol production, especially in processes development to C6 sugar obtainment and fermentation have consolidated this technology worldwide. On the other hand, the fermentation of C5 five sugars is not well-established, which is critical for the implementation of biorefinery concept to biofuels production plants. In this study the kinetics of culture growth and ethanol production from Pichia stipitis (ATCC 58376) and Pachysolen tannophilus (ATCC 32961) were assessed by fitting a modified Gompertz equation. The estimated parameters were lag-phase (λ), maximum growth/production (P) and growth/production rate (Rm). The hemicellulosic sources employed as substrates included: Saccharum spp (sugarcane bagasse), Typha domingensis, Vetiveria zizanioides, Canna x generalis and Cyperus papyrus. Prior to utilization, these biomass were pretreated with a solution containing 1% H_2SO_4 (v/v) for 0.5 h at 120ºC (1.05 atm) in order to provide pentoses hydrolyzates. The tests were performed in five batch reactors (in triplicate). The reactors were placed in an orbital shaker at 150 rpm and temperature was adjusted to 28°C. Liquid samples were periodically taken from the reactors for analysis. Regarding the maximum cellular concentrations reported with Pichia stipitis (ATCC 58376), Saccharum spp, Typha domingensis, Vetiveria zizanioides, Canna x generalis and Cyperus papyrus presented 1.61, 2.54, 2.50, 1.13 and 1.21 g L-1, respectively. The ethanol production using Pichia stipitis was also higher than that obtained with Pachysolen tannophilus (ATCC 32961). The utilization of this culture yielded 3.6 g L-1 (Saccharum), 1.2 g L-1 (Typha), 2.6 g L-1 (Vetiveria), 1.9 g L-1 (Canna) and 1.6 g L-1 (Cyperus) as the maximum ethanol concentration. Mostly, the culture of Pichia stipitis (ATCC 58376) overcame the performance of Pachysolen tannophilus (ATCC 32961) in all parameters estimated, thus indicating that Pichia stipitis (ATCC 58376) can be a versatile microbiological source for different pentoses fermentation.

Poster Session

BAM-PT1055 - Production of value added products by biotin prototrophic Corynebacterium glutamicum strains

Petra Peters-Wendisch[1], Susanne Götker[1], Sabine A. E. Heider[1], Gajendar Komati Reddy[1], Anh Q. Nguyen[1], K. Corinna Stansen[1], Volker F. Wendisch[1]
[1]*Faculty of Biology & CeBiTec, Bielefeld University, Bielefeld, Germany*

Corynebacterium glutamicum is a Gram-positive biotin auxotrophic bacterium originally isolated for its ability to secrete glutamate. This bacterium is a workhorse of amino acid production, especially of L-glutamate and L-lysine, and is known for decades of safe production in the food and feed industry. C. glutamicum has also been engineered for the production of other amino acids or organic acids. Moreover, also diamines or carotenoids can be produced by recombinant C. glutamicum strains. Besides a biotin uptake system (BioYMN) (1) and a transcriptional regulator (BioQ) (2), this bacterium possesses functional enzymes for converting pimeloyl-CoA to biotin, but is unable to synthesize pimeloyl-CoA. Heterologous expression of bioF from E. coli enabled biotin synthesis from pimelic acid added to the medium, but when bioF was expressed together with bioC and bioH from E. coli did not reconstitute the E. coli pathway for pimeloyl-CoA biosynthesis from acetyl-CoA. However, the biotin synthesis pathway from B. subtilis could be reconstituted in C. glutamicum by heterologous expression of bioWAFDBI from this bacterium and resulted in biotin prototrophy. Stable growth of the recombinant strain was observed without biotin addition for eight transfers to biotin-depleted medium while the empty vector control stopped growth after the first transfer. Expression of bioWAFDBI from B. subtilis in C. glutamicum strains overproducing the amino acids L-lysine and L-arginine, the diamine putrescine, and the carotenoid lycopene, respectively, enabled formation of these products under biotin-depleted conditions. Thus, biotin-prototrophic growth and production of value added products in biotin-free media by recombinant C. glutamicum was achieved. (1) Schneider J, Peters-Wendisch P, et al. (2012) BMC Microbiol 12: 6. (2) Brune I, Götker S, et al. (2011) J Biotechnol 159(3): 225–234. (3) Peters-Wendisch P, Götker S, et al. (2014) J Biotechnol, doi.org/10.1016/j.jbiotec.2014.01.023.

Poster Session

BAM-PT1057 - Evaluation of antibacterial activity of Nigella sativa, Lawsonia inermis, Pimpinella anisum, extracts on the selected human pathogenic bacteria

Mona Alwahibi[1], Kahkashan Perveen[1]
[1]*King Saud University, Riyadh, Saudi Arabia*

Thus the study was carried out to evaluate antibacterial potential of water and ethanolic extracts of Nigella sativa, Lawsonia inermis, Pimpinella anisum against Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The ethanolic extract was prepared with help of Soxhlet extractor. Dried powder of the plant (15 g) and 96% ethanol (200 ml) were placed in the extraction tube and the apparatus was run for 24 h to get the extract of the plant material. The liquid extract was dried in the oven for 24 h at 50°C. The dried residue was scraped and dissolved in 100 ml distilled water. The so obtained extracts were used for further study. The agar well diffusion method was employed to screen the effect of extracts against the human pathogenic bacteria. In this method, 24 h old culture of test bacteria was evenly streaked over the entire surface of the sterilized nutrient agar plates to obtained uniform inoculums. With the help of sterile cork borer four well were made in each plate and plant extract (50 µl) was inoculated in respective wells. Doxycyline was used as positive control.In general extracts of L. inermis were positively effective against the tested strains. However, the E. coli was found to be resistant against all the ethanolic extracts. Water extract of P. anisum exhibited the highest zone of inhibition against S. aureus (30 mm) and at par of it was the water extract of L. inermis against E. coli (30 mm). Whereas, ethanolic extract of L. inermis showed the 20 mm zone of inhibition against B. cereus and S. aureus. Thus it can be concluded that the plants evaluated in the present study have the antibacterial potential.

Poster Session

BAM-PT1059 - Bioremediation of wastewater by rhizobacteria and their application in artificial wetlands

Irina Salgado Bernal[1], Mario Cruz Arias[1], María Elena Carballo Valdés[1], María del Carmen Durán Domínguez de Bazúa[2]
[1]Department of Microbiology and Virology, Biology Faculty, University of Havana, Havana, Cuba, [2]Chemistry Faculty, Mexican Autonomous National University, D.F., México

The treatment of wastewater, before their dumping to the aquatic ecosystems, constitutes a topic of current importance. The objective of this investigation was to evaluate the physiologic and metabolic diversity of the rhizosphere bacteria from hydrophyte plants Typha domingensis (Pers.) Kunth of natural wetlands. Individual bacteria strains isolated from natural ecosystems were tested in front of pollutants of different nature. Microbial consortia were faced to water simulating a domestic effluent and synthetic water similar to an industrial effluent. This allowed the selection of the autochthonous strains Bacillus sp. (T-119, T-117 and T -229), Acinetobacter sp. (T -118), Pseudomonas sp. (T -1111) and Exiguobacterium sp. (T -316) for their capacities in the elimination of organic matter, ammonium and phosphate. Also, the strains Bacillus sp. (T-119, T-117, T-1115 and T -1113) were selected for the lead, chromium and mercury biosorption, from synthetic residual waters. Two consortia designed with these strains achieved 75%, 85% and 100% removal of organic matter, ammonium and phosphate and 70%, 9% and 17% removal of lead, chromium and mercury, respectively. The effect of factors associated with the process of pollutant decontamination was determined, which contributed to the design and bioaugmentation, with the consortia, of artificial wetlands to laboratory scale. With these treatment systems were reached 100%, greater than 70% and 55% removal of organic matter, ammonium and phosphate and over 95%, 80% and 50% elimination levels of lead, mercury and chromium, respectively. It was settled down an integral strategy, for the obtaining of effective bacterial consortia in the bioaugmentation of constructed wetlands, applied in the removal of inorganic and organic pollutants and it was achieved effluents with the quality required for the disposition in the water bodies. The group of results has repercussion in the current strategies for the preservation of water.

Poster Session

BAM-PT1061 - Quantitative real-time PCR of qacJ gene in Staphylococcus aureus linked to quaternary ammonium compound resistance

Marisa Coetzee[1], Arina Jansen[1], Charlotte Boucher[1], Robert Bragg[1]
[1]University of the Free State, Bloemfontein, South Africa

Bacterial infections are a major problem in the poultry industry. These infections are generally controlled through the use of antibiotics. However, the increase in antibiotic resistance has caused restrictions for the use in animals. In certain countries the use of antibiotics as growth promoters have been banned (Dibner & Richards, 2005); thus the poultry industry is slowly heading for a post antibiotic era. The use of disinfectants can possibly be the last resort in the fight against bacterial infections. Quaternary ammonium compound (QAC) based disinfectants are frequently used in the poultry industry to control the spread of bacteria (Bragg & Plumstead, 2003). However, efflux mediated resistance has been observed in bacteria, and some of these resistance genes have been isolated and identified (McDonnell & Russell, 1999; Bjorland et al., 2005). The small multidrug resistance genes smr, qacH, qacG, qacJ, code for QAC resistance and are generally found on plasmids (Bjorland et al., 2005). This study focused on determining the expression of the qacJ gene in Staphylococcus aureus using quantitative real-time PCR. The expression of the qacJ gene was determined when induced with different concentrations of didecyldimethylammonium chloride (DDAC). Quantitative real-time PCR was used to determine the expression of this gene. The expression was normalized and quantified against reference genes: proC, rho and gyrA using the Pfaffl model (Pfaffl, 2001). There was no significant difference in the expression of qac resistance gene when cells were treated with different concentrations of DDAC. This suggests that resistance to QACs is more complex as an increase in the gene expression pattern with the increase in DDAC concentration was expected. Future research includes investigating the expression of the known qac resistance genes simultaneously. This is in order to determine whether there is a relationship between the qac genes in conferring resistance.

Poster Session

BAM-PT1063 - Microbiological quality and biosafety of a daily mortality compost produced at the university veterinary hospital in Brazil.

Sonia Villamizar[1], Janaina Fonseca[2], Juan Carlos Caicedo[1], Lucia Carareto[1]

[1]Departamento de Tecnologia, Faculdade de ciencias agrarias e veterinarias- UNESP, [2]COSTSA Proreitoria UNESP.

Daily and outbreaks mortality composting have been identified as the best method for final disposal of carcasses, but the potential risk of pathogens transmission seriously limits its use. In this study we assessed the microbiological quality and biosafety of a compost produced in experimental unit composting daily mortality at the university in the state of Sao Paulo, Brazil. Mature compost sample was evaluated to determine the presence of pathogenic bacteria E. coli (STEC) and E. coli (EPEC) using molecular techniques, the presence and counting of coliforms and Salmonella sp and several soilborne phytopathogenic fungi was also estimated, the evaluation was conducted using selective and differential microbiological culture media. The presence of STEC, EPEC, Salmonella and phytopathogenic fungi were negative. Coliform levels were 1.1 log10/kg. The results show that daily mortality composting method is effective to reduce pathogenic microorganisms, but so the product can be applied on crops or plants such as vegetables that are for direct human consumption, additional tests must be performed to assess the presence of viral pathogens such as viruses avian influenza and newcastle, endospores forming bacteria like Bacillus anthracis and prions.

Poster Session

BAM-PT1065 - Abundance and diversity of halophilic Archaea in Kuwait tidal flats

Anwar Al-qattan[1], Huda Mahmoud[1]
[1]*Kuwait University, Al-zahraa, Kuwait*

Tidal flats are productive marine ecosystems that occupy a strategic logistic position as intermediate zones between land and sea therefore; they are expected to harbor unique microbial groups. Among the least studied group of microbes in the tidal flats are the archaea thus; the current study focuses the attention to the archaeal population inhabiting tidal flat north of the Arabian Gulf. During the study, archaeal diversity and abundance was investigated in Kuwait Bay and non-Bay stations during summer and autumn. Sediment samples from the tidal flats were collected using core sampler and the total number of archaea was determined using fluorescent in situ hybridization (FISH). The culturable and viable but unculturable archaea were investigated using 7 types of media and PCR-DGGE-Sequencing technique respectively. The results showed that Haloferax sp., Halogeometricum sp. and Natrinema sp. dominated the culturable archaea while the Halorhabdus sp., Halomicrobium sp. and Halorussus sp. dominated the viable but not necessary culturable archaea. In addition, the FISH results showed that the total numbers of archaea were relatively higher in summer than in autumn and in Bay-stations than in non-Bay station. In addition, the archaea number decreased as the depth of the sediment increased and sediment of muddy nature found harboring higher numbers of archaea than sandy sediments. In conclusion, the halophlic archaeal diversity in the uppermost surface of the tidal flats north of the Arabian Gulf is limited.

Poster Session

BAM-PT1067 - Microbial source tracking of fecal contamination in the urban waters of the Greater Montreal Area

Marc-André Blais[1], Karla Vazquez[1], Guy Deschamps[2], Sarah Dorner[3], Pierre Payment[1], Richard Villemur[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada, [2]Réseau de suivi du milieu aquatique, Montreal, Canada, [3]École Polytechnique de Montréal, Montreal, Canada

Many Canadian municipalities have a heavily degraded and aging sewage infrastructure. Sanitary sewers, which normally convey wastewater to treatment plants, can leak or be connected to storm sewers that flow directly to nearby urban streams and rivers. The presence of pathogenic microorganisms in these surface waters poses a public health risk for drinking water treatment plants and prevents the development of recreational activities. Urban animals and wildlife such as racoons can inhabit sewage infrastructures which can also be a factor in the fecal contamination of urban waters. This research project, done in collaboration with l'École Polytechnique de Montréal, seeks to assist local municipalities by identifying sources of fecal contamination using microbial source tracking methods (MST). Several urban streams and drainage basins in the Greater Montreal Area that were known to receive water from storm sewers were sampled during the summers of 2012 and 2013. Particulates in suspension were concentrated by filtration. DNA was extracted and amplified with PCR reactions that targets human mitochondrial DNA and Bacteroides 16S rRNA markers to identify samples with fecal contamination. These markers were quantified in the positive samples by qPCR. Analysis of chemical markers (ex. : caffeine) was also done by the research consortium. Human-specific fecal contamination markers were detected in most of the streams and drainage basins, which is indicative of probable human fecal contamination which can be attributed to leaky or cross connected sanitary sewers. The quantification of these markers demonstrated a variable level of fecal contamination depending on the sampled site which could allow for the prioritisation of repairs for the most problematic areas. A correlative study between microbiological and chemical markers will lead to the development of an Indices of Sanitary Contamination. New fecal contamination markers targeting human, dog, and raccoon mitochondrial DNA were developed and tested during this project.

Poster Session

BAM-PT1069 - Isolation and taxonomic characterization of a novel bacterium Palleronia sp. from the solar saltern in Jeungdo Island, South Korea

Suhk-Hwan Park[1], Geon-Hyoung Lee[1]
[1]*Dept. of Biology, Kunsan National University, Gunsan, Korea*

A novel Gram-negative, strictly aerobic bacterial strain was isolated from solar saltern in Jeungdo island of Jeollanam-do, South Korea. This bacterial strain, designated as KMB80, was halophlic, non-motile and non-spore forming rods. Colonies were circular, red-pink pigmented and smooth edge bacteria. The KMB80 colony was measured approximately 0.5~1mm in diameter after 10 days of growth on marine agar at 30℃. Oxidase-positive and catalase-positive. Strain KMB80 grew at the pH range of 6.0~9.5 with optimal growth at pH 7.0 and at temperatures of 20~40℃ with optimal growth at 30℃ and salinities of 0.5~20%(w/v) NaCl with optimal growth at 5% (W/V). Based on the 16S rRNA gene sequence analysis, the strain KMB80 belong to the genus Palleronia within the family Rhodobacteiraceae and is closely related to the type strain of Palleronia marisminoris with 95.9% of 16S rRNA gene sequence similarities, respectively. Molecular evidence and phenotypic characteristics suggest that the strain KMB80T (KCTC32522T = JCM19390T) represents a novel species within the genus Palleronia.

Poster Session

BAM-PT1071 - Examination of bacterial polar lipid compositions and host environment reveals relationships between host ecology and taxonomy

Denice C. Bay[1], Raymond J. Turner[1]
[1]University of Calgary, Calgary, Canada

Bacterial phospholipid membrane compositions, such as fatty acid length, saturation and polar head group variation, can differ considerably when surveying bacterial phyla, classes and even species and often serve as chemotaxonomic markers. The causes for such lipid diversity are thought to be driven by environmental physical pressures such as temperature, pH, and salinity and/or due to limitations of the host's innate physiology. The aim of this study is to determine what extent bacterial environment and taxonomy influence lipid compositions when considering its environment. To accomplish this three lipid composition parameters (phospholipid head group charge, fatty acid chain length, and acyl chain unsaturation) were surveyed using an assembled dataset of published experimentally determined polar lipid plasma membrane compositions from over 300 bacteria (both Gram- positive and Gram- negative). Lipid parameters were compared to host environmental parameters which included pH, temperature, and salinity. Lipid dataset matrices were examined using multivariate and network statistical analyses to explore phylum and class specific associations between each membrane parameter and environmental and/or phylogenetic affiliation. The results of this study confirmed that particular membrane compositions were related to the physical environment of its host and/ or its phylogenetic relationship to other members and identified lipid- trends within a number of unexamined bacterial classes. This outcome of this study provides a useful lipid dataset resource for other comparative membrane studies and provides insights into the influences of lipid and host ecology.

Poster Session

BAM-PT1073 - Extracellular electron transfer by Shewanella oneidensis MR-1 follows proton-coupling mechanism

Shafeer Kalathil[1], Akihiro Okamoto[1], Kazuhito Hashimoto[1]
[1]The University of Tokyo, Tokyo, Japan

Extracellular electron transport (EET) by microbes is critical for geochemical cycling of metals, bioremediation and bio-catalysis. A clear understanding of how microbes exchange electrons with electrode is highly important and several mechanisms for the microbial EET have been proposed including indirect and direct electron transfer to solid electron acceptors. But the whole picture of mechanism for the EET is still lacking and further studies are needed to accomplish this target. In the EET process, it has been considered that protons permeate outer membrane or are used for driving ATP synthesis. Thereby free energy of electron has been a main issue, and the role of proton in the EET kinetics is largely neglected so far. We here report that proton-coupled electron transfer (PCET) takes place in the EET process of Shewanella oneidensis MR-1 via flavin cofactor in outer-membrane bound c-type cytochromes (OM c-Cyts). PCET reactions refer to transfer of an electron and a proton in a single step without any stable intermediate which is kinetically more favorable than a multi-step reaction. Whole-cell differential pulse voltammetry (DPV) measurement showed that decrease in pH shifted redox potential of flavin peak to more positive region while increase in pH shifted the potential to more negative region. This redox potential shift of 40 mV/pH well matches with the characteristics of PCET process reported for numerous quinone enzymes and this result is consistent with the recent finding related with semiquinone formation of bound flavin in OM c-Cyts in the EET process as semiquinone formation usually follows PCET in flavoproteins. Furthermore, the addition of heavy water (D2O) reduced anodic current as D2O can slow down the electron transport by interfering with PCET process, indicating that EET by S. oneidensis MR-1 follows PCET.

Poster Session

BAM-PT1075 - Release of engineered nanomaterials in estuarine waters : toward a selection of resistant bacterial strains?

Karine Lemarchand[1], Kim Doiron[1], Lyria Berdjeb[1], Jean-Pierre Gagné[1], Émilien Pelletier[1], Mathieu Millour[1]
[1]*Institut des Sciences de la Mer de Rimouski, UQAR, Rimouski, Canada*

Over the last decade, industrial production and commercial use of engineered nanomaterials (ENMs), such as silver nanoparticles (AgNPs) and carbon nanotubes (CNTs), have significantly increased worldwide. ENMs can enter aquatic environments from their manufacturing processes to their disposal. As a consequence, the USEPA has included them among the emerging aquatic contaminants since 2009. Nevertheless, potential toxic effects of these emerging contaminants on natural microbial communities inhabiting estuarine coastal ecosystems are not well understood. The first aim of this study was to report on changes in a complex marine bacterial community (richness and abundance) in marine microcosms exposed to 20 nm polymer-coated AgNPs and ionic silver (Ag^+). Our results clearly demonstrated that at low concentrations (5 and 50 µg/L total silver), polymer-coated AgNPs and dissolved Ag^+ contamination produced similar effects: a longer lag phase suggesting an adaptation period for microorganisms. In addition, richness decreased in the treated samples and suggests the selection of a fraction of the initial community that is insensitive to silver contamination. Our second objective was to investigate the potential toxic effects of carboxylated single-walled carbon nanotubes (SWNT-COOH) on the cell growth and viability of four marine bacterial strains (S. pomeroyi, O. beijerinckii, V. splendidus, V. gigantis). We observed different sensitivity levels of these strains toward SWNT-COOH exposure. A bactericidal effect of SWNT-COOH has been observed for Vibrio species, with cell loss viability ranging from 86% to 98% when cells were exposed 2 hours at 100 µg/mL. However no significant effect of SWNT-COOH concentration or incubation time had been observed on S. pomeroyi, suggesting a stronger resistance of this strain. Overall, our results demonstrate a strain-dependent toxic effect of ENMs toward marine bacteria and that dissemination of ENMs in estuarine and marine systems may significantly impact ecosystemic services through the modification of natural microbial communities composition.

Poster Session

BAM-PT1077 - Evaluation of antimicrobial activities of essential oils in vapor phase on pink slime-forming microorganisms

Nozomi Ihara[1], Jin Sakamoto[1], Tetsuaki Tsuchido[1, 2]
[1]Kansai University, Suita, Japan, [2]TriBioX Laboratories, Takatsuki, Japan

Antimicrobial activities of essential oils (EOs) against pink slime-forming microorganisms occurring in wet areas of living houses were evaluated. Objective microorganisms were isolated from 31 samples of pink-colored contamination, which were collected from the wet areas of 31 households, and then identified. These microorganisms were found to belong to Methylobacterium as a bacterial genus, Rhodotorula as a yeast genus, and Fusarium as a fungal genus. Antimicrobial activities of EOs, rosemary, teatree and peppermint were examined against Methylobacterium mesophilicum, Rhodotorula mucilaginosa, and Fusarium oxysporum, as representative pink slime-forming organisms. Escherichia coli, Staphylococcus aureus and Candida albicans were selected as the respective control microorganisms, and the agar vapor assay in a petri dish was used as the test method. Upon treatment for one day with the 20µl EOs, the inhibition zone for all EOs tested was not detected against E. coli or S. aureus, but complete inhibition was observed against M. mesophilicum. No inhibition zone for all 20µl EOs was detected against C. albicans, R. mucilaginosa, or F. oxysporum, but the colony color of R. mucilaginosa and F. oxysporum changed to white by treatment with peppermint. Upon treatment for five days with the 20µl EOs, no inhibition zone for all EOs tested was detected against C. albicans, but complete inhibition was observed against R. mucilaginosa. In conclusion, the antibacterial effects of all EOs tested against M. mesophilicum, and the antifungal effects of peppermint against R. mucilaginosa were established. EOs may be potential tools for controlling pink slime-forming microorganisms that occur in the wet areas of living houses such as bathrooms and washing rooms.

Poster Session

BAM-PT1079 - Distribution of antimicrobial lipopeptides in Bacillus and Pseudomonas spp., two genera with antagonistic effects against plant pathogens

Rowida Mohamed[1], Emma Groulx[1], Tyler Avis[1]
[1]Carleton University, Ottawa, Canada

Composts and soils are a source of numerous microorganisms with potential beneficial traits for plant production. Our laboratory has previously isolated bacteria from plant disease suppressive soils, composts, and compost teas. Among the identified antagonistic bacteria, species of the genera Bacillus and Pseudomonas were generally the most suppressive to growth of plant pathogens. Other work showed that some of these isolates provided inhibition of plant pathogens by the production of extracellular antimicrobial compounds, including antimicrobial lipopeptides. These lipopeptides mainly cause antimicrobial effect through their pore-forming ability in plant pathogen membranes and each lipopeptide has different effectiveness and specificity toward plant pathogens. The aim of the study was to determine the number and distribution of antimicrobial lipopeptides produced by the most antagonistic bacteria from suppressive soils, composts and compost teas. In this study, 16 strains of Bacillus spp. and Pseudomonas spp. were investigated for antimicrobial lipopeptide production. Lipopeptide extracts were prepared from liquid cultures in medium for optimal lipopeptide production. Lipopeptides were isolated on purified using ammonium sulfate precipitation and reverse-phase column chromatography. Lipopeptide families were separated by LC-MS and identified using authentic standards and comparison with published data. Results showed that Bacillus spp. produced one or more of the following lipopeptide families: fengycins, iturins, and surfactins. Results also showed that Pseudomonas spp. produced a broader spectrum of antimicrobial lipopeptides including known lipopeptides such as syringomycin, viscosin, as well as putisolvin I and II. Results show that different isolates produce variable lipopeptides profiles within a same genus or species. This may explain, in part, the variable efficacy of these isolates on different target plant pathogens.

Poster Session

BAM-PT1081 - Classification of Aeromonas popoffii strains isolated from fresh water sources

Ivo Sedlacek[1], Stanislava Kralova[1], Michaela Sedlackova[1], Eva Kroupova[1], Tereza Gelbicova[1], Pavel Svec[1]
[1]Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic

Mesophilic aeromonads are widespread in the environment and some species are pathogens of poikilotherm animals and/or humans. Aeromonas popoffii occurring in aquatic habitats is a member of the Aeromonas hydrophila complex. Although A. popoffii has never been reported from clinical samples it possesses certain virulence traits present in pathogenic aeromonads. The aims of our study were to compare A. popoffii identification achieved using automated ribotyping and MALDI-TOF MS with multiplex PCR identification and to determine presence of virulence traits among the environmental A. popoffii strains. A group of 45 A. popoffii strains isolated from water sources was sampled in the Czech Republic and Slovakia during 2008-2012. The isolates were initially identified by biotyping, further characterized using automated ribotyping, cpn60 gene sequencing, and MALDI-TOF MS and finally characterized using multiplex PCR. The presence of β-haemolysin and act gene (cytotoxic enterotoxin) was investigated. Obtained results showed inconvenience of biotyping and ribotyping for the classification of A. popoffii to the species level. In contrast, MALDI-TOF MS and the multiplex PCR were shown as good tools for rapid and reliable identification of A. popoffii. Phenotype profiles of some isolates were different from the original species description. They produced acid from turanose (55.5%), L-arabinose (35.3%) and inositol (20%) as well as gas from glucose (33.3%). Many strains shared virulence factors. The act gene was present in 38 strains out of 45 analysed (84.5%). Sixteen strains (35.3%) showed β-haemolytic activity; all these strains carried also the act gene. Our results demonstrated that biotype identification of A. popoffii is unreliable and showed genotyping and MALDI-TOF MS as good tools for A. popoffii identification. The occurrence of virulence factors among the environmental isolates implies potential pathogenicity of tested strains and suggests A. popoffii as moderately pathogenic species. This work was supported by the project CZ.1.07/2.3.00/20.0183.

Poster Session

BAM-PT1083 - Newly isolated plant growth promoting rhizobacteria, Tetrathiobacter sp. NZRP3 from wild plant, Tetragonia tetragonoides inhabited Australian coastal sand dune

Eun Young Lee[1], Sun Hwa Hong[1], Min Hwan Oh[1], Ji Seul Kim[1]
[1]*Suwon University, Gyeonggi-do, Korea*

Coastal sand dunes serve as a border between the sea and land, and are considered an important zone for the protection and conservation of costal plant resources. For environmental remediation, a potential strategy is phytoremediation using the symbiotic relationship of plants and microbes in the rhizosphere, which has proven ecologically sound, safe, and cost effective. In this study, we evaluated the plant growth-promoting ability of newly isolated bacteria from wild plant inhabited in coastal sand dune of Great Ocean Road, Victoria, Australia. And new bacterium isolated from the rhizosphere soil (RS) or rhizoplane (RP) of Tetragonia tetragonoides was studied to assess the potential use of phytostabilization. Bacteria was then tested for plant growth promoting activities (PGPAs) such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, indole acetic acid production(IAA) and siderphores synthesis ability. The bacterium was identified by comparison of the 16S rDNA gene sequences. This isolate has shown capacities for IAA production (136.51 ± 11.48 mg/L of IAA accumulated during 5 days) and ACC deaminase (Absorbance value: 0.20 ± 0.00 by 49 hours incubation). The isolated bacterium was identified Tetrathiobacter sp. In addition, quantification of strains was constructed to real-time PCR techniques. These results indicate that Tetrathiobacter sp. NZRP3 can serve as a promising microbial inoculant for increased plant growth in coastal sand dune environment to improve the phytostabilization efficiency.

Poster Session

BAM-PT1085 - Screening of bacteria and fungi for antimicrobial activity against Staphylococcus aureus and Pseudomonas aeruginosa

Snizhana Olishevska[1], Éric Déziel[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada

Repeated and improper use of antibiotics against bacterial and fungal infections is the primary cause of development antibiotic-resistant strains. For instance, multidrug resistant strains of the important opportunistic pathogens Staphylococcus aureus and Pseudomonas aeruginosa are becoming worldwide clinical problems in medical practice. Therefore, there is an emergency to discover novel molecules with high antimicrobial activity and functioning through novel mechanisms of action. The main goal of this research is to isolate from various environmental samples and to screen microorganisms able to produce extracellular metabolites inhibiting the growth of S. aureus and P. aeruginosa. A total of 612 bacterial and 124 fungal strains were isolated from 123 environmental samples (various vegetable organs and soils) collected from different region of Québec (Canada) during 2011-2013. Antimicrobial activities of isolated bacterial and fungal strains against clinical and multidrug-resistant S. aureus ED711 (SARM) and P. aeruginosa ED416A (kanamycin, gentamycin, carbenicillin) were estimated using well-diffusion assay based on measuring of the inhibition zone of pathogens around the wells containing their cell-free supernatants. Antimicrobial activity against S. aureus ED711 and P. aeruginosa ED416A was produced by 83 and 22 bacterial isolates, respectively. The best bacterial candidates were identified through sequencing of the 16S rRNA gene. Among them, cell-free filtrate of Paenibacillus sp. 329 formed the biggest growth inhibition zones of S. aureus ED711 (33.0±0.48 mm) and P. aeruginosa ED416A (23.2±0.63 mm) respectively. Furthermore, 13 fungal strains demonstrated antimicrobial activity against S. aureus ED711. Among them, the largest clear inhibition zone (44.0±1.00 mm) was formed by Paenicillium sp. VFr37. Only the cell-free supernatant of Aspergillus niger 8PT formed a growth inhibition zone (13.8±0.31 mm) of P. aeruginosa ED416A. In conclusion, a large scale screening of microorganisms allowed us to isolate the most active strains capable to produce secondary metabolites with antimicrobial activity against S. aureus and P. aeruginosa.

Poster Session

BAM-PT1087 - Plasmid transfer in defined donor-recipient biofilms

Farhana Shamsad[1], Martina Hausner[1]
[1]*Ryerson University, Toronto, Canada*

Conjugation of plasmids is an important mechanism of horizontal gene transfer (HGT). Plasmid-harbouring cells which may act as potential donors have been detected in various environmental niches, including the human gut and aquatic or soil ecosystems. In such environments, microorganisms predominantly exist in the form of biofilms or other bioaggregates, where they may be in a state of active growth or exhibit reduced activity. Biofilms provide favourable conditions for HGT. Biofilms promote plasmid stability, and, on the other hand, experimental evidence suggests that plasmids enhance biofilm formation. Our objective is to investigate the effect of growth phase and biofilm age on plasmid transfer in planktonic cultures and dual species biofilms, respectively. Modified Pseudomonas putida BBC443 strain carrying a GFP-tagged TOL plasmid pWWO and Pseudomonas putida ATCC12633 were used as plasmid donor and recipient, respectively. GFP expression is repressed in the donor due to the action of lacI gene. GFP is expressed in transconjugants. To study the effect of growth phase on plasmid transfer, plate matings were carried out on LB plates using standard procedures utilizing planktonic cell cultures grown to exponential or stationary phase. Plasmid transfer frequency was evaluated microscopically and by detecting growth of transconjugants on agar plates. Plasmid transfer frequencies, evaluated microscopically, were 1.0×10^{-1} and 1.7×10^{-1} when both of the donor and recipient cells were harvested from exponential and stationary growth phase, respectively. Next, biofilms will be cultivated in continuous flow through cells. Donor cells harvested from exponential or stationary growth phase will be added to 1 day and 1-2 week old recipient biofilms. Transconjugant abundance and location will be determined in situ using confocal microscopy and digital image processing. This study could be of importance to understand the mechanism of acquisition and spread of antibiotic resistance in environments by plasmid mediated horizontal gene transfer.

Poster Session

BAM-PT1089 - Involvement of c-di-GMP in small colony variant (SCV) formation and related protein appearance when Pseudomonas aeruginosa is grown on hexadecane

Thuy Nguyen[1], Eric Déziel[1], Richard Villemur[1]
[1]*INRS - Institut Armand Frappier, Laval, Canada*

Pseudomonas aeruginosa is found in a wide variety of environments, such as soil, water or associated with plants and animals. One of its features is formation of small colony variants (SCV) when grown on hydrophobic substrates such as long-chain linear alkanes. In previous study, we reported that SCV cells have a number of phenotypic characters that are different from those of parent wild type (WT) cells. The SCV cells are hyperpiliated and able to grow without initial lag phase on n-alkanes. Although phase variation controls SCV formation in many bacteria, but the precise mechanism is still unknown in *P. aeruginosa*. However, the secondary messenger c-di-GMP promotes the transition from the planktonic to the sessile lifestyle of bacteria. To study further the involvement of c-di-GMP in SCV formation, we have generated two derivatives of the WT environmental strain 57RP constitutively expressing a phosphodiesterase (PDE) (RP401) or a diguanylate cyclase (DGC) (RP402), enzymes involved in the hydrolysis and synthesis of c-di-GMP, respectively. Results obtained from the comparative study, carried out on 57RP-SCV, 57RP-401S and 57RP-402S strains, suggest the existence of the intercellular concentration threshold of c-di-GMP for switching the phase variation. Interestingly, our data showed that modification of intracellular concentrations of c-di-GMP resulted in modulation of the phase variation switching. In the comparative analysis of proteome profiles of SCV and WT cells, we found differences in the protein expression pattern which represent a part of the changes promoting SCV growth on n-alkanes.

Poster Session

BAM-PT1091 - Study of the biodegradation potential of glyphosate present in native microorganisms isolated from different ecosystems of North-East Mexico

Luis Alberto Aguilar-Acosta[1], Alejandra Jimenez-Martinez[1], Martha Alicia Gonzalez-Gloria[1], Ulrico Javier Lopez-Chuken[1], Maria Elena Cantu-Cardenas[1], Karim Acuna-Askar[2], Juan Francisco Villarreal Chiu[1]
[1]Universidad Autónoma de Nuevo León, Facultad de Ciencias Químicas, San Nicolas, Mexico, [2]Universidad Autónoma de Nuevo León, Facultad de Medicina, Monterrey, Mexico

Phosphonates are molecules characterized by the presence of a direct bond between a carbon and phosphorus atoms. These compounds are used as antibiotics, herbicides and antivirals due to the close structural similarities to their more common analogues, phosphate esters. N-phosphonomethylglycine, or glyphosate, is the active ingredient of the herbicide Roundup. This formulation is considered the world's biggest-selling chemical used for weed control. Glyphosate is an analogue of the natural amino acid glycine, which acts as a potent inhibitor of the enzyme 5-enol-pyruvylshikimate-3-phosphate synthase. This affects directly the synthesis of aromatic amino acids. Glyphosate, as other phosphonates, is difficult to metabolize by natural microbiota due to the distinctive phosphonic bond, which is chemically and thermally more stable than the phosphate ester bond. To date, only the multi-enzyme system called C-P lyase is known to be capable to cleave this bond. However, it is subjected to the Pho regulon, provoking the improbable mineralization of glyphosate due to the presence of high concentrations of inorganic phosphate present in the environment. In this study, we screened the ability of different native microorganisms from North-East Mexico to biodegrade the herbicide glyphosate, which has been detected in wastewater discharges that reach streams connected to water supplies. Results indicate that near 35% (20 out of 58) of isolated bacteria were capable of utilizing glyphosate as sole phosphorus source, but not as nitrogen or carbon sources. Additionally, no inorganic phosphate release was observed, suggesting that glyphosate was used as scavenge source of phosphorus, and this mechanism is ruled by the Pho regulon. RT-PCR confirmed the expression of the gene phnJ, which is responsible for the cleavage of the carbon-phosphorus bond in E. coli. Despite positive results, no bacterial isolate was capable of mineralizing glyphosate, suggesting that new strategies of isolation or acclimatization should be considered.

Poster Session

BAM-PT1093 - Isolation of several marine bacteriophages including the SAR116 phage and their distribution revealed by virome binning

Ilnam Kang[1], Ahyoung Choi[1], Jang-Cheon Cho[1]
[1]*Department of Biology, Inha University, Incheon, Republic of Korea*

Recent ocean metagenomic studies have revealed the numerous novel genetic repertoires of marine viromes but most genome fragments in the viromes are hard to be categorized into known viral groups. The reason of the poor assignment of virome sequences to viral genomes has been thought to be the low number of phage isolates infecting major bacterial groups. Since cultivation of bacterial strains is the only way of isolating bacteriophages, there is an urgent requirement for the isolation of phages that infect important marine bacterial groups. In this presentation, we summarize the isolation of several bacteriophages from the euphotic zone of the Yellow Sea and the East Sea of Korea, genome characteristics, and distribution of their genome sequences by virome binning. We obtained 7 phages infecting 5 strains of bacteria: HMO-2011 from Ca. "Puniceispirillum marinum" IMCC1322, P12024L and P12024S from Persicivirga sp., P12026 from Marinomonas sp., P12053L from Celeribacter sp., and P2559S and P2559Y of Croceibacter atlanticus. Except for HMO-2011, genome sequences of all other phages showed limited similarity to other bacteriophage genomes in public databases. In this study, the genome sequence of HMO-2011 is compared to virome sequences retrieved from varying depths of diverse regions in the Pacific Ocean using metagenome binning approaches. HMO-2011 was confirmed to be one of the most highly assigned viruses in the virome binning, with a maximum of 7.6% of total reads assigned to HMO-2011. HMO-2011 type phages represented a depth-specific distribution, showing prevalence in the marine euphotic zone of coastal, transition, and open ocean regions but not in the dark ocean. Overall distribution pattern of HMO-2011 type phages was similar to that of the SAR116 clade, suggesting that the dynamics of SAR116 bacteria may be under the influence of infection by phages coexisting in their habitats.

Poster Session

BAM-PT1095 - Synergistic relationship between two bacterial isolates from a denitrifying biofilm treating seawater at the Montreal Biodome

Christine Martineau[1], Jonathan Cloutier[1], Karla Vazquez[1], Céline Villeneuve[1], Florian Mauffrey[1], Geneviève Payette[1], Richard Villemur[1]
[1]*Centre INRS-Institut Armand Frappier, Laval, Canada*

A methanol-fed bioreactor naturally colonized by a denitrifying biofilm was installed at the Montreal Biodome to reduce nitrate levels in a 3 million litre seawater aquarium. In previous studies, about fifteen different bacterial species were identified in the biofilm and the two most abundant bacteria, Methylophaga sp. JAM1 and Hyphomicrobium sp. NL23, were isolated. Strains JAM1 and NL23 were identified as major contributors to the denitrifying activity in the biofilm and were selected for further characterization, with the objective of improving denitrification in seawater through a better understanding of how these strains evolve and interact. In the first part of this project, both strains were characterized and identified as new bacterial species (Methylophaga nitratireducenticrescens strain JAM1 and Hyphomicrobium nitrativorans strain NL23). Closed and fully annotated genomes were also obtained for both strains. The growth of strain JAM1 under denitrifying conditions led to the accumulation of nitrite, a toxic compound for strain JAM1. The incapacity of strain JAM1 to reduce nitrite could be related to the presence in its genome of a truncated nitrite reductase gene. On the opposite, genes encoding the full spectrum of reductases for a complete denitrification pathway were identified in strain NL23 genome. Correspondingly, growth of strain NL23 under denitrifying conditions without significant nitrite accumulation was observed over a wide range of initial nitrate concentrations. However, strain NL23 was sensitive to >1.0% NaCl concentrations and its abundance in a bioreactor treating seawater (~2.5% NaCl) is surprising. Cultivation of strains JAM1 and NL23 in co-cultures led to higher growth and denitrification rates than what was observed in mono-cultures of each strain. We suggest that strains NL23 and JAM1 have developed a synergistic relationship in the biofilm allowing them to evolve under conditions that would be detrimental to each strain alone. Further characterisation of this synergistic relationship is ongoing.

Poster Session

BAM-PT1097 - Isolation of VBNC Vibrio cholerae from environmental water sample, Kolkata, India, 2013

Tamaki Mizuno[1], Daisuke Imamura[1], Shin-ichi Miyoshi[2], Asish Kumar Mukhopadhyay[3], Sumio Shinoda[1]
[1]Collaborative Research Center of Okayama University for Infectious Diseases in India, Kolkata, India, [2]Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan, [3]Division of Bacteriology, National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India

The viable but non-culturable (VBNC) state is defined that bacteria is alive but cannot grow on the routinely bacteriological media. More than 60 species including Vibrio cholerae O1/O139 causing cholera are known to become this dormancy form to survive under unsuitable conditions. In Kolkata, India, in spite of the presence of so many cholera patients, only a few numbers of V. cholerae O1/O139 is detected in aquatic environments. Therefore, it has been assumed that the river or pond water contaminated not only with the culturable cells, but also with VBNC cells is the infectious source of cholera. We recently reported that the VBNC cells recovered the culturable state by the addition of the lysate from HT-29 cells and the converted factor contained in the lysate was catalase. And so, we carried out to isolate the VBNC V. cholerae by using the lysate from HT-29. Several water samples collected from some ponds in Kolkata, were inoculated on the selective agar plates supplemented with or without the lysate from HT-29 cells. After cultivation, the V. cholerae-like yellow colonies were picked up, and to verify to be V. cholerae, the presence of the species-specific gene ompW encoding the outer membrane protein were tested by PCR. Thereafter, to confirm to be V. cholerae O1/O139, the agglutination test and PCR to detect ctxA gene encoding cholera toxin and rfb gene related in O-antigen biosynthesis were carried out.

Poster Session

BAM-PT1099 - Adapting established wastewater treatment bioreactor technology for use with Alberta oilsands process water

Joe Lemire[1], Marc Demeter[1], Matt Frankel[1], Howard Ceri[1], Ray Turner[1]
[1]*University of Calgary, Calgary, Canada*

Surface mining of oilsands is a water intensive process, and produces large quantities of waste tailings - dense clay, sand, and fluidic oilsands process water (OSPW). The waste tailings are collected in ponds – large engineered dam and dyke systems – that currently cover an area of 176km2 in Northern Alberta, Canada. OSPW is highly alkaline and rife with heavy metals, polyaromatic hydrocarbons and naphthenic acids (NAs). The latter is managed under a zero discharge policy due to its toxicity to aquatic and terrestrial organisms. Here we describe the capacity of OSPW-derived microbes to be harvested as biofilms, and used in a lab scale ex-situ wastewater treatment bioreactor to remediate NAs. We established the proof of principle that OSPW microbes can be grown as mixed-species biofilms, capable of degrading model NAs. Comparative analysis to single species biofilms demonstrated that mixed species biofilms are more efficacious at degrading model OSPW pollutants. Reactor volumes were scaled up by 4 orders of magnitude and used to assay various industry approved, bioreactor biofilm support materials. OSPW biofilms grown in these reactors removed over 80% of the target compounds in 28 days. Our results suggest that the most commonly utilized biofilm support materials demonstrated the poorest performance in OSPW remediation. Co-contamination of organics with heavy metals frequently hinders the biodegradation of organic pollutants. Thus, it was critical to assess whether OSPW-derived biofilms exhibited a strong tolerance to a wide assortment of heavy metals that are found to accumulate in OSPW. Using the metal tolerant bacterium Cupriavidus metallidurans as a comparative control, we demonstrated that OSPW mixed species biofilms are highly tolerant to many of the metals found within oilsands tailings. Findings from our proof of principle study suggest that our facile process can be fine-tuned for various contaminated water and soil treatment applications.

Poster Session

BAM-PT1101 - Stable-isotope probing reveals that the rate of cellulose decomposition and diversity of cellulose degraders in forest soils are reduced by timber harvesting

Roland Wilhelm[1], Hilary Leung[1], Andras Seitz[1], William W. Mohn[1]
[1]University of British Columbia, Vancouver, Canada

Cellulose decomposition by fungi and bacteria is a major contribution to carbon cycling in forest soils, yet the diversity and structure of the responsible communities is poorly understood due to the incredible complexity of soil. Stable isotope probing (SIP) was used to target cellulose degraders in forest soils with the goal of better defining these communities and the effects of forest harvesting. The experimental site in California compared an unharvested reference plot to three harvested treatments varying in intensity of organic matter removal. Soil from the organic and mineral horizon was collected 15 years after harvesting and replanting. Samples were incubated with 13C-labeled cellulose and decomposition rates were assessed based on respiration rate and incorporation of 13C into phospholipid fatty acids (PLFAs). 13C-enriched DNA, attributable to active cellulose decomposers, was isolated and bacterial and fungal phylogenetic marker amplicons were sequenced to identify cellulose-degrading populations. Results demonstrate a consistently lower respiration rate and lower 13C-enrichment of PLFAs in harvested versus reference soils. With increasing harvesting intensity, the ratio of fungal to bacterial PLFAs increased, while the diversity of PLFAs declined. Analysis of the phylogenetic marker amplicons supported this decline in diversity and revealed a surprising number of unclassified organisms putatively involved in cellulose decomposition. The research presented is preliminary evidence suggesting that alterations to soil nutrient cycling persist 15 years after timber harvesting. The implications of these changes for the regenerative potential of soil in secondary forest ecosystems should continue to be researched to better inform long-term agro-forestry practices.

Poster Session

BAM-PT1103 - The epidemiology of extended spectrum beta lactamase producing Acinetobacter baumannii in Kuwait

Leila Vali[1], Ali A. Dashti[1], Andres Opazo[1], Khaled Obaid[2], Sebastian G.B. Amyes[3]
[1]Kuwait University, Kuwait, [2]Edinburgh Univerisity, Edinburgh, UK, [3]Amiri Hosptial, Kuwait

In recent years in Kuwait the prevalence of resistance to cephalosporins in Acinetobacter baumannii has risen. Resistance to cephalosporins is mainly caused by chromosomal AmpC or by extended spectrum beta-lactamases, such as PER. We investigated the epidemiology of PER-like enzymes among the clinical A. baumannii. Ninety non-duplicate A. baumannii isolates were collected from July 2011-August 2012 from patients who had been previously treated with cephalosporins. Antibiotic susceptibility testing was performed and examined according to the CLSI guidelines. gyrB multiplex PCR was performed to identify Acinetobacter species. PCR was used to amplify bla (OXA-types) carbapenemases, insertion elements, bla(NDM), bla(PER), bla(GES), bla(VIM) and bla(IMP). Pulsed-field gel electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) were used to genotype the isolates. Six (7%) of the A.baumannii contained a PER-like enzyme and OXA-23. These strains belonged to ST type 2 but to two different PFGE types Two point mutations on the Ω-loop of the PER-like protein were also detected which may be significant in increasing resistance. These point mutations may have occurred following administrating cephalosporins to the patients. The PER-like gene has been shown to be located on multi-resistance plasmids; therefore it is important to monitor the horizontal transfer to prevent the spread of resistance.

Poster Session

BAM-PT1105 - Microbial community dynamics of the Great Lakes

Mohammad Mohiuddin[1], Radhey S. Gupta[1], Tom A. Edge[2], Herb E. Schellhorn[1]
[1]McMaster University, Hamilton, Canada, [2]Environment Canada, Burlington, Canada

Microbial water communities are complex consortia of bacteria, viruses and protozoa. Upon lysis, these microorganisms release their nucleic acid as free form. We estimated that free DNA accounts for ~60% of the total DNA present in water while bacteria and viruses comprise the rest (~23% and ~17% respectively). Here, we present a simplified approach to examine the lower Great Lakes microbiome using free DNA in combination with bacterial and viral DNA. To characterize the Great Lakes microbiome, we collected samples (both water and sand-pore) from six different locations around Lake Ontario and Lake Erie from June to September of 2012 and separated them into three distinct fractions: bacterial, viral and free DNA. DNA was extracted from all three fractions and sequenced. Genotypic richness and diversity of the microbial communities were estimated from each fraction and a comparative analysis was performed between the water and sand-pore samples' microbiome. Our analysis suggests that free DNA, though containing sequences of higher organisms, are rich in sequences of bacterial and viral origin. Therefore, exhaustive sequencing of free DNA offers the potential to generate a comprehensive profile of all microbial components of a given water sample. This free DNA can also be used to identify novel diagnostic sequences such as conserved insertions or deletions (indels) that are specific for targeted pathogens.

Poster Session

BAM-PT1107 - Biocontrol against Fusarium head Blight and mycotoxin accumulation in wheat and barley

Vladimir Vujanovic[1]
[1]*University of Saskatchewan, Saskatoon, Canada*

Fusarium Head Blight (FHB) causes severe yield losses in wheat and barley in North America, thus greatly impacting agriculture sector and bioeconomy. Saskatchewan prairies represent approximately 40% of the overall cultivated land in Canada and are one of most important cereal crop regions worldwide. Fungi can counteract Fusarium species causing FHB and hence prevent the appearance of the disease and accumulation of associated mycotoxins (deoxinivalenol, nivalenol and zearalenone). Optimal biocontrol seems to rest within Fusarium-specific Sphaerodes mycoparasites. These are considered environmentally friendly or green solutions for feed and food safety, as well as economic profitability of cereal crops.

Poster Session

BAM-PT1109 - Microbiological and Physiological Analyses of Okpuloumuobo River, Aba, Nigeria

Uchechi Ekwenye[1], Ifeanyi Nwachukwu[1]
[1]*Michael Okpara University of Agriculture,Umudike,Umuahia,Nigeria*

Microbiological and Physicochemical analyses were carried out on the Okpuloumuobo River, an extension of the Aba River in Aba, Abia State, Nigeria. The results obtained were compared with World Health Organization (WHO) standards for drinking and recreational water. Total viable count was by pour plate technique while most probable number (MPN) counts were by the multiple tube fermentation technique. The water sample was found to harbour coliform organisms in numbers greater than the WHO standards for water with counts ranging from 1.3 x105+– 4.3 x105 cfu/ml. The total aerobic plate counts were generally high exceeding the limit of 1.0 x 102 cfu/ml for water and this ranges from 4.6 x105 - 1.6 x106 cfu/ml while the fungal count ranges from 3.7 x 104 - 6.8 x 104 cfu/ml. Seven bacterial isolates were obtained from the study and were identified as Staphylococcus aureus, Pseudomonas aeruginosa, Proteus species, Salmonella species, Shigella species, Escherichia coli and Klebsiella species. The fungal isolates were identified as Aspergillus species, Mucor species, Penicillium species and Rhizopus species. The physicochemical parameters which included temperature, total nitrogen, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Dissolved Oxygen (DO) did not fall within the WHO standards values with mean values of 33.50OC, 55.82mg/l, 15.90mg/g, 23.85mg/l and 5.27mg/l respectively. However, parameters such as Turbidity (17.80 NTU) Total Dissolved Solid (TDS) (215.30mg/l) and Electrical Conductivity (EC) (51.49 μs/cm) were within the standard value without the detection of lead. These results indicated the unwholesomeness of the river for domestic and recreational activities. Hence, effort should be made to treat the water before being used for the above mentioned purposes as well as creating awareness on the associated potential health risk.

Poster Session

BAM-PT1111 - Denitrification gene expression and abundance of denitrifier Pseudomonas mandelii PD30 in the wheat rhizosphere

Philip Formusa[1], Dr. Marc Habas[1], Dr. Hung Lee[1], Dr. Jack Trevors[1]
[1]*University of Guelph, Guelph, Canada*

Denitrification, the stepwise enzymatic reduction of nitrate to dinitrogen, allows energy generation in microorganisms under anoxic or anaerobic environments. Previous research showed microbial communities are influenced by the rhizosphere and cause an increase in denitrification. Our research investigated the influence of the early wheat rhizosphere on expression and abundance of nitrate and nitric oxide reductases (encoded by narG and norB, respectively) in Pseudomonas mandelii PD30. Primers for narG and norB were designed using genome sequence data. Wheat plants were grown for 2 weeks in soil, which had been inoculated with 0, 105, or 107 CFU/g-dry-soil of P. mandelii cells. Following plant growth, the soil water content was increased to 70% of its capacity with one of water, or water containing 500 µg/g-dry-soil nitrate, or water containing 500 µg/g-dry-soil nitrate and glucose. Soil samples (taken at 4 and 24 hours after increasing the water content) from the soil-air interface, rhizosphere, and bulk soil at the bottom of each pot were analyzed for gene expression and abundance. Pots provided with water only had the highest narG expression in samples taken from the bulk soil at 4 hours. Expression of narG at 24 hours of all samples were not statistically different. Expression of norB was highest in the bulk soil of all treatments at 4 hours, while expression of norB at 24 hours showed no influence of sampling location or concentration of cells added. The results suggest that expression of individual denitrifying genes may not be as significant as abundance for the rhizospheric influence on denitrification.

Poster Session

BAM-PT1113 - The role of tetrahydrofolate in the regulation of dimethylsulfoniopropionate metabolism in Ruegeria pomeroyi DSS-3

Hannah Bullock[1], Andrew Burns[2], Mary Ann Moran[2], William Whitman[1]
[1]Department of Microbiology, University of Georgia, Athens, USA, [2]Department of Marine Sciences, University of Georgia, Athens, USA

The phytoplankton produced organosulfur compound dimethylsulfoniopropionate (DMSP) plays a key role in the global sulfur cycle and marine microbial sulfur and carbon metabolism. The marine Roseobacter Ruegeria pomeroyi DSS-3 is capable of metabolizing DMSP via two competing pathways, the cleavage or demethylation pathway. The cleavage pathway forms the volatile gas dimethylsulfide (DMS), the largest natural source of sulfur to the atmosphere. The demethylation pathway produces methanethiol (MeSH), which is readily assimilated or oxidized. A model for the regulation of DMSP metabolism has been developed for R. pomeroyi. The availability and turnover of methyl-tetrahydrofolate (THF) formed by the demethylation pathway DMSP demethylase DmdA appears to be a major regulatory point. RNA-Seq expression data shows that R. pomeroyi genes involved in THF biosynthesis and methyl-THF metabolism are significantly up-regulated during growth on DMSP as compared to acetate. Inhibition of THF biosynthesis using the dihydrofolate reductase inhibitor trimethoprim also increased the production of the cleavage pathway product DMS, indicating elevated use of the cleavage pathway over the demethylation pathway when THF biosynthesis is inhibited. The two R. pomeroyi DmdB methylmercaptopropionate CoA ligases, RPO_DmdB1 and RPO_DmdB2, are secondary regulatory points. Both DmdB enzymes are inhibited by cellular concentrations of DMSP. RPO_DmdB1 regains activity in the presence of increasing ADP concentrations, indicating it responds to changes in cellular energy charge. RPO_DmdB2 is responsive to changes in the concentration of MMPA. This response is likely related to the availability of free THF and the turnover rate of DmdA. High levels of methyl-THF metabolism allow DmdA to function at its maximal rate and sufficient MMPA to accumulate to overcome the DMSP-based inhibition of RPO_DmdB2. Based on the current knowledge of DMSP metabolism, the regulation of the two pathways in R. pomeroyi appears to be complex and multifaceted.

Poster Session

BAM-PT1115 - Dramatic alteration of metabolic processes under the cell-to-cell interaction

Kenshi Suzuki[1], Fatma Azwani[2], Yuma Inuzuka[1], Hajime Kobori[3], Weitao Qui[3], Yosuke Tashiro[1], Hirokazu Kawagishi[3], Hiroyuki Futamata[1]
[1]Engineering, Shizuoka University, Hamamatsu, Japan, [2]Institute of Tropical Agriculture, University Putra Malaysia, Malaysia, [3]Agriculture, Shizuoka University, Shizuoka, Japan

Dynamic equilibrium of bacterial population and function in bacterial ecosystem was investigated by using interdisciplinary methods. As the model ecosystem, a phenol-feeding chemostat culture was constructed with Pseudomonas sp. C8, Comamonas testosteroni R2, and Ralstonia sp. P-10. Interestingly, although the supernatant of strain C8 (C8SP) repressed significantly the growth of strains R2 and P-10, strains C8 and R2 became dominants simultaneously in the mixed culture after starvation stress. Of the C8SP fractionated with different molecular weight, the fraction less than 5000 Da in the H2O-soluble components repressed the growth of strain R2. Since the only supernatant of strain C8 incubated under the presence of phenol as sole carbon source exhibited the growth repression, it was seemed that the growth repressing compound(s) is secondary metabolites in phenol degradation. Reporter strains for detection of C4-8-AHL and C10-16-AHL did not respond to strain C8. No genes coding N-acyl homoserine lactone (AHL) and Pseudomonas quinolone signals (PQSs) were found in genome sequence of strain C8. These results suggested that the growth repressing compound(s) from strain C8 was not a kind of AHL and PQS but a novel hydrophilic compound. Interestingly, the growth of strain R2 repressed with C8SP was almost completely recovered by addition of ferric chloride. Metabolomic analyses revealed that metabolic processes under the control condition, the presence of the C8SP and C8SP with ferric chloride were different from each other. These results suggested that ferric chloride plays role in a trigger for release of the growth repressing conditions. On the other hand, since a medium used in the mixed culture does not include ferric compounds, it suggested that there is a novel system for strain R2 to become dominant with strain C8 in the mixed culture.

Poster Session

BAM-PT1117 - Transcriptome analysis of sulfur metabolism in Acidithiobacillus thiooxidans

Rodolfo Frazao[1], Christian Baron[1]
[1]*Université de Montréal, Montréal, Canada*

The mining industry is a very important business worldwide. It is a key factor in today's society and its expansion around the world is increasing reflecting the needs for metals and other extracted resources by industry. However, an environmental problem called "Acid Mine Drainage" (AMD) occurs principally in left over materials after extraction of the metals from ores. This reaction producing sulfuric acid contributes to the acidification of rivers, lakes and all mining surroundings sites. This process happens because of microorganisms capable of oxidizing sulfur in mine tailings. The metabolism leading to the oxidation of sulfur is still poorly characterized because of the disproportionation of the different sulfur compounds produced and used in enzymatic reactions. During this process, spontaneous reactions also take place further complicating the understanding of this metabolic pathway. In order to characterize sulfur metabolism by AMD bacteria we are conducting a high-throughput analysis of the transcriptome of the reference bacterium Acidithiobacillus thiooxidans (AT), using RNA-seq. In addition, we analyze candidate genes coding enzymes involved in its sulfur metabolism using qPCR. Also, AT's growth on different sulfur compounds will be assessed by fluorescence microscopy and scanning electron microscopy (SEM). The results from these multiple techniques will lead to a better understanding at the transcriptome level of its sulfur metabolism and its contribution to acid formation in AMD. Finally, we plan to define a biomarker capable of identifying at which moment this microorganism is producing acidity. This will help the mining industry to better manage the AMD process in tailings.

Poster Session

BAM-PT1119 - Quantifying patient bacterial exposure risk from re-useable phlebotomy tourniquets

Cameron Schauer[1], David Hammer[1]
[1]*Northland DHB, Whangarei, New Zealand*

The risk of transmission of nosocomial pathogens from re-useable phlebotomy tourniquets to patients is debated. This study aimed to quantify the number of bacteria to which patients are exposed with each blood collection episode and assess for the presence of Multi-Drug Resistant Organisms (MDROs) on tourniquets. Tourniquets were randomly sampled in a 246-bed, secondary level, New Zealand hospital, which is currently non-endemic for MDROs. A six centimetre length of each tourniquet sampled was applied to the surface of an agar plate and the Colony Forming Units (CFUs) were enumerated. All colonies were then screened for MDROs using standard methods. CFU counts per centimetre were multiplied by a range of patient arm circumference measurements. Comparison was also made between uncleaned tourniquets left on the wards and phlebotomy service tourniquets after daily cleaning with a proprietary disinfectant. The average exposure risk from non-cleaned tourniquets was 173 CFUs per collect (95% CI: 104 to 861). None of the general ward tourniquets grew any MDROs but four out of five dedicated, single-patient re-useable, isolation room tourniquets grew MRSA. Cleaned tourniquets had few, if any CFUs and CFU counts were significantly lower than non-cleaned tourniquets (p=0.0001). Quantitative risk from re-useable tourniquets appears low in the setting of MDRO non-endemicity, with the application of sensible infection control practices.

Poster Session

BAM-PT1121 - Mars-like soils in an Upper Dry Valley, Antarctica and the cold-arid limit of life

Jackie Goordial[1], Alfonso Davila[2], Denis Lacelle[3], Charles Greer[4], Chris McKay[2], Lyle Whyte[1]
[1]McGill, Ste. Anne de Bellevue, Canada, [2]NASA Ames Research Centre, Moffett Field, USA, [3]University of Ottawa, Ottawa, Canada, [4]BRI, National Research Council, Montreal, Canada

Characterized as the most old, cold and arid environment on Earth, the Upper McMurdo Dry Valleys (UDV) in Antarctica, are one of the harshest environments on earth for life. The UDVs are the only place on Earth where there is the presence of permanently frozen ice-cemented ground (permafrost) overlain with a layer of dry, ice-free permafrost soil, similar to the Pheonix landing site on Mars. University Valley (UV) is an UDV, with air temperatures that never rise above 0°C, and soils with extremely low water activity. At this site, ice in the ice-table is deposited via water vapour diffusion rather than liquid water. We investigated the potential for dry and ice-cemented permafrost in UV to support active microbial life. Direct cell counts showed biomass of 10^3 cells/g wet soil, orders of magnitude below what is found in permafrost from the Arctic and the lower dry valleys of Antarctica. Culturable isolates in permafrost were extremely low, 6 isolates were obtained, 2 of which (a Rhodococcus and Rhodotorula) displayed sub-zero growth. Sensitive radiorespiration assays at sub-zero temperatures revealed no microbial activity in UV permafrost over 700 days incubation, while permafrost from coastal Antarctica displayed sub-zero activity at rates comparable to the Canadian high Arctic. Pyrosequencing was carried out on two permafrost cores, and high diversity and heterogeneity was found in Bacterial communities, but low diversity in Archaeal and Fungal communities. Many of the Bacterial taxa were similar to taxa associated with cryptoendolithic, or marine communities, and may represent cells deposited via Aeolian processes. The extremely low biomass, low culturable bacteria, and lack of detectable microbial activity indicates that University Valley is at the edge of the cold and arid limit of life on Earth, and that it is unlikely to find extant life under the more extreme conditions of Mars.

Poster Session

BAM-PT1123 - The evaluation of heat stability of amylases from new thermophilic species

Mahnaz Farahmand[1], Nour Amirmozafari[2], Mehrdad Memarian[3], Hooman Baghery[4]
[1]Department of Biology, Faculty of Basic Science, East Tehran Branch, Islamic Azad University, Ghiamdasht, Tehran, Iran, [2]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran, [3]Department of Micobiology, Islamic Azad University of Qom, Qom, Iran, [4]Department of Biology, Faculty of Basic Science,Center Tehran Branch, Islamic Azad Universiy, Tehran, Iran

Background: Thermophilic bacteria are valuable sources of thermostable hydrolytic enzymes. Amylases are important in industry and are used for a variety of commercial-industrial processes. Objectives: The aim of this study was to determinate the stability of thermophilic amylases in different conditions, specially in the presence of Ca2+. Methods: Water samples were collected from three hot spring sources in Iran. A total of four bacterial strains were isolated which were later identified by 16SrRNA gene ribotyping using PCR followed by the amplicon sequencing. Alpha-amylase activity was tested by DNS(Dinitrosalisilic acid) method. Conclusions: The four isolates were identified to be Geobacillus thermocatenulatus La-2, Bacillus licheniformis KH-5, Aneurinibacillus aneurinilyticus SH-3 and Anoxybacillus pushchinoensis KH-3. Studies on crude alkaline amylases from La-2 and KH-3 characterization revealed that optimum activity was at 90ºC and 80ºC respectively. The amylase produced by the KH-5 and KH-3 strains retained 66% and 55% of their original activities with placed for 30 min at 90ºC, respectively. The amylase produced by the SH-3 and La-2 strains retained 44% and 60% of their original activities with placed for 30 min at 100ºC, respectively. The purified enzyme from La-2 strain was fully stable after 150 min incubation at 90°C and retained 50% maximal activity after 3 h of treatment at 90°C. The effect of calcium on enzyme stability of all thermophilic strains was studied. The activity of four thermophilic enzymes was increased 50% by Ca2+.

Poster Session

BAM-PT1125 - Microbial Diversity of hypersaline lagoons in Puerto Rico using culture-independent techniques

Coralis Rodriguez-Garcia[1], Rafael Montalvo-Rodriguez[1]
[1]University of Puerto Rico, Mayagüez, Puerto Rico

Hypersaline lagoons are classified as extreme environments due to their high salt content, especially NaCl. These lagoons have specific communities of microorganisms, which are responsible of performing the necessary processes to keep life on them. Diversity studies of halophilic archaea and bacteria in this type of environment using culture-dependent techniques are limited and biased by media restrictions. Metagenomics as an emergent science presents a helpful tool for these analyses. Even though the technique does not recover all the diversity present in the environment, it provides a more comprehensive alternative than the traditional culture-dependent methods. Our aim in this study is to construct environmental gene libraries using metagenomic DNA from the lagoons Candelaria and Fraternidad, with moderate salinity of 14% and 24% NaCl (w/v), respectively. Both natural lagoons are localized at the Cabo Rojo salterns in Puerto Rico, a tropical island in the Caribbean. Because of its location, the island maintains template weather through the whole year and low precipitation frequency. Those factors provide particular characteristics to the lagoons of interest in comparison with other salterns in the world. Water samples were taken from each lagoon, and the metagenomic DNA was extracted. This DNA was used for PCR reactions with specific primers for the amplification of the 16S rRNA gene from bacteria (27F/1492R) and archaea (7F/927R). Amplicons were cloned using the pGEM®-T vector system. A total of 40 clones were obtained. We are currently analyzing additional clones from both libraries to determine OTU frequency, abundance and diversity.

Poster Session

BAM-PT1127 - Isolation and characterization of microorganism with agarase activity from the Cabo Rojo, Puerto Rico solar salterns

Miguel X. Oyola-Rivera[1], Eduardo Tosado-Rodriguez[1], Rafael Montalvo-Rodriguez[1]
[1]University of Puerto Rico, Mayagüez , Puerto Rico

Agarases are enzymes that are able to hydrolyze agar, which is a phycocolloid product extracted from the cell wall of red algae (Rhodophyceae) including Gelidium and Gracilaria. These types of enzymes are classified in two groups (α-agarase and β-agarase) according to the cleavage pattern. Several agarases have been isolated from seawater and marine sediments. Recent studies have demonstrated that the agarase gene (agaV) could be useful in two aspects; first, as an agarolytic enzyme, and also, as a reporter in the construction of a secretion signal trap which proved to be a simple and efficient molecular tool for the selection of genes encoding secretion proteins from both gram-positive and gram-negative bacteria. Molecular studies of agarases have not been conducted in Puerto Rico; therefore the purpose of this study is to isolate prokaryotic strains having agarase activity from saline environments in the Island. Water samples were taken from marine (3.5% - 5.0% NaCl), moderate saline (10.0% - 15.0% NaCl) and hypersaline (20.0% - 25.0% NaCl) environments from the Cabo Rojo salterns. These samples were then filtered using a 0.45μm nitrocellulose membrane. Membranes were inoculated in a modified version of the Synthetic Crenarcheota medium. Isolated strains were grown in medium with yeast extract, without yeast extract, with yeast extract and agarose and without yeast extract and agarose to demonstrate their capability of agarase production in media with or without nutrients. A total of 51 isolates were obtained from the samplings and 15 of these had agarase activity. The isolates are being characterized using physiological, molecular and morphological properties. Analysis of a 16S rDNA amplicon revealed that the isolates belong to the Archaea and Bacteria domains. This study can contribute in understanding the diversity and function of the enzyme agarase in saline environments from Cabo Rojo, PR.

Poster Session

BAM-PT1129 - Microscopic characterization of the cell envelope of Planococcus halocryophilus Or1 during subzero growth at -15°C

Nadia Mykytczuk[1], John Lawrence[2], Christopher Omelon[3], Gordon Southam[4], Lyle Whyte[5]
[1]Laurentian University, Sudbury, Canada, [2]Environment Canada, Saskatoon, Canada, [3]University of Texas at Austin, Austin, USA, [4]University of Queensland, Brisbane, Australia, [5]McGill University, Montreal, Canada

Planococcus halocryophilus strain Or1, isolated from high Arctic permafrost active layer, divides at temperatures as low as -15 ºC under high concurrent salinity of 18% NaCl. Initial studies of P. halocryophilus Or1 identified that under subzero conditions the cell envelope appeared to have some unusual changes composition, favouring a higher proportion of saturated fatty acids and distinct changes in cellular appearance at subzero temperatures. Further analyses using electron microscopy (SEM, TEM), confocal laser scanning microscopy (CLSM), and scanning transmission X-ray microscopy (STXM) were used to better characterize the changes in cell envelope features and total composition occurring over a range of temperatures from optimal (25ºC) down to subzero growth (-15ºC). S/TEM and CLSM images illustrate that -15 ºC growth temperatures coincide with increasing hydrophobicity of the cell and distinct encrustations surrounding cells that are closely associated with the cell wall and are not composed of extracellular polysaccharide observed to coat cells at 25 and 0 ºC. STXM analyses resolved the cell composition to favour higher amounts of protein and polysaccharide at higher temperatures while cells grown at -15 ºC were most strongly distinguished by 20% calcium carbonate and choline (29%) in/on the cell envelope. Complementary analyses of the P. halocryophilus sequenced genome were used to describe genes and their relative expression and found the presence of several copies of carbonic anhydrase, a protein responsible for the mineralization of calcium carbonate and found its expression to increase at -15 ºC along with the synthesis of peptidoglycan. The unusual cell features of P. halocryophilus Or1 at -15ºC indicate a distinct cellular response and physiology in response to, and perhaps enabling, growth at subzero temperatures.

Poster Session

BAM-PT1131 - Regulation of the Rhodobacter capsulatus gene transfer agent at single-cell resolution

Hao Ding[1], J. Thomas Beatty[1]
[1]The University of British Columbia, Vancouver, Canada

The α-proteobacterium Rhodobacter capsulatus produces bacteriophage-like particles of a gene transfer agent (RcGTA) to mediate an unusual type of horizontal gene transfer. RcGTA resembles a small tailed bacteriophage and mediates gene transfer via a generalized transduction-like mechanism. However, RcGTA differs from bacteriophage in two main aspects. First, RcGTA only contains random segments of host DNA in its head. Second, RcGTA packages a smaller amount of DNA (~4 kb) than the RcGTA gene cluster encoding the particle (~ 15 kb). The expression of the RcGTA gene cluster is culture growth phase-dependent, with increased expression in the stationary phase, regulated by the quorum-sensing proteins GtaI and GtaR. The R. capsulatus cellular histidine kinase CckA and response regulator CtrA, which are thought to participate in a signal transduction phosphorelay pathway, appear to play a central regulatory role in RcGTA production. In addition, the expression of RcGTA genes is heterogeneous and bistable within a clonal population. Less than 1% of wild type cells express the RcGTA gene cluster; whereas in a wild type-derived overproducer strain, more than 25% of cells express the RcGTA gene cluster. Through single-cell gene expression analyses, including fluorescence-activated cell sorting and time-lapse fluorescence microscopy, we demonstrated that the bistable expression of RcGTA genes arises from stochastic processes rather than from genetic changes, such as mutation or phase variation. Using transposon mutagenesis combined with a high throughput screening approach, we discovered that disruption of a gene encoding a hemolysin-type calcium-binding repeat family protein led to the RcGTA overproduction in the wild type strain. It is further confirmed that a single point mutation within the overproducer strain, homolog of this gene is responsible for the "hyper-production" phenotype. We are in the process of determining the molecular mechanism behind these phenomena.

Poster Session

BAM-PT1133 - The Ll.LtrB group II intron from the gram-positive bacterium Lactococcus lactis excisses as circles and generates double-stranded head to tail DNA junctions in vivo

Caroline Monat[1], Cecilia Quiroga[1], Benoit Cousineau[1]
[1]*Dept of Microbiology & Immunology, MDTC, McGill University, Montreal, Canada*

Group II introns are large ribozymes that require the assistance of intron-encoded or free-standing maturases to splice from pre-mRNAs in vivo. The branching pathway, releasing group II introns as lariats, is the major and most studied splicing pathway. However, group II introns can also splice through secondary pathways like hydrolysis and circularization that are not as well characterized as branching. The Ll.LtrB intron from the gram-positive bacterium Lactococcus lactis is the best model to study group II intron splicing and mobility. Here, we assessed splicing of the Ll.LtrB group II intron in L. lactis by amplifying the splice junction of its ligated exons and released introns. Ligated exons revealed that different mutants of Ll.LtrB undergo alternative splicing using the same remote 5' and 3' alternative splice sites. We also found that the maturase activity of LtrA controls 5' and 3' splice site selection and the balance between accurate and alternative splicing. The study of excised introns revealed the presence of lariats, circles and alternatively spliced products for all Ll.LtrB variants studied albeit at different ratios. Removal of the branch point residue prevented Ll.LtrB excision through the branching pathway but did not hinder circle formation and alternative splicing. A number of circular and alternatively spliced introns were found harboring extra non-encoded nucleotides at the splice junction. The presence of identifiable mRNA fragments at the junction of some intron RNA circles provides insights into the circularization mechanism of group II introns. Complete intron RNA circles were found associated with LtrA but forming inactive RNPs. Traces of double-stranded head to tail intron DNA junctions were also detected from L. lactis total RNA and nucleic acid extracts. This work unveils that Ll.LtrB excises not only as lariats but also as circles in vivo and shed light on the circularization pathway of group II introns.

Poster Session

BAM-PT1135 - Effect of headspace pressure and dissolved carbon dioxide on growth kinetics and plasmid copy number in plasmid variants of beer-spoilage organism Lactobacillus brevis BSO 464

Jordyn Bergsveinson[1], Barry Ziola[2]
[1]Department of Health Sciences, Unversity of Saskatchewan, Saskatoon, Canada, [2]Department of Pathology, Unversity of Saskatchewan, Saskatoon, Canada

Few microorganisms have the ability to grown in the harsh environment of beer, due to the presence of several growth-inhibiting factors. In addition to the antimicrobial affect of hops and ethanol, there are also inhospitable levels of nutrients, oxygen and carbon dioxide. As the ability to grow in and spoil beer is an isolate-specific trait, it has long been suspected that beer-spoilage organisms possess a considerable degree of genetic specialization in order to deal with each of these stress factors. Thus far, little attention has been devoted to exploring the relationship between, and extent to which, dissolved carbon dioxide and pressure has on the physiology and stress response of beer-spoilage organisms. We now show that the presence of pressure, and resulting increase in dissolved carbon dioxide, significantly alters the growth kinetics of the virulent beer-spoilage organism Lactobacillus brevis BSO 464 (Lb464) in beer. Notably, there is an increased lag time when Lb464 is grown in the presence of dissolved carbon dioxide and an increase in generation time, relative to Lb464 growth kinetics in beer that has been removed of all carbon dioxide. Ten unique plasmid-variants of Lb464 were also assessed for alterations in growth kinetics and it was found that the loss of multiple plasmids resulted in significant changes in growth ability of Lb464 under pressure conditions. The plasmid copy number of several plasmid-variants were then evaluated in pressurized beer and non-pressurized beer, revealing an important association between plasmid copy number of specific plasmids, the coding capacity of these plasmids, and the overall growth success in the hostile pressurized-beer environment. This data will be useful for comparison against other beer-spoilage organisms of different beer-spoilage capability, and provides further insight into the relationship between growth conditions and the plasmid profile of beer-spoilage organisms.

Poster Session

BAM-PT1137 - Effects of antibiotics on natural transformation in SigH-expressing Staphylococcus aureus

Le Thuy Thi Nguyen[1], Kazuya Morikawa[1]
[1]*University of Tsukuba, Tsukuba, Japan*

In Staphylococcus aureus, development of competence for natural transformation requires a sigma factor SigH that is responsible for competence machinery expression. The overexpression of SigH by plasmid allows us to detect transformation. However, the transformation efficiency varies depending on growth conditions, suggesting that certain environmental factor is necessary in competence development in addition to SigH expression. In this study, we tested effects of antibiotics or chemicals on transformation in SigH expressing S. aureus. Cell wall-targeting antibiotics exhibited variable effects depending on their mode of action. Ciprofloxacin and mitomycin C suppressed transformation. Protein synthesis inhibitors such as streptomycin and spectinomycin had no effect on competency. Our results suggest the complex response of S. aureus to activate or inactivate competence for DNA transformation.

Poster Session

BAM-PT1139 - Comparision of MIC with MBEC assay for invitro antimicrobial susceptibility testing in biofilm forming Clinical Bacterial isolates

Summaiya Mulla[1], AbdulVahed Mulla[2], Sangita Revdiwala[3]
[1]Govt. Medical College, Surat, India, [2]Private,Surat,India, [3]Govt. Medical College, Surat, India

Introduction: A number of factors have been postulated for resistance of antimicrobial agent by biofilm producing organism. MIC does not provide a true estimation of the concentration of antibiotics required to treat a bacterial biofilm. The Minimum Biofilm Eradication Concentration (MBEC) measure the determination to be made for a biofilm. In this study, biofilms developed in an in vitro model system, those grown adherent to microtiter wells early in the process of biofilm formation. Antibiotic susceptibility of planktonic bacteria and biofilms in killing by the antibiotics were also measured. We compared the MIC and MBEC assays to evaluate differences in the antibiotic sensitivity patterns of different isolates from patients implanted with medical devices. Material & Methods: The study was carried out on 50 positive bacteriological cultures of medical devices which were inserted in hospitalized patients. Biofilm forming strains were identified by tissue culture plate method & tube method. Biofilm-producing reference strains of Acinetobacter baumanni (ATCC 19606) and Pseudomonas aeruginosa (ATCC 27853) and non-biofilm forming reference strain of Staphylococcus aureus (ATCC 25923) and E.coli (ATCC 25922) were used as controls. Assay has been developed for use with flat bottom, 96-well microtiter plates. Sterile autoclaved PCR tubes were used as pegs which provide surface for the biofilm formation. Amikacin, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Vancomycin, Cefoperazone/sulbactam, Gentamycin were tested for MIC and MBEC assay. Results: Colonization by Klebseilla, Acinetobacter and Pseudomonas were prevalent with maximum positivity in biofilm forming assay; 45%, 77% & 78% respecticely. MBEC was higher for all the antibiotics as compared to MIC except Amikacin MBEC for Pseudomonas was less then MIC. Conclusion: MIC is not a right test for biofilm forming bacterial isolates in drug susceptibility testing. These can result in to therapeutic failure.Cost effective novel systems for testing such isolates is a need so should be developed.

Poster Session

BAM-PT1141 - Shifts in microbial community composition and function accompanying the perturbation of soil structure in a microcosm system

Nicole Sukdeo[1], Ewing Teen[1], Michael Rutherford[1], Hugues Massicotte[1], Keith Egger[1]
[1]*Natural Resources and Environmental Studies Institute, University of Northern British Columbia, Prince George, Canada*

Disruption of soil structure, horizon order and location of reservoirs of organic matter are all potential consequences of mechanically homogenizing soils. Soil excavation procedures that lead to disorganization and re-organization of soil structure include industrial trenching operations and related activities associated with building underground pipelines. These changes to soil structure may potentially impact the community structure of indigenous microbes, the observed level of organic matter decomposition and change the profile of community members exhibiting litter decomposition activities. Our investigation uses a repeated measures, time series soil microcosm experiment to examine the effects of mechanical disturbance of soil layers (organic and mineral) on microbial community composition and on the magnitude of litter decomposition enzyme activities in soil suspensions. The mechanical disturbance treatment of organic horizons in this study result in the deposition of litter components beneath the soil surface, which we anticipate may be a key determinant of community structure and observed enzyme activities. Changes in bacterial/archaeal and fungal diversity were monitored by Illumina sequencing of 16S and internal transcribed spacer 2 (ITS2) amplicons, respectively. Activities of the decomposition enzymes N-acetylglucosaminidase, β-D-glucosidase, and cellobiohydrolase were quantified for soil suspensions by fluorometric assay, as a measure of litter decomposition functions. With these data, we evaluate the resilience of community composition and stability of biochemical activities important to organic matter degradation, in response to perturbation of soil structure.

Poster Session

BAM-PT1143 - Molecular characterization by ARDRA analysis of Microbial Diversity Associated to Three Species of Fern in "Penjamo" Guanajuato, México

Cesar Eduardo Vargas Perez[1], Victoria Hernandez Hernandez[1], Gustavo Alberto de la Riva de la Riva[1], Claudia Isela Gonzalez Lopez[1], Juan Gualberto Colli Mull[1]

[1]Instituto Tecnologico Superior de Irapuato, Irapuato Guanajuato, Mexico

In natural ecosystems, plants can interact with a wide range of bacteria, which can have pathogenic, neutral, or beneficial effects on their hosts. The majority of bacteria are epiphytic and are known to colonize the rhizosphere. Some strains isolated from plant reproductive organs were also isolated from the rhizosphere or other plant parts under natural conditions, indicating a potential pathway ofcolonization. The objective of this study was analyze, which culturable bacteria colonize reproductive organs, rhizosphere, burk soil and leaf of three species of fern under natural conditions, and to compare them with bacterial communities are similar on the three especies of the fern. We have different isolates of the tree especies of fern, actually we determinated by ARDRA analysis the microbial diversity and communities structure of bacteria associated to plants.

Poster Session

BAM-PT1145 - Nitric oxide scavenger hunt: discovery of novel differential inhibitors for ammonia-oxidizing archaea and bacteria

Ashley Ross[1], Laura Sauder[1], Josh Neufeld[1]
[1]University of Waterloo, Waterloo, Canada

Ammonia-oxidizing archaea (AOA) are thought to possess a different respiratory pathway than their bacterial counterparts. Nitric oxide (NO) has been proposed to function as an electron shuttle in the thaumarchaeotal ammonia oxidation pathway, whereas ammonia-oxidizing bacteria (AOB) transfer electrons from a quinone pool. These differences suggest that NO scavengers would be generally effective at inhibiting ammonia oxidation by AOA, but not AOB. Despite this possibility, only carboxy-PTIO has been identified as an NO scavenger for AOA inhibition, yet this compound is expensive (e.g., >$1200 per g). In this study, we investigated the ability of alternative NO scavengers to inhibit archaeal and bacterial ammonia oxidation. Pure cultures of Nitrosopumulis maritimus SCM1 (AOA) and Nitrosomonas europaea (AOB) were analyzed alongside two novel AOA enrichment cultures, Candidatus Nitrosofontus exaquare and Candidatus Nitrosopurus aquariensis, which are group I.1a and I.1b representatives, respectively. Ammonia oxidation and nitrite production rates were compared for five NO scavengers: PTIO, caffeic acid, curcumin, methylene blue hydrate, and Trolox. At specific concentrations all scavengers inhibited ammonia oxidation of AOA cultures and had no effect on ammonia oxidation by N. europaea. Inhibition of AOA ammonia oxidation by caffeic acid (100-300 µM) and methylene blue hydrate (10 µM) was comparable to inhibition by carboxy-PTIO. Trolox (300 µM) and curcumin (50 µM in DMSO) inhibited N. maritimus and Ca. N. aquariensis, although only partial ammonia inhibition was observed for Ca. N. exaquare. These tested alternative AOA inhibitors are orders of magnitude less expensive than carboxy-PTIO. This study further substantiates NO as an electron shuttle in the thaumarchaeotal ammonia-oxidizing pathway and reflects metabolic differences between AOA and AOB. Although additional incubations with environmental samples would confirm ideal alternative inhibitors, our culture-based results suggest that tested alternative NO scavengers are suitable and inexpensive alternatives to carboxy-PTIO for differential inhibition of AOA and AOB.

Poster Session

BAM-PT1130 - Archael community in production waters of Algerian oilfields revealed by 16S rRNA gene amplicon 454 pyrosequencing

Nesrine Lenchi[1], Özgül Inceoğlu[2], Salima Kebbouche-Gana[1], Mohamed lamine Gana[3], Marc Llirós[4], Pierre Servais[2], Tamara García-Armisen[2]

[1]Laboratoire Valorisation et Conservation des Ressources Biologiques, Université M'Hamed Bougara de Boumerdes, Boumerdes, Algérie, [2]Ecologie des Systèmes Aquatiques, Université Libre de Bruxelles, Campus de la Plaine, Brussels, Belgium, [3]Centre de Recherches et de Developpement, laboratoire de biocorrosion, Sonatrach, Boumerdes, Algeria, [4]Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Spain

The microorganisms inhabiting many petroleum reservoirs are multi-extremophiles capable of surviving in environments with high temperature, pressure and salinity. To study these archaeal assemblages, four different Algerian oilfields were studied through 16S tag-encoded FLX-titanium amplicon pyrosequencing. The analysed wells differ from other wells already reported in the literature by their location, depth, temperature, salinity, and stratigraphic distribution. To the best of our knowledge, all the available studies so far on microbial communities in oilfields have focused on high- and low-temperature petroleum reservoirs; and none of them present an array of samples covering a range of different salinities and pH. Furthermore, previous studies pointed towards the dominance of methanogenic Archaea and their indigenous behaviour in oilfields. In the present study, phylogenetic analyses of the archaeal community composition (ACC) revealed a unique ACC for each production well with retrieved phylotypes mainly belonging to Methanobacteria, Methanomicrobia, Thermoprotei and Halobacteria classes. It is important to note that potential methanogens were also detected in three samples, which contained high sulfate concentrations (range: 371 mg/L – 503 mg/L). However and in comparison with previous studies, no methanogens were detected in saline and/or acidic wells, suggesting that the ACC in the analysed reservoirs was determined by the physico-chemical characteristics of production waters of oil reservoirs. It's not worth to mention that a large proportion of unclassified archaeal sequences were found in the samples analyzed, highlighting the need of further research about these multi-extremophiles species, their ecological functioning and metabolic capabilities.

Poster Session

BAM-PT1000 - Comparative study of Proteus mirabilis biofilms grown on organic and inorganic aquatic surfaces

Milagro Fernandez-Delgado[1, 2], Zoilabet Duque[3], Paula Suarez[2], Hector Rojas[1, 4], Monica Contreras[1], Maria Garcia-Amado[1], Carlos Alciaturi[3]

[1]Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela, [2]Universidad Simon Bolivar, Caracas, Venezuela, [3]Fundacion Instituto Zuliano de Investigaciones Tecnologicas, Zulia, Venezuela, [4]Instituto de Inmunologia - Universidad Central de Venezuela, Caracas, Venezuela

P. mirabilis is a human pathogen able to form biofilms on the surface of urinary catheters. Besides clinical surfaces, little is known about biofilms formed by this bacterium in organic and inorganic aquatic surfaces. The main aim of this work was to study and compare the biofilm structures of clinical and environmental Proteus mirabilis isolates over surfaces of chitin from shrimp exuviae and stainless steel for 4 to 96h by using Environmental Scanning Electron Microscopy (ESEM). The biofilms of the clinical isolate grown on chitin at 4h showed to possess a greater aggregation, thickness and extracellular matrix production than the stainless steel, whereas the biofilms of the environmental isolate on both surfaces had less aggregation. Between 24 and 96h, P. mirabilis biofilms of any origin formed on chitin were observed with highly structured organization, such as pillars, mushrooms, channels and crystalline-like precipitates, in contrast with flat-layer biofilms produced on stainless steel. Significant differences ($p<0.05$) were found by the non-parametric Mann-Whitney test in the frequency of pillars and channels formed by the clinical and environmental isolates on chitin during this period. Sessile cells obtained by detaching from the surfaces showed for 96h membrane integrity (104–105 cells/cm2) by LIVE/DEAD BacLight stain in the biofilms of both isolates. In conclusion, P. mirabilis from different origins are able to form dissimilar biofilms structures under chitin and stainless steel surfaces. This represents the first study that provides evidence of P. mirabilis biofilms in non-clinical surfaces and set the basis for future researches using ESEM. The biofilm formation of this pathogen in aquatic and industrial systems could have epidemiological and economic implications.

Poster Session

BAM-PT1002 - The ability of Listeria monocytogenes to produce biofilms on different materials used in the dairy industry

Sarah Hwa In Lee[1], Drucila Cristina Factor Carandina[1], Carlos Augusto Fernandes de Oliveira[1]
[1]USP, Faculty of Animal Science and Food Engineering, Pirassununga, Brazil

Listeria monocytogenes is an important food-borne pathogen capable of forming biofilms on surfaces of equipment in the food industry. Biofilms can be a source of continuous contamination of food, especially in dairy products, as the bacteria embedded in biofilms are more difficult to be eliminated. The aim of this study was to evaluate the biofilm production ability of L. monocytogenes strains isolated from the environment of dairy plants located in São Paulo state, as well as from brine and cheese samples. Thirty-eight pulsotypes previously identified by PFGE were used in the study. Each pulsotype stored in ultra- freezer at -80 °C was re-suspended in tubes containing Tryptone Soy Broth, incubated at 37°C/24 h and diluted until a concentration of 0.5 in MacFarland scale was reached. Triplicate aliquots of 200 µl of each bacterial suspension were transferred to three wells of a sterile 24-well flat-bottomed plastic microplate with an inert material (stainless steel, rubber and silicone) and incubated at 37°C/48 h. From 38 pulsotypes analyzed, 17 (44.7 %) isolates were able to produce biofilm on the surface of stainless steel, while only 1 (2.6%) produced biofilms on silicone and 5 (13.1%) in rubber. In conclusion, the ability to form biofilms on different surfaces is an important virulence factor for L. monocytogenes, indicating the potential for persistence of this pathogen in several sites of the environment in the dairy plants studied, especially on stainless steel.

Poster Session

BAM-PT1004 - Activity of selected disinfectants and antiseptics against bacterial biofilms analyzed by the MBEC assay

Stefan Tyski[1,2], Angelika Abramczuk[2], Ewa Bocian[2], Wanda Grzybowska[2]
[1]Medical University of Warsaw, Warsaw, Poland, [2]National Medicines Institute, Warsaw, Poland

Several different methods have been established to monitor the microbial biofilms formation and to measure the antibiofilm activity of antimicrobial agents. Minimum Biofilm Eradication Concentration assay allows bacteria to grow on 96 identical pegs protruding down from a polystyrene lid of microplate. The system was applied to compare the antibacterial biofilm efficacy of 14 disinfectants and 2 antiseptic preparations available on the market in Poland. These products contained active substances from different chemical groups: alcohols, aldehydes, bigunidins, quaternary ammonium compounds, phenols, amines derivatives, oxidizing agents. Biofilms were created by bacterial strains from ATCC, representing the following species: Staphylococcus aureus, S.epidermidis, Enterococcus feacalis, E.hirae, Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens, Stenotrophomonas maltophilia. The susceptibility of 24h and 48h biofilms of Gram-positive cocci and 4h, 24h and 48h biofilms of Gram-negative rods to selected preparations in recommended concentrations, was determined. The density of bacterial biofilm growing on microplate peg, before antibacterial agents treatment varied 105-107 CFU/mL, depending on strain and biofilm age. Bacterial biofilm on peg was washed to remove planctonic cells and incubated with antibacterial preparation for 1 min. Pegs with biofilm were transferred to neutralizer for 5 min, then to dilution solution and finally sonicated for 10 min to remove the biofilm from peg surface. Obtained material was cultured on Tryptic Soya Agar and incubated at 37oC for 24-48h. Number of colonies was counted and reduction of microbial count was calculated. 3 preparations containing alcohols were mostly effectively killing biofilm bacteria in 100% regardless of strain and biofilm age. Aldehyde and peroxide preparations were less effective especially against old biofilm. Preparation containing enzymes, beside biguanidin and amines derivatives, was effective only against S.epidermidis and E.faecalis biofilms.

Poster Session

BAM-PT1006 - Requirement of the lipopolysaccharide O-antigen for biofilm formation by Actinobacillus pleuropneumoniae

Skander Hathroubi[1], Yannick D.N. Tremblay[1], Josée Labrie[1], Mario Jacques[1]
[1]Groupe de Recherche sur les Maladies Infectieuses du Porc, Université de Montréal, Canada

Actinobacillus pleuropneumoniae (APP) is a Gram-negative bacterium belonging to the Pasteurellaceae family and the causative agent of porcine pleuropneumonia, a highly contagious lung disease causing important economic losses. APP has the ability to rapidly form a biofilm rich in a N-acetyl-D-glucosamine polymer (PGA). Surface polysaccharides including lipopolysaccharides (LPS) and capsular polysaccharides (CPS) are implicated in the adhesion and virulence of APP but their role in biofilm formation is still unclear. In this study we investigated the requirement of surface polysaccharides in biofilm formation by A. pleuropneumoniae serotype 1. Well characterized Tn10-transposon mutants were used in this study: an O-antigen LPS mutant 44.1, a truncated core-LPS mutant CG3 with an intact O-antigen and a capsule mutant 33.2. We compared the amount of biofilm produced by the parental strain and the mutants using a polystyrene microtiter plate assay and a continous flow system, characterized the biofilm architecture and composition using microscopic techniques, and evaluated the relative expression of pgaA and the relative amount of PGA associated with the biofilm cells. Only the biofilm of mutant 44.1 was dramatically reduced compared to the parental strain or the other mutants. PGA, proteins and eDNA were confirmed as components of the biofilm matrix. Detection of PGA by immunoblot demonstrated that the O-antigen mutant had less cell-associated PGA than the parental-strain or the other mutants. Real-time PCR analyses revealed a 6-fold reduction in the level of pgaA mRNA in the biofilm cells of mutant 44.1 compared to the wild-type. In conclusion, truncation of the LPS core oligosaccharide or the absence of the capsule polysaccharide did not have an effect on biofilm formation. The absence of the O-antigen reduces the ability of APP to form a biofilm and this is associated with a reduction in the expression and the production of PGA.

Poster Session

BAM-PT1008 - Biofilm formation in the surface and drinking water distribution systems in Mafikeng, North West Province, South Africa

Suma Mulamattathil[1], Carlos Bezuidenhout[2], Moses Mbewe[1]
[1]University of Limpopo, Polokwane, South Africa, [2]North West University, Potchefstroom, South Africa

Poor quality source water and poorly treated reused waste water may result in poor quality drinking water that has a higher potential to form biofilms. This study was conducted to investigate the biofilm growth in the drinking water distribution systems in the Mafikeng area, North-West Province, South Africa. Analysis was conducted to determine the presence of faecal coliforms, total coliforms, Pseudomonas spp., and Aeromonas spp. in the biofilms. Biofilms were grown on a device that contained copper and galvanized steel coupons. Furthermore, a mini tap filter, a point-of-use (POU) treatment device which can be used at a single faucet was also used. Scanning electron microscopy demonstrated that multi-species biofilms developed on all the coupons as well as on the point-of-use filters. Galvanized steel and the carbon filters had the highest density of biofilm. Total coliforms, faecal coliforms and Pseudomonas sp were isolated only from raw water biofilm coupons. Aeromonas sp. and Pseudomonas sp. were isolated from filters. The susceptibility of selected isolates was tested against 11 antibiotics of clinical interest. The most prevalent antibiotic resistance phenotype observed was KF-AP-C-E-OT-K-TM-A. Furthermore, isolates were tested for the presence of virulence genes using PCR. Pseudomonas species were screened for the presence of the exoA, exoS and exoT virulence gene determinants while Aeromonas species screened for aerA and hylH virulence gene. Aeromonas sp. that were isolated from Modimola dam raw water biofilm and mixed water harboured the hylH gene. The exoA gene was detected in Pseudomonas sp. from the raw water biofilm and biofilm isolates from the treated dam water. Isolates from the biofilm from all sites harboured exoT genes. These results indicate that bacteria persistent in the water have the ability to colonize as biofilms and drinking water biofilms may be a reservoir for opportunistic bacteria including Pseudomonas and Aeromonas species.

Poster Session

BAM-PT1010 - Shiga toxin-producing E. coli (STEC) of serotype O157:H7 have a better ability to form biofilms than other serotypes

Philippe Vogeleer[1], Yannick Tremblay[1], Grégory Jubelin[2], Mario Jacques[1], Josée Harel[1]
[1]Centre de Recherche en Infectiologie Porcine et Aviaire (CRIPA), Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Canada, [2]UR454 Microbiologie, INRA Clermont-Ferrand Theix, France

Shiga toxin-producing Escherichia coli (STEC) are food-borne pathogens that cause diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome, which may result in death. A major priority for the food industry is to avoid the presence of STEC in the food production chain. In this sector, biofilm represents a real problem by contaminating the facility and being resistant to traditional cleaning and disinfection protocols. Bacterial factors, such as proteins, cellulose or poly-N-acetylglucosamine (PGA) have been associated with biofilm formation in E. coli. The aims of the study were to evaluate the ability of human STEC isolates representing the most pathogenic seropathotypes to form biofilms and to characterize the matrix composition of some of these STEC biofilms. To evaluate biofilm formation, overnight cultures were diluted in M9 broth supplemented with 0.4% glucose and were inoculated in polystyrene microplate which was incubated under static condition for 24h at 30° C. Bacterial biomass fixed to the bottom of wells was then quantified by crystal violet staining. Matrices of some STEC biofilm were also observed by using confocal microscopy. Importance of matrices components in the integrity of mature (24h) biofilm was then investigated by enzymatic digestion of DNA, proteins, cellulose or PGA. We have shown that biofilm formation was variable among STEC isolates (DO595nm: 0.04 to 2.0). Interestingly, strains belonging to seropathotype A (O157:H7) have significantly higher potential to form biofilm than other STEC seropathotype. By using confocal microscopy, PGA was only detected in seropathotype A biofilms. In two of these seropathotype A biofilms, cellulose was also detected. Furthermore, enzymatic treatments indicated that proteins appear to play an important role in STEC biofilm integrity while DNA, PGA and cellulose do not. This is the first study that described a more important potential of biofilm formation for seropathotype A (O157:H7) isolates than other STEC seropathotypes.

Poster Session

BAM-PT1012 - Use of a microfluidic system to study biofilm formation in pathogenic Escherichia coli

Yannick Tremblay[1], Philippe Vogeleer[1], Mario Jacques[1], Josée Harel[1]
[1]*Groupe de recherche sur les maladies infectieuses du porc, Université de Montréal, St-Hyacinthe, Canada*

Biofilms are typically studied using static 96-well microtitre plates and this system offers several advantages such as high throughput screen and small volumes (e.g. 200 µl). However, the microtitre plate is a closed system and there is an absence of shear force. It is generally accepted that in vivo biofilms develop in the presence of shear force. Several biofilm reactors incorporate shear forces but these systems often require large volume of media and are prone to contamination. Recently, a microfluidic system, which incorporates shear force and very small volumes (e.g. 20 µl), has been tested for the development of bacterial biofilms. Therefore, the objective of this study was to determine if the Bioflux 200 microfluidic system could be used to grow biofilms of pathogenic Escherichia coli. Type strains of enterohemorrhagic, enteropathogenic, enteroaggregative, diffuse-adherent, adherent-invasive, extraintestinal uropathogenic and extraintestinal septicemic E. coli were selected for our investigation. Initially, biofilm formation was assayed using a static 96-well plate assay. Every strain tested formed a biofilm. For the Bioflux 200 biofilms, different parameters for optimal biofilm formation were tested and these included bacterial density and shear force level (0.5 to 2.0 dynes/cm²). Pathogenic E. coli strains were able to form a biofilm in the Bioflux 200 system if the initial density (OD600) was greater than 0.5, the shear force was below 1.0 dyne/cm², the growth medium was M9 with glucose and the temperature was 30°C. Biofilm formation was strain dependent and did not always correlate with the results obtained with the microtitre plates. For example, some strains such as the enteropathogenic strain, formed larger biofilms in the Bioflux system than the microtitre plate system. In conclusion, conditions have been optimized to allow the study of biofilm formation of various pathotypes of E. coli in the presence of shear force in a microfluidic system.

Poster Session

BAM-PT1014 - Participation of flagella in the interactions of an O157:H7 Shiga toxin-producing Escherichia coli strain with different biotic and abiotic surfaces

Cecilia Matheus-Guimarães[1], Cecilia Mari Abe[2], Suely Ferreira Sampaio[1], Tania A T Gomes[1], Beatriz E C Guth[1]
[1]Universidade Federal de São Paulo, São Paulo, Brasil, [2]Instituto Butantan, São Paulo, Brasil

Shiga toxin-producing *Escherichia coli* (STEC) strains are important food-borne pathogens associated with human diseases including mild diarrhea, hemorrhagic colitis and hemolytic uremic syndrome. Ruminants, specially cattle, are considered to be its major reservoir. The ability to colonize the host intestinal epithelium and to survive and persist in the environment are crucial features in the STEC pathogenesis and epidemiology. Biofilm formation has been also described as an important characteristic for their maintenance and persistence in the host. The aim of this study was to evaluate the participation of flagella during adherence and invasion processes of an O157:H7 STEC strain isolated from cattle carcass. Deletion of *fliCH7* gene from this O157 STEC strain was obtained by homologous recombination using the Lambda Red System. Wildtype (wt) and mutant (Δ*fliC*) strains were evaluated regarding the ability to form biofilm in polystyrene and glass surfaces, to adhere to HeLa cells and rocket leaves, and to invade Caco-2 and T84 cells. Decreased rates of biofilm formation on abiotic surfaces were detected for the Δ*fliC* strain when compared with the wt strain. When adherence to HeLa cells was tested, no statistical difference was observed, but the ability of Δ*fliC* strain to invade human cells was reduced 2-fold on Caco-2 cells and over than 6-fold when assays where carried out with T84 cells. Interestingly, the ability of the mutant strain to interact with rocket leaves increased when compared with the wt strain. Thus, flagellum is associated with the ability of the O157 STEC strain to interact with abiotic surfaces as well as its capacity to invade human cells, specially colonic tissues. However, the increased ability of ΔfliC strain to interact with rocket leaves suggests a compensatory mechanism when this gene is deleted.

Poster Session

BAM-PT1016 - Purification and characterization of BcsC; a critical protein in bacterial cellulose export

Emily Wilson[1], Deanna MacNeil[1], Christina Notte[1], Joel T. Weadge[1]
[1]*Wilfrid Laurier University, Waterloo, Canada*

The majority of bacteria are thought to grow as multicellular communities living in a biofilm. The structure of a biofilm consists of bacterial cells thriving inside a self-produced matrix that surrounds and protects these bacteria. The predominant component of the biofilm matrix is long carbohydrate chains, such as cellulose. Cellulose is produced by a number of bacteria, including *Escherichia coli*. Cellulose-containing biofilms dramatically increase bacterial resistance to antibiotics, disinfectants and host defense mechanisms. Recently, BcsA and BcsB were characterized for their coordinated ability to synthesize and transport cellulose across the cytoplasmic membrane. The goal of the present research has been to purify and begin characterizing BcsC; the enzyme that is predicted to be responsible for the last steps in export of cellulose from bacteria. Based on its homology to other polysaccharide export proteins in the alginate and poly β-1,6-N-acetylglucosamine systems, BcsC has been hypothesized to consist of an outer membrane β-barrel connected to a periplasmic tetratricopeptide repeat (TPR) region. Expression constructs of each predicted domain and the full length protein have been generated in order to gain a better understanding of the structure and function of BcsC. High yields of soluble protein for the predicted TPR construct have been obtained and are now being used to structurally characterize (circular dichroism and X-ray crystallization trials) this domain. Concurrently, we are also testing the ability of the TPR construct to bind cellulose and/or to form protein-protein interactions with other periplasmic proteins involved in cellulose synthesis and export in *E. coli* (i.e. BcsG). The results of this research will be key to revealing unique approaches to circumvent the biofilm barrier by interrupting the export process. These results will also provide valuable insight into the role of similar carbohydrates that contribute to biofilm development in other bacterial species.

Poster Session

BAM-PT1018 - Biofilm production and antibiotic resistance of oral Streptococcus spp. isolated from healthy children, Turkey

Alp Kayhan Kivanç[1], Merih Kıvanç[2], Zuhal Kırzıoğlu[3]

[1]Yeditepe University, Faculty of Dentistry, Department of Restorative Dentistry,İstanbul, Turkey., [2]Anadolu University, Science of Faculty, Department of Biology, Eskişehir, Turkey., [3]Süleyman Demirel University, Faculty of Dentistry, Department of Pediatric Dentistry, Isparta, Turkey.

Mutans streptococci are microorganisms associated with the development of caries and Streptococcus mutans is the most frequently isolated member of this group in humans. Because of its heterogeneous colonization of the oral cavity, it may detected on some teeth and surfaces but not on others in the same mouth. This study aims to identify the predominant streptococcal species in the mouths of healthy children and investigate the biofilm production and antibiotic susceptibility of oral Streptococcus spp. The mouth subjects were healthy school children aged between 10 and 16 years. The oral flora was sampled dental plaque, and dental caries. Spread- plated onto Mitis salivarus agar (MSA), 5% defibrinated sheep's blood agar and M17 agar (Oxoid). The plates incubated at 37oC for 2-3 days in an atmosphere of 10% CO2. Growing colonies were subcultured from MSA and M17 agar plates. Presumptive isolates identified by morphological, cultural and biochemical tests. Automated EcoRI Ribotyping performed with a RiboPrinter® Microbial Characterization System. The resistance of the isolates to 9 different antibiotics determined by Kirby–Bauer disc diffusion test with commercial disks. The isolates were evaluated for biofilm production according to a modified microtiter plate method. Slime production in Streptococcus strains was determined by cultivation on Congo Red Agar (CRA) plates. The predominant species were Streptococcus mutans. Resistance to Oxacillin and Penicillin were found in these Streptococcus spp. The presence of resistant bacteria in the mouth can be the major cause of dental antibiotic prophylaxis failure. Particular attention should be paid to antibiotics that are most frequently used in dental practise. Majority of streptococcus strains are shown to form biofilms on dental plaques. Antibiotic multi-resistance and strong biofilm production abilities are an important equipment.

Poster Session

BAM-PT1020 - Effects of low concentrations of vancomycin on biofilm production by eye isolated

Sertac Argun Kivanc[1], Merih Kivanc[2]
[1]Department of Ophthalmology, Medical Faculty, Uludağ University, Görükle/Bursa, Turkey, [2]Department of Biology, Faculty of Science, Anadolu University, Eskisehir, Turkey

A biofilm is a functional consortium of microorganisms organised within an extensive exopolymer matrix. Organisms within a biofilm are difficult to eradicate by antimicrobial therapy. Biofilm can cause indolent infections. We investigated the biofilm stimulation effect of low dose of vancomycin on Coagulase-negative staphylococci (CoNS). The isolates were evaluated for biofilm production according to a modified microtiter plate method. Slime production in CoNS strains was determined by cultivation on Congo Red Agar (CRA) plates. Non-Biofilm producer CoNS isolates (2 S. epidermidis, 1 S. hominis, 2 S. warnei, 3 S. lugdunensis) were tested. Isolates were both ica A and ica D positive, genotypicly. Newly biofilm production was noticed, after vancomycine concentrations approaching the minimum inhibitory concentration applied. Biofilms increase antibiotic resistance and it may cause the failure of therapy. Vancomycin is a drug, which is also use as fortified ophthalmic drop in calcitrant infections. However, it was showed that low concentrations of vancomycin may increase the density of newly forming S epidermidis biofilms may indicate an area of potential concern in the use of vancomycin in ocular infections.

Poster Session

BAM-PT1022 - Seasonal changes in microbial diversity and abundance in water and biofilms collected from a pilot scale water treatment plant

Nick Dimas[1], David Scott[2], Marthinus (Otini) Kroukamp[1], Gideon Wolfaardt[1], Martina Hausner[1]
[1]Ryerson University, Toronto, Canada, [2]City of Toronto, Canada

The purpose of this study is to gather data in order to re-evaluate the function and efficiency of active and passive biofilters as well as predict how biofilters act under different water quality conditions. The diversity and abundance of microorganisms in water and biofilms found on granulated activated carbon (GAC) and anthracite used in biofiltration units in a pilot scale water treatment plant will be characterized. Two sampling events will be carried out (winter and summer 2014) to investigate seasonal changes in the microbial populations. Chemical analysis of total organic carbon (TOC) removal and dissolved oxygen (DO) fluctuations indirectly indicate microbial activity. Preliminary results showed that TOC and DO were reduced to a greater extent in GAC biofilters than in those filled with anthracite. Samples will be collected at various sites within the pilot plant, including raw water, biofilms residing on GAC and anthracite in biofiltration units and backwash and treated water. This will help give an idea on microbial population dynamics within the biofilms. Confocal microscopy of both samples will be carried out to visualize the thickness and structure of biofilms. PCR-amplification of 16S rRNA fragments from water and biofilms samples followed by denaturing gradient gel electrophoresis will be carried out to obtain and compare microbial community fingerprints. Standard culture based-techniques for heterotrophic plate counts and coliform enumeration will complement DNA-based and microscopic studies. Finally this research will also take an ecological perspective and analyze the predator-prey relationships which take place on the biofilters. Metezoa including organisms such as rotifers, feed on many of the organisms housed in these biofilms and therefore lessen the microbial diversity downstream throughout the distribution facility.

Poster Session

BAM-PT1024 - Differential protein expression of Leptospira biflexa during biofilm formation

Priscyla Ribeiro[1], Bruno Bastos[1], Larissa Vasconcelos[1], Áurea Almeida[1], Tania Silva[1], Suzana Lima[1], Gregorio Iraola[2], Mitermayer Reis[3], Hugo Naya[2], Paula Ristow[1,3]

[1]Laboratório de Biologia Molecular Carmem Lemos, Instituto de Biologia, Universidade Federal da Bahia, Salvador, Brazil, [2]Unidad de Bioinformática, Institut Pasteur, Montevideo, Uruguai, [3]Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil

Leptospirosis is a widespread zoonosis and an important public health problem, particularly in developing countries. We have previously demonstrated that pathogenic and saprophytic Leptospira species form biofilms in vitro. Biofilms are a common survival strategy for prokaryotes. For Leptospira, biofilm formation might improve survival in the environment or play a role during kidney chronic colonization. We aimed to compare the differential protein expression between Leptospira biflexa sorovar Patoc grown in biofilms and planktonic cells. Cells were cultivated in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium at 29 ºC in glass tubes (biofilms) and in anti-adherent polypropylene tubes (planktonic cells). Leptospires were harvested at 12 h (early biofilm formation), 48 h (mature biofilm) or 120 h (declining biofilm formation), washed, lysed, and sonicated. Protein extracts were analyzed through 10-12.5% SDS-PAGE and stained with silver. We detected 16 bands, ranging from 24 kDa to 160 kDa, for both protein extracts. We observed marked differences in the total protein profile from biofilm and planktonic conditions. The most striking difference was a band with approximately 64 kDa, which was 110% (± 46%) more expressed in biofilm than in planktonic extracts. This protein was expressed in biofilms as soon as with 12 h, and increased its expression with 48 h and 120 h of cultivation. This is evidence that this molecule may play an important role on biofilm formation and maintenance. Leptospires present an immunodominant stress-related chaperone of 60 kDa (GroEL). Orthologs of GroEL are up-regulated during biofilm formation in Streptococcus mutans. Further studies are under way to characterize this protein of 64 KDa, as well as others found in the differential proteome. We hope to contribute to the understanding of the molecular mechanisms involved in biofilm formation of Leptospira, as well as to the biology of this spirochete and the pathogenesis of leptospirosis.

Poster Session

BAM-PT1026 - Isolation, identification and characterization of photosynthetic microorganisms from biofilms of Yohualichan, an archaeological site in Puebla, Mexico

Roxana Olvera-Ramírez[1], César Hugo Hernández-Rodríguez[2], Isela Aguila-Maldonado[1]
[1]Botánica, Esc. Nacional de Ciencias Biológicas, IPN, Mexico, Mexico, [2]Microbiología, Esc. Nacional de Ciencias Biológicas, IPN, Mexico, Mexico

Microbial interaction with the materials and their environment can lead to biodeterioration, a common problem in monuments and archaeological sites. Microorganisms form biofilms, which are communities structured by bacteria, algae, cyanobacteria, fungi and protozoa that are embedded in a polymeric matrix. The aim of this work was the isolation, characterization and identification of photosynthetic microorganisms from biofilms of Yohualichan, an archaeological zone in Puebla, Mexico. Biofilm samples were collected from archaeological monuments in the area known as “Juego de Pelota” (Ballgame), as well as in the East, West and “Las Grecas” buildings. The strains were isolated by using BG-11 medium, solidified with 1.3% bacteriological agar, until obtaining “monoalgal” cultures along with the propagation of isolates in liquid BG-11. Twenty eight “monoalgal” cultures were obtained (eighteen from “Juego de Pelota”, one from the East Building, two from "Las Grecas" and seven from the West Building) grouped into six different orders and twelve genera. Among the microalgae found were Chlorella, Chlorococcum, Chlorokybus, Desmodesmus, Elakatothrix, Fernandinella, Fottea, Klebsormidium and Oocystis; and the cyanobacteria found were Komvophoron, Lyngbya, and Phormidium. In order to detect the presence of exopolysaccharides (EPS) in the seven isolated cyanobacteria toluidine blue staining was used and therefore, it was demonstrated that these strains contained exopolysaccharides which give them protection from environmental changes and which also participate in the biofilms´ formation on rocky materials. Finally, the extraction and quantification of phycobiliproteins was performed in seven cyanobacteria, four of which had a higher amount of phycoerythrin (0.0544 to 0.0663 mg / mL), all of the above suggests that cyanobacteria can grow in conditions of lower light intensity. To conclude it can be said that biofilms found in this archaeological site are primarily constituted by microalgae (75%), and cyanobacteria that produced EPS in order to protect the microbial community.

Poster Session

BAM-PT1028 - Structure-activity relationships for potent broad-spectrum anti-biofilm peptides

Evan Haney[1], Sarah Mansour[1], Kelsey Lee[1], César de la Fuente-Núñez[1], Fany Reffuveille[1], Robert Hancock[1]
[1]*University of British Columbia, Vancouver, Canada*

Biofilms constitute at least 65% of all infections and are adaptively resistant to most classes of antibiotics. The recent observation that short cationic peptides possess potent anti-biofilm activity has spurred interest in these short polypeptide sequences as potential anti-biofilm agents. Not only can these peptides prevent the formation of bacterial biofilms on a surface, but they can also eradicate pre-formed biofilms caused by both Gram positive methicillin resistant Staphylococcus aureus (MRSA) and MDR Gram negative bacteria, including Klebsiella, Pseudomonas, Acinetobacter and E. coli. They also demonstrate strong synergy with conventional antibiotics. Conceptually, anti-biofilm peptides share certain characteristics with antimicrobial peptides including a net positive charge and high content of hydrophobic amino acids. However, anti-biofilm peptides work against Burkholderia sp. which are completely resistant to antimicrobial peptides, and there is no correlation between anti-biofilm activity and antimicrobial activity versus planktonic cells. As a result, little is known about the sequence/structure requirements that contribute to the anti-biofilm activity of a peptide. To address this issue, we used SPOT synthesis of peptides on cellulose sheets to systematically perform single amino acid substitutions of nine different residues into three different 12 amino acid peptides: 1018, 1002 and HH2. In total, more than 300 peptide sequences were evaluated for their anti-biofilm activity against MRSA. The amount of biofilm grown in the presence of peptide was quantified using crystal violet staining. Those substitutions that resulted in enhanced anti-biofilm activity compared to the parent sequence were used to inform the synthesis of second generation peptides with enhanced anti-biofilm activity. Additionally, this second generation of peptides were structurally characterized and their interactions with various biomolecules were measured to understand the factors that contribute to their anti-biofilm activity.

Poster Session

BAM-PT1030 - Antimicrobial activity and inhibitory effect of alveolar bone resorption by a toothpaste containing Pycnogenol®

Takenori Sato[1], Toshizo Toyama[1], Shun-suke Takahashi[2], Nobushiro Hamada[1]
[1]Department of Microbiology, Kanagawa Dental University Graduate School, Yokosuka, Japan, [2]Department of Oral Science, Kanagawa Dental University Graduate School, Yokosuka, Japan

Objectives: Pycnogenol® (PYC) is a phytochemical extracted from the bark of the French maritime pine tree (*Pinus maritima* Aiton). In this study, we investigated antimicrobial activities of a toothpaste containing PYC against *Streptococcus mutans* and *Porphyromonas gingivalis*. In addition, we evaluated the inhibitory effect of alveolar bone resorption in rat. Materials and Methods: The antimicrobial activities of the toothpaste containing PYC against *S. mutans* and *P. gingivalis* were assessed by using an agar diffusion test. Bacterial growth inhibition assay of PYC was evaluated the optical density of bacterial suspensions at 3, 6, 12 and 24 hours. The inhibitory effect of alveolar bone resorption was determined as follows. Seven-week-old male Sprague Dawley rats were orally administered with the toothpaste containing PYC. Each rat received 0.5 ml of the toothpaste by oral gavage at 24-hours interval. Sham-infected control rats were administered 5% carboxymethylcellulose only. The levels of horizontal alveolar bone resorption around the maxillary molars were examined by digital high-definition system and micro-computed tomography at 15 weeks of age. The osteoclasts around maxillary molars were evaluated by a tartrate-resistant acid phosphatase (TRAP) staining. The experimental procedures of this study were reviewed and approved by the Committee of Ethics on Animal Experiments at Kanagawa Dental University Graduate School and were carried out under the guidelines for animal experimentation. Results: The toothpaste containing PYC have antimicrobial activities against *S. mutans* and *P. gingivalis*. The level of alveolar bone resorption in rats administered with the toothpaste containing PYC was significantly lower than that in sham-infected control. The number of osteoclasts at the maxillary molars was decreased by the toothpaste containing PYC. Conclusion: The toothpaste containing PYC had an antibacterial activity against oral bacteria and inhibitory effects on osteoclastogenesis, thus it may be useful for oral health and prevent alveolar bone resorption.

Poster Session

BAM-PT1032 - Contribution to the phenotypic and genotypic study producing bacteriocins strain isolated from Algerian raw goat milk

Noura Benhamouche[1]
[1]*Universite Ustomb*

Lactic acid bacteria are known for their capacity to produce inhibitory substances against the unwanted bacteria involved in food poisoning such Listeria innocua , St.aureus. The search for species of lactic acid bacteria can inhibit harmful germs is the subject of this work. Microbiological techniques of confrontation on solid medium and the effect of the substance on the growth of Listeria innccua were performed. The search for bacterial antagonism was achieved in the solid medium by the method of double-layer method and the wells, the whole experience was conditioned in a buffered medium to eliminate the effect of acidity Results shows that 7 isolates gave an antagonistic action vis-à-vis of Listeria innocua. The strain of Lactococcus lactis (8b), isolated from raw goat's milk showed an anti-listeria in vitro and was selected because of its ability to inhibit as Staphylococcus aureus. With a diameter of 15mm, the action of proteolytic enzymes, trypsin, chymotrypsin is shown that the substance was a protein, the kinetics of growth in milk medium showed that the number of St. aureus after 24 h incubation was reduced to 7.68 log cfu in mixed culture with strain 8b which was comparable to the control of 9.14 log cfu, which signifies that the survival percentage was 3.4%, phenotypic characteristics (biochemical and physiological) showed that the strain was a Lactococcus lactis sub sp lactis against the results based on molecular ADNr16Ss showed that the strain is an Enterococcus faecium, duran, hirae. The sequencing results show that Leuconostoc mesenteroides and Enterococcus faecium were the dominant summers anti-Listeria species in samples of goat milk Algerian. The isolates had the potential of multiple bacteriocin production and do not present some important elements of virulence. Key words: Bacteriocins, goat milk, Listeria, St. aureus,ADNr16s,PCR/RAPD

Poster Session

BAM-PT1034 - Poly-gamma-glutamic acid (γ-PGA) – a promising biosorbent for removal of heavy metals

Adetoro Ogunleye[1], Iza Radecka[1], David Hill[1], Craig Williams[1]
[1]*University of Wolverhampton, Wolverhampton,UK*

Poly-gamma-glutamic acid (γ-PGA), an unusual natural anionic biopolymer composed of D- and/or L-glutamic acid units polymerized through amide linkages between α-amino acid and γ–carboxylic acid groups, was synthesized by three bacterial strains – Bacillus subtilis (natto), Bacillus licheniformis 9945a and Bacillus licheniformis 9945. Three culture media - one containing glycerol, citric acid and L-glutamic acid as carbon sources, another, having citric acid, sucrose and L-glutamic acid as carbon sources and the third one with sucrose and L-glutamic acid as its sources of carbon were used in this study. Each strain produced γ-PGA extracellularly when grown aerobically in one or all three media. The biopolymers produced were identified as γ-PGA by Fourier transform infrared spectroscopy (FTIR). The effects of different fermentation temperatures (37℃ and 50℃) and media on bacterial growth, production and molecular weight of γ-PGA were investigated. The metal binding affinity of γ-PGA was also studied and it was found that it binds heavy metals. The optimal γ-PGA yield of 11.45g/l as well as the highest molecular weight of 1,650 kDa was obtained when Bacillus subtilis (natto) was grown aerobically at 37℃ for 96 hours in a culture medium having citric acid, sucrose and L-glutamic acid as carbon sources.

Poster Session

BAM-PT1036 - Stimulation by yeast extract on growth of fungi MGS-2 in defined medium containing organic acids of peatsoil

Diana Nurani[1], Koesnandar Sastrawijono[1]
[1]*Agency for the Assessment and Application of Technology, Jakarta, Indonesia*

Some organic acids as a result of lignin degradation during peatsoil formation has been shown to have significant role in lowering pH of peatsoil and has phytotoxic effect on plants. Some microbes have been selected to grow in the medium containing organic acids extracted from peatsoil. The focus of the study was to optimize the medium that favorable for the growth of fungi in organic acids. The selected fungi MGS-2 was grown in defined medium containing organic acids of peatsoil, nitrogen sources and salt. At initial pH of 3.8. The cell growth, culture pH and content of organic acid were analyzed. The identification of isolated fungi was based on the 28S rDNA. Molecular identification revealed that the MGS-2 was closed to Hypocrea sp. The organic acid and nitrogen optimum for the growth were 33.1 mN-NaOH and 2 g NH2SO4 per liter medium, respectively. The interaction between carbon and nitrogen source was found to be significantly influenced by the increment of culture pH, however did not effect to the cell growth and decrease of organic acids. The carbon source affected significantly the cell growth and acid metabolism by MGS-2. The fungi could not grow well in the medium without yeast extract , but grew well in the limitation of NH2SO4, suggested that yeast extract was not only use as amino acid but also metabolized as nitrogen source. The mass production of MGS-2 for peatsoil treatment is under investigation.

Poster Session

BAM-PT1038 - The role of NUDIX hydrolase in biosynthesis of Poly-β-Hidroxybutyrate and alkylresorcinols in Azotobacter vinelandii

Leidy Bedoya[1], Soledad Moreno[1], Guadalupe Espin[1]
[1]*University National Autonomous of Mexico, Biotechnology Institute, Cuernavaca-Morelos, Mexico*

Azotobacter vinelandii is a nitrogen-fixing soil Gram-negative bacterium that synthesizes polyhydroxybutyrate (PHB), a biopolyester of industrial interest to produce biodegradable plastic. The enzymes necessary for PHB synthesis are encoded by phbBAC operon and its transcription is activated by PhbR. Furthermore, A. vinelandii can undergo a differentiation process to produce resistant desiccation cysts. During the encystment, the bacterium synthesizes phenolic lipids called alkylresorcinols (ARs) which replace the membrane phospholipids. Synthesis of PHB and ARs is abrogated in strain carrying a ptsP mutation, ptsP gene encoding EI-NTR of PTS-NTR regulatory system. Upstream and transcribed in the same direction of ptsP is nudH gene that encoding NudH, a member of NUDIX - nucleoside diphosphate linked to another moiety X- hydrolase superfamily, that occur in all kingdom of life and are most commonly pyrophosphohydrolases active on substrates with the structure NDP-X and remove pyrophosphate present in 5' of some messenger RNAs, allowing their degradation by RNase E. Our aim is to characterize the role of NudH in the biosynthesis of PHB and ARs in A. vinelandii. By using RT-PCR we determined that nudH and ptsP genes are transcribed as operon. A mutation on nudH (allowing transcription of ptsP) reduced the accumulation of PHB and ARs as compared to the wild type strain. Additionally, using transcriptional and translational fusions of phbB and phbR with gusA reporter gene, we determined that the nudH mutation had a negative effect mainly at post-transcriptional level of both phbB and phbR expression. These findings, allowed us hypothesize that NudH contributes to the degradation of a mRNA that encodes a negative regulator of phbB and phbR expression.

Poster Session

BAM-PT1040 - Identification of Pseudomonas fluorescens for the degradation of chitin

Azhar Alhasawi[1], Vasu. D Appanna[1]
[1]*Laurentian University, Sudbury, Canada*

Chitin, the second most abundant bio-polymer in nature after cellulose, composed of β (1→4) linked N-acetylglucosamine (GlcNAc), is a major structural component in the cell walls of fungi and the shells of crustaceans. Chitin and its derivatives are gaining importance of economic value due to its biological activity and its industrial and biomedical applications. There are several methods to hydrolyze chitin to NAG, but they are typically expensive and environmentally unfriendly. Chitinase which catalyzes the breakdown of chitin to NAG has received much attention owing to its various applications in biotechnology. The presented research examines the ability of the versatile soil microbe, Pseudomonas fluorescens grown in chitin medium to produce chitinase and a variety of value-added products under abiotic stress. We have found that with high pH, Pseudomonas fluorescens enable to metabolize chitin more than with neutral pH and the overexpression of chitinase was also increased. P-dimethylaminobenzaldehyde (DMAB) assay for NAG production will be monitored and a combination of sodium dodecyl and blue native polyacrylamide gels will be used to monitor the proteomic and metabolomic changes as a result of the abiotic stress. The bioreactor of chitinase will also be utilized.

Poster Session

BAM-PT1042 - Development and analysis of a mathematical model for growth of anaerobic acidogenic microbial communities

Gustavo Mockaitis[1], Guilherme Peixoto[2]

[1]São Carlos Engineering School, University of São Paulo (EESC/USP), São Paulo, Brazil, [2]ulty ofDepartment of Bioprocesses and Biotechnology, Fac Pharmaceutical Sciences, São Paulo State University (FCFAR/UNESP), São Paulo, Brazil

The present work shows the development and analysis of a mathematical model to describe the growth of selected anaerobic acidogenic communities. Usually, the pattern of the growth curve for microbial populations has a sigmoidal form (as observed in known models like Boltzmann, logistic, Gompertz, Richards, Schnute, and Stannard). However, these models are not always accurate, and only describe an aspect and phase of the growth behavior of some mixed cultures, especially when there is another growth pattern after the first one (as having another community growing afterwards). The developed model parameters allow for determining parameters like start and end time for the exponential growth phase and the maximum growth rate could be obtained from the values of the parameters of the model. This model could be a powerful tool to analyze the microbial growth and associated processes such as substrate consumption or product production when two different growth patterns occurs in the same culture.

Poster Session

BAM-PT1044 - Two transketolase and transaldolase isogenes from Moniliella megachiliensis respond in a different way to osmotic and oxidative stress

Hisashi Iwata[1], Daiki Mizushima[1], Yuki Ishimaki[1], Jun Kato[1], Jun Ogihara[1], Takafumi Kasumi[1]
[1]Department of Chemistry and Lifescience, Nihon University, Fujisawa, Japan

Moniliella megachiliensis SN-124A is an extremely osmo-tolerant basidiomycetous yeast that can grow even in 60% glucose solution. Unlike other yeasts such as Saccharomyces cerevisiae that produce glycerol, M. megachiloiensis produces considerable amounts of erythritol when growing in an osmotic stress environment. In this study, we investigated the gene structure and specific function of two transketolase and transaldolase isoforms derived from M. megchiliensis (MmTKL and MmTAL). Specifically, we focused on stress response and polyol biosynthesis in order to better understand microbial carbohydrate metabolism. We cloned and sequenced two transketolase (MmTKL1, MmTKL2) and two transaldolase (MmTAL1, MmTAL2) genes from M. megachiliensis. The amino acid sequences encoded by these two genes showed 68% homology between the two MmTKLs and 72% homology between the two MmTALs. An AP-1 (ap response element) associated with oxidative stress was present in the promoter region of MmTKL2 and MmTAL1. STRE (stress response element) is involved in osmotic stress response. No AP-1 was present in the promoter region of MmTKL1 and MmTAL2. In early-stage response cultivation (up to 2 h), MmTKL2 and MmTAL1 were specifically expressed in response to oxidative stress (0.15 mM menadione), whereas MmTKL1 and MmTAL2 were expressed in response to osmotic stress (1.2 M NaCl). Erythritol accumulated intracellularly in response to oxidative stress, and glycerol accumulated intracellularly in response to osmotic stress. From these results, we concluded that M. megachiliensis selectively uses two isogenes and produces either erythritol or glycerol during early-stage response to stress, depending on the type of environmental stress.

Poster Session

BAM-PT1046 - Improvement of counterselection technique following intergeneric conjugation with actinomycetes using a donor strain auxotrophic for diaminopimelic acid (DAP)

Nancy Allard[1], Dominic Poulin-Laprade[1], Mariana Gabriela Ghinet[1], Vincent Burrus[1], Ryszard Brzezinski[1], Sébastien Roy[1]

[1]*Centre d'étude et de valorisation de la diversité microbienne, Université de Sherbrooke, Sherbrooke, Canada*

Actinomycetes comprise a large diverse group of Gram-positive, filamentous soil bacteria renowned as the most important producers of antibiotics and other secondary metabolites. In view of the ecological, medical, biotechnological and economic importance of actinobacteria, the development of methodologies and techniques for genetic manipulation of a wide range of these industrially important microorganisms is needed. Genetic analysis and manipulation of *Streptomyces* species have been greatly facilitated by the development of protoplast transformation procedures. However there is no universal procedure for protoplast transformation and protocols must be empirically optimized for each species. As an alternative, the intergeneric conjugation with methylation-deficient *Escherichia coli* has often been used more efficiently to introduce foreign DNA in a variety of actinomycete species. Unfortunately, despite the progress made in these conjugation-based methodologies, there are several inherent limitations, such as the ability to counterselect the recipient exconjugants against the donor strain. Nonetheless, the natural resistance of many actinobacteria against nalidixic acid is generally exploited to eliminate the sensitive *E. coli* donor strain following conjugation. Since some species of actinobacteria are sensitive to nalidixic acid, we developed a post-conjugational conterselection technique using an E. coli donor strain auxotrophic for diaminopimelic acid (DAP), to circumvent the use of antibiotic. These *dap*-negative mutants require exogenously added DAP, since it is an essential peptidoglycan cross-linking agent in the bacterial cell wall. As a result, counterselection can be easily achieved on most rich, complex media without unsettling the recipient strains. This method has been used successfully with several *Streptomyces* and Frankia strains and could be easily used with a wide range of other Gram-positive bacteria of biotechnological potential.

Poster Session

BAM-PT1048 - Cell growth and alcohol production is differently dependent on the level of agitation and glucose concentration in Dekkera bruxellensis

Beatriz Cristina Pecoraro Sanches[1], Ana Paula Guarnieri Bassi[1,2], Vanda Renata Reis[2], Anna Lívia Paraluppi[1], Sandra R. Ceccato-Antonini[1]

[1]DTAiSER Centro de Ciências Agrárias, Universidade Federal de São Carlos, Via Anhanguera, São Paulo, Brazil, [2]Post-Graduation in Food Science and Technology, ESALQ/USP, São Paulo, Brazil

Strains of Dekkera bruxellensis have been found to impair the ethanolic fermentation in Brazilian industrial conditions. Recent reports pointed out for their growth ability in fermentation conditions, acid production and poor fermentative capacity, resulting in low ethanol productivity. In this work we evaluated the effect of different glucose concentrations (50, 100 and 150 g/L) and level of flask agitation (0, 150 and 250 rpm) in a synthetic medium upon the growth (monitored by optical density), acid (variation of pH) and alcohol production utilizing a strain of D. bruxellensis (CCA155) isolated from the fermentation tank. The flasks containing the yeast (108 cells/mL) and 200 mL of medium were maintained at 30oC for 96 h. The growth was higher in concentrations of 50 and 100 g/L of glucose and in shaken flasks (150 and 250 rpm) by 48 h of cultivation. By the other hand, the alcohol production was higher in concentrations of 100 and 150 g/L of glucose but without flask agitation. However, the highest alcohol concentration was achieved in 96 h of fermentation. The pH varied from 4.5 to 2.0-2.5 in 24 hours of cultivation regardless the sugar concentration and level of agitation. Glucose was totally consumed after 48 hours of cultivation at initial concentration of 50 g/L. Considering the conditions of Brazilian industrial fermentation for fuel alcohol production, i.e., cell recycling after a period of 8-12 h of fermentation, without aeration, with sugar concentration ranging from 200-250 g/L, the contribution of D. bruxellensis for the ethanol production would not be relevant but the growth would be greatly enhanced due to the cell recycling along with the starter yeast Saccharomyces cerevisiae. The acids produced by D. bruxellensis may affect the fermentation as well. The work will proceed using sucrose as carbon source. Support: FAPESP (2011/17928-0 and 2012/16258-4) and CNPq.

Poster Session

BAM-PT1050 - Change in carbon and nitrogen source; effect on Aspergillus carbonarius morphology and raw starch digesting amylase production

Onyetugo Amadi[1], Bartho Okolo[1]
[1]*Microbiology Department, University of Nigeria, Enugu, Nigeria*

In submerged fermentation filamentous fungi are able to exhibit diverse morphological forms, which subsequently influence metabolite yield. Change in medium composition is one of the several factors that influence morphology and metabolite production. Aspergillus carbonarius a fungus isolated from rotten cassava is able to produce copious amount of the enzyme raw starch digesting amylase. Studies were carried out to determine the effect of change in carbon and nitrogen source on morphology of Aspergillus carbonarius and raw starch digesting amylase production. Cassava was substituted for corn while yeast extracts for KNO3. Mean convex perimeter of clumps/pellets was used to characterize mycelia morphology. Amylase activity, protein content, biomass concentration and pH were determined. Change in medium composition greatly influenced the morphology of the fungus. Mycelial trees were entangled to form clumps without a compact structure and visible vacuoles. Mean convex perimeter of clump increased with time and dropped by 96h. Maximum RSDA activity of 40.9U/ml was indicated at 72h and 18.2U/ml at 144h of fermentation. There was a slight increase in protease activity at 48h; biomass level increased continuously all through the fermentation. While total protein increased steadily reaching a peak of 0.035mg/ml at 120 h, pH of the medium was acidic at 3.39 within 48h after an initial pH value of 5.32. On the other hand medium with cassava and yeast extract revealed mycelia morphology to be predominantly pellets RSDA yield was higher with a maximum yield of 403U/ml. Medium composition influenced morphology and yield of RSDA.

Poster Session

BAM-PT1052 - Purification and characterisation of two distinct amylases of Geotrichum species

Rosemary Ugwuanyi[1], Odibo Ferdric[2]
[1]*Microbiology, Enugu, Nigeria*

Two distinct forms of amylases were produced by Geotrichum sp in a medium containing 1.5% (w/v) cassava starch and 1% (w/v) soybean meal. Optimum amylase production occurred during the late logarithmic phase (54h) and corresponding with a culture pH of 5.4. Amylases I and II were purified 10.27- and14.27-fold, respectively by dialysis against 5M sucrose solution, ion-exchange chromatography on Q-Sepharose and gel filtration on Biogel P4. Both enzymes were optimally active at 40oC and retained 90% of their original activity at 70oC for 1h. Amylases I and II were maximally active at pH 5 and 6, respectively. Both enzymes were activated by Mn2+ but were markedly inhibited by Hg2+ and EDTA. Amylases I and II had km values of 9.8x10-2 and 6.7x10-2 mg/ml, respectively. Both enzymes hydrolysed various amylaceous polysaccharides producing maltooligosaccharides. Both enzymes also had inulinase activity.

Poster Session

BAM-PT1054 - Purification, characterization and preliminary crystallization of a basic peroxidase from a Nigerian sorghum variety, SK 5912

Chukwudi Nnamchi[1], Bartholomew Okolo[1], Anene Moneke[1], Chima Nwanguma[2], Gary Parkin[3], Igor Efimov[3], Jaswir Basran[4]

[1]Department of Microbiology, University of Nigeria, Nsukka, Nigeria, [2]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria, [3]Department of Chemistry, University of Leicester, Leicester, UK, [4]Department of Biochemistry, University of Leicester, Leicester, UK

Towards the determination of its properties, a cationic peroxidase was purified to homogeneity from SK5912, an important brewing grade sorghum variety obtained from Nigeria. Pure fractions of the enzyme were obtained by sequential fractional precipitation with ammonium sulphate and thereafter, anion and cation exchange chromatography. The peroxidase had a soret peak at 402 nm (ε = 119 mm-1 cm-1) and was found by SDS PAGE to have a molecular mass of about 35.5 KDa. A similar molecular mass was obtained when the purified protein was subjected to mass spectroscopy with the spectrum from MALDI-tof peaking at 35.571 while that from ESI peaked at 35.65 KDa. Web based mowse search using these molecular weights confirmed the sorghum peroxidase identity of the enzyme. The enzyme showed optimum activity at pH 5; was active at a wide pH range of 3 to 11 and remained remarkably stable at pHs 6 to 11. It was also stably active at temperatures of up to 80oC. Steady state kinetics study of the enzyme with several organic compounds including guaiacol (Kcat/Km value = 6.76 ± 0.1 mMS-1), ortho-dianisidine (Kcat/Km value = 449 ± 4 mMS-1), pyrogallol (Kcat/Km value = 43.941 ± 0.8 mMS-1), ABTS (Kcat/Km value = 12.56 ± 0.2 mMS-1) showed varying levels of preference for the compounds. Sk5912 sorghum peroxidase had a low reduction potential E of -266 mV NHE with phenosafranin. It bound to the inhibitors potassium cyanide (Kd = 11.9 μM) and sodium azide (Kd = 19. 4 μM) but not to sodium fluoride. Stopped flow experiments with the enzyme and H2O2 produced an intermediate with the features of compound I. Epr data obtained for the peroxidase showed that it has a high spin. Tentative crystal structure of the peroxidase was obtained after initial crystal screening and subsequent refining using standard methods.

Poster Session

BAM-PT1056 - Identification of thrasustochytrid strain 12B as a new species of Aurantiochytrium

Ahmad Iskandar Bin Haji Mohd Taha[1], Hitomi Ando[1], Zakia Perveen[1], Kiyohito Yoshida[1], Mamiko Sato[2], Motoaki Tojo[3], Kazuhiro Kogame[1], Tadashi Nagamine[4], Hidetoshi Okuyama[1]
[1]Hokkaido University, Sapporo, Japan, [2]Japan Women's University, Tokyo, Japan, [3]Osaka Prefecture University, Sakai, Japan, [4]Rom Company Limited, Sapporo, Japan

The thraustochytrid strain 12B, which was isolated from the mangrove area of Okinawa, Japan, has been recognized as an unidentified microorganism producing very high levels of docosahexaenoic acid (DHA). It had a lipid content of 58% with DHA at 43% of total fatty acids and its DHA production rate is approximately 3 g/L/day, when grown in commonly used culture media containing glucose, peptone, and yeast extract in 50% seawater. Here we conducted molecular, physiological, and morphological studies to identify strain 12B. Strain 12B exhibited the presence of amoebic stage, the disintegration of the cell wall during zoospore releasing, and the rare presence of ectoplasmic network, all indicating that strain 12B belongs to the genus of Aurantiochytrium. Moreover, phylogenetic analysis of the 18S rRNA gene and mitochondrial cytochrome oxidase II gene sequences showed that strain 12B was grouped together well with other Aurantiochytrium species such as A. limacinum and A. mangrovei and that strain 12B was highly related to A. limacinum strain SR21. However, there were several phenotypic differences between strain 12B and A. limacinum strain SR21: the coloration of colonies on agar plate; the rate of glucose utilization; and the structure of tubular hair on the anterior flagellum of zoospore. In addition, cell fusion, which had never been reported in any other thraustochytrid species, was observed in strain 12B. Taken together we conclude that strain 12B could be identified as a novel species of Aurantiochytrium. Productivity advance of DHA in this Aurantiochytrium species when cultured in modified media is also presented.

Poster Session

BAM-PT1058 - Application of Rhodobacter sphaeroides in the treatment of corn pap wastewater for reduction of environmental pollution

Ifeoma Mbachu[1], Chima Mbachu[2]
[1]Department of Microbiology, Anambra State University, Uli, Nigeria, [2]Department of Electrical and Electronic Engineering, Anambra State University, Uli, Nigeria

Abstract: Corn pap processed from corn starch fermentation is a staple food in Nigeria. The wastewater from its processing is always disposed into gutters where it ferments further, producing offensive odor. Rhodobacter species have been isolated from these gutters and reports have shown they possess ability to convert sulfide to sulfate during photolithoautotrophic growth. We hypothesize that Rhodobacter sphaeroides may reduce chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of corn pap wastewater (CPW) and that nutritional content of corn pap wastewater is capable of supporting increase in biomass of Rhodobacter sphaeroides. In determining these facts, 125ml of mineral salts-succinate broth was inoculated with 6.25ml of water sample collected from gutter in Awka, Nigeria. Cultural characteristics included reddish turbidity in broth culture and rosette bunch colonies on solid agar medium. Motility was negative, Gram stain negative and sulfide oxidation was sulfate, confirming the isolate to be Rhodobacter sphaeroides by Bergey's criteria. Initial BOD and COD of the CPW are 5,100mg/l and 8,250mg/l respectively. Four treatments were given to the CPW. The first treatment, sterile corn pap wastewater (SCPW) without inoculation with R. sphaeroides, served as control and the percentage reductions of its BOD and COD are 0% each. In the other treatments, percentage BOD and COD reductions are 60% and 48% for raw corn pap wastewater (RCPW) without inoculation with R. sphaeroides, 96% and 90% for RCPW inoculated with R. sphaeroides, 96% and 90% for SCPW inoculated with R. sphaeroides respectively. Based on an initial biomass dry weight of 0.25g, increase in biomass dry weight was observed and it was 64%, 66%, and 69% for RCPW, RCPW inoculated with R. sphaeroides and SCPW inoculated with R. sphaeroides respectively. R. sphaeroides is effective for reducing environmental pollution. Keywords: R. sphaeroides, corn pap wastewater, treatment.

Poster Session

BAM-PT1060 - Enzymatic and antimicrobial screening of Bacillus isolated from Bataan mangrove soil against human pathogens

Imelda Quiban[1], Reynaldo Estacio[1], Lilibeth Lumapas[1]
[1]*DepEd Makati, National Capital Region, Philippines*

Bacillus isolated from mangrove soil samples were collected from the different locations in Barangay Tortugas, Balanga City, Bataan, Philippines. These were screened for their enzyme-producing capacity and their antimicrobial activity against most common human pathogens. An Amylase activity was detected after growth on soluble starch agar plates by a hydrolysis zone around the colony using iodine staining. Lipase activity was detected after growth on peptone agar media by formation of whitish precipitate-zone using copper sulfate staining. Futhermore, the screening of thirty-five bacteria isolated from Bataan mangrove soil for specificity they exhibited relative to the reactions they catalyzed shows 27 of the isolates have amylolytic activity while only 6 isolates show a potential for lipase production in vitro, which illustrate their potential use in commercial applications. Moreover, primary antimicrobial screening by Agar Plug method was done on a new thirty-five isolates against Gram-positive Staphylococcus aureus, Gram-negative Escherichia coli, and yeast fungus Candida albicans as test organisms. As observed five isolates were active against Gram-positive bacteria, nine against yeast and none against Gram-negative bacteria. Ten of the putative isolates were further subjected to secondary screening by Cup Cylinder method to test their capabilities on inhibiting these pathogenic microorganisms on the basis of zone of inhibition. The results show a positive zones of inhibition of bacillus which is an indication of its antimicrobial effects against S. aureus and C. albicans. Thus, considering all of the above evidences and comparing with the current literature, these screening studies open an avenue to work with some of the potent strains of bacteria from Bataan mangrove soil for the different biotechnological application at large scale using microbial enzymes in lieu of synthetic chemicals. The antimicrobial capacity of bacillus could also be a potential source of a broad-spectrum in treating different diseases caused by these pathogenic microorganisms.

Poster Session

BAM-PT1062 - Isolation and characterization of a potent N2O-quencher, Chitinophaga sp. from an oil palm plantation on peat in Sarawak, Malaysia

Sharon Yu Ling Lau[1,2], Lulie Melling[1], Yasuyuki Hashidoko[2]
[1]Tropical Peat Research Laboratory Unit (Chief Minister's Department of Sarawak), Malaysia, [2]Graduate School and Research Faculty of Agriculture, Hokkaido University, Japan

Nitrous oxide reductase (N₂OR) encoded by *nos* gene is the enzyme responsible in reduction of nitrous oxide (N₂O) in the final step of denitrification and is reported to be the only conduit for N₂O degradation. The biochemistry of this process has been studied extensively in denitrifiers such as *Paracoccus denitrificans* and *Bradyrhizobium japonicum*. With the potential of biology-based strategies for N₂O mitigation, search is also extended to non-denitrifiers such as *Anaeromyxobacter* sp. that possess the *nosZ* gene. We successfully isolated potent N₂O-quenchers, identified as *Burkholderia* sp. and *Chitinophaga* sp. from soils collected at various depths from an oil palm plantation on peat in Sarawak, Malaysia. *Chitinophaga* sp. showed an extraordinary N₂O quenching activity and was able to eliminate as much as 3000 ppmv (atmospheric level at 300 ppbv) of the supplemented N₂O in the headspace (22.57mL) within 3 days. Furthermore, when subjected to the 10% acetylene inhibition assay, no acceleration of N₂O production was observed. Additionally, the *nosZ* gene was not detected by PCR assay, using degenerate primer sets for both typical and atypical *nosZ* genes suggesting that its ability of N₂O quenching may not be through the exclusive N₂OR. Therefore, the whole-genome sequence of *Chitinophaga* sp. was determined using an Illumina HiSeq pyrosequencer and unassigned ORFs were annotated to conduct a BLAST search for N₂O-quenching genes, including the periplasmic N₂OR family proteins, gas transporters and channel proteins that may be involved in N₂O quenching. The pyrosequencing study will reveal whether N₂O quenching activity of *Chitinophaga* sp. is associated with unknown-type N₂O reductase or other redox mechanisms.

Poster Session

BAM-PT1064 - Impact of H2 on soil microbial diversity and physicochemical properties

Sarah Piché-Choquette[1], Philippe Constant[1]
[1]*INRS Institut Armand-Frappier, Laval, Canada*

Nitrogen enrichment of soil by nodules isn't the only advantage of crop rotation. Studies showed that H2 (dihydrogen), one of nitrogenase's obligate by-products, also enhances plants growth. It is suggested that H2 diffusion in soil enriches H2-oxidizing bacteria, including species affecting plant growth positively. This implies that H2 has direct effects on plant-bacteria relationships. However, we suspect that H2 also has indirect effects on plant growth. H2-oxidizing bacteria would be beneficial to plants by interacting with other microorganisms involved in soil biogeochemical cycles. The objective of this study is to determine the impact of H2 on the microbiogeochemical structure of soil. Microcosms have been exposed to either 500ppmv H2, to mimic the exposure near nodules, or 0,5ppmv H2, to simulate exposure to atmospheric concentrations. Microbiological and physicochemical analyses were performed throughout the 35-day incubation. The activity of H2-oxidizing bacteria has been evaluated by gas chromatography. Exposure to high concentrations of H2 led to a lower capacity to oxidize low concentrations of H2 over time. Bacterial species most favored by 500ppmv H2 are probably not able to oxidize low concentrations of H2. Afterwards, the use of 31 carbon sources was measured with Ecoplates. Distances matrixes were formed to compare metabolic profiles between samples. Dendrograms and heatmaps were created from these results to demonstrate that microorganisms exposed to low concentrations of H2 partly lost their catabolic diversity while microorganisms exposed to 500ppmv H2 didn't. DNA samples extracted from the microcosms are currently being sequenced with the Illumina platform for ribotyping. Molecular Ecological Networks (MENs) generated with these results will allow the identification of microbial mutualistic and competitive interactions under the influence of H2, leading to a better understanding of biogeochemical changes beneficial to plants.

Poster Session

BAM-PT1066 - Microbial community responses to simulated N deposition at the Mer Bleue Bog, Eastern Ontario

Galen Guo[1], Tim R. Moore[2], Jill L. Bubier[3], Tuula Larmola[3], Nathan Basiliko[1]
[1]Laurentian University, Greater Sudbury, Canada, [2]McGill University, Montreal, Canada, [3]Mount Holyoke College, South Hadley, United States

Peatlands accounts for just 3% of the Earth's terrestrial surface but have stored up to 550 pg of carbon over the Holocene. Flooded, anoxic conditions and nutrient poor soils that have facilitated sequestration of CO2 also lead to the production and emissions of the very potent greenhouse gas, CH4. Human activities such as agriculture and fossil fuel burning have increased reactive atmospheric N to record levels over the past century. N deposition can provide a limiting nutrient to these systems, and although net primary production increases, high N deposition has also been associated with plant community shifts and increased decomposition rates due to less chemically recalcitrant and nutrient poor litters, as well as potentially greater rhizosphere activity. In other wetlands, excess mineral soils N have been shown to enhance nitrification and denitrification as well as affect methane oxidation. Therefore increased N deposition has the potential to lead to greater emission of multiple greenhouse gases (CO2, CH4 and N2O) via the disruption of natural microbial communities and functioning. By examining peat soils from replicated plots in the longest running simulated chronic N deposition experiment in a peat bog (Mer Bleue, near Ottawa where 0, 5, 10, and 20 times ambient N has been added over the past 14 years with and without other nutrients), our objective is to characterize how nitrogen deposition and resulting observed vegetation shifts impact diversity and abundance of broad-spectrum microbial decomposers using community fingerprinting (T-RFLP), high throughput DNA sequencing, and qPCR of SSU rRNA genes. Functional genes involved in nitrification, denitrification, and methane oxidation will also be characterized with qPCR and DNA sequencing, and linkages established between functional-gene-defined communities and activities measured in short-term controlled soil incubations.

Poster Session

BAM-PT1068 - Effect of the liquid to solid cultivation shift on the viability ofEscherichia coli OW6 cells

Jin Sakamoto[1], Kota Nishitani[1], Hiroyuki Sasaki[1], Yusuke Inoue[1], Tetsuaki Tsuchido[1,2,3], Masakazu Furuta[2]
[1]Kansai University, Suita, Japan, [2]Osaka Prefecture University, Sakai, Japan, [3]TriBioX Laboratories Ltd., Kyoto, Japan

When cells of E. coli NBRC 106482 (OW6), a proline-requiring derivative of K12 W3110 were grown to logarithmic phase in EM9 (M9 medium supplemented with 0.1% Casamino acids) broth and placed on EM9 plates containing 1.5% agar with the same composition after washing, the resultant viability was slightly decreased. We designated this phenomenon as the liquid to solid cultivation shift effect (L-S shift effect). Under the same conditions, the viability of its oxyR mutant was much more decreased by the shift. However, no or almost no reduction in viability for either strain was observed when plates containing agar at a low concentration of 0.75% was used. The addition of sodium pyruvate to the 1.5% agar plates suppressed the reduced viability. Furthermore, the L-S shift effect was not observed in cells grown anaerobically. These facts suggest that the L-S shift may cause oxidative stress on E. coli OW6 cells. When using the E. coli oxyR strain of the NBRP (National BioResource Project) Keio collection, the degree of the reduction in the viability on 1.5% agar was rather decreased, indicating that the degree of the L-S shift effect is dependent upon the strain. We also examined the L-S shift effect using the OW6 strain with knockouts in genes for the oxyR regulon, but found none. In conclusion, the L-S shift effect should be taken into consideration for the estimation of viable E. coli cells.

Poster Session

BAM-PT1070 - Identification of a biomarker for the soil uptake of atmospheric carbon monoxide

Isabelle Lalonde[1], Philippe Constant[1]
[1]*INRS Institut Armand-Frappier, Laval, Canada*

Carbon monoxide (CO) is considered as an indirect greenhouse gas because it is competing against methane for hydroxyl radicals (OH•), the « cleansing molecule » of the troposphere. Indeed, an increase in the concentration of CO would decrease the amount of OH• available for methane oxidation, resulting to a positive feedback on global warming. OH• is the main sink for CO, eliminating 85% of global emissions in the atmosphere, but soil bacteria also play a significant role by eliminating the remaining 15%. Identification and characterization of these bacteria is of critical importance to predict if the capacity of soil to consume CO will continue to mitigate CO emissions in future. For this purpose, we propose to develop a molecular marker for CO oxidation activity in soil. There are two different forms of coxL gene encoding for the large subunit of the CO- dehydrogenase (CODH) catalyzing CO oxidation in bacteria, namely OMP- and hypothetical BMS-CODH. Compelling evidence suggests that BMS-type is non-functional and thus, couldn't be a suitable marker for CO uptake activity. In order to test this hypothesis, we analyzed CO oxidation activity in Burkholderia xenovorans LB400, possessing genes encoding for both OMP- and BMS-CODH isoenzymes. This strain demonstrated the ability to oxidize atmospheric CO and expressed both OMP- and BMS-coxL genes during the stationary phase. Inactivation of coxL-BMS gene in the bacterium is in progress and will confirm whether BMS-CODH confers or not high affinity CO uptake activity. This genetics approach is complemented by bioprospection of high affinity CO-oxidizing bacteria in soil, using an innovative dynamic microcosm chamber approach. So far, our results suggest that coxL-OMP gene is the best candidate to predict CO oxidation activity in soil and improve our ability to predict the fate of this important ecosystem service in response to global change.

Poster Session

BAM-PT1072 - Degradation of tetrachloroethylene by microbiological substance(s) from activated sludge

Katsuya Ohno[1, 2], Kohei Nakamura[2], Kazuhiro Takamizawa[2]
[1]IBIDEN CO., LTD., Ogaki, Japan, [2]Gifu University, Gifu, Japan

Tetrachloroethylene (PCE) has been widely used in dry-cleaning or cleaning of e-infrastructure which found subsurface or ground water as a pollutant. PCE is harmful for human health and non-degradable in nature; particularly under aerobic condition. Our research detected a novel mechanism of PCE degradation by microbiological substance(s) from activated sludge under aerobic condition. Activated sludge collected from wastewater treatment tank of e-infrastructure manufacturer was acclimatized with PCE at initial concentration of 1000 mg/l, for 4 years. Results showed 100 mg/l of PCE decreased to 31 mg/l within 24 hr. Filtrates of activated sludge which were passed through the GS-25 glass fiber filter (pore size: 1 μm) and membrane filter (pore size: 0.2 μm) showed PCE-decreasing activity as well. Furthermore, when the membrane filtrates were subjected to heat treatment or charcoal adsorption, their PCE-decreasing activity was lost. Separation and characterization of microbiological substances into various molecular weight cut-offs (MWCO) of 10000, 5000 and 3000, showed that, fraction of less than 3000 MWCO decreased by 0.38 mg of PCE. Microbiological substance(s) was quantified as proteinous substance(s) by Qubit system and BCA kit. The PCE-decreasing amount was 0.08 mg per protein, which was twice as that of crude membrane filtrate. These results suggest that low molecular substance(s) such as peptide are likely involved in the PCE degradation observed. Although PCE degradation products were not able to be detected by GC-MS, FID, and TCD and ion chromatography but, we found that, C-Cl bounds in PCE were cleaved and chlorides were being ionized after reaction of PCE with microbiological substance(s) by analysis of x-ray absorption fine structure (XAFS) with synchrotron. A novel PCE degradation mechanism would be shown in our study.

Poster Session

BAM-PT1074 - An atypical high-affinity hydrogenase scavenging atmospheric H2 is implicated in the persistence of Streptomyces avermitilis

Quentin Liot[1], Patrik Hallenbeck[2], Philippe Constant[3]
[1]INRS Institut Armand Frappier, Laval, Canada, [2]Université de Montréal, Montréal, Canada, [3]INRS Institut Armand Frappier, Laval, Canada

Reactivity toward hydroxyl radicals makes H2 an indirect greenhouse gas, and anthropogenic emissions of H2 are increasing. Despite, the atmospheric level of this trace gas remains stable due to the contribution of soil actinobacteria responsible for 80% of the global loss of atmospheric H2. Compelling evidence suggests these microorganisms possess a hypothetical high affinity group 5 NiFe-hydrogenase conferring the ability to scavenge atmospheric H2. Despite the unique proprieties of this enzyme, virtually nothing is known about its regulation and physiological role. Hydrogenases catalyse the interconversion of H2 into protons and electrons. These enzymes are involved in many different processes, such as respiration, cofactor oxydoreduction and H2 sensing coupled to gene regulation. However, considering the free energy of atmospheric H2 oxidation, we proposed that actinobacteria use the energy potential of H2 to supply maintenance energy, potentially implying this enzyme in bacterial persistence, especially in Streptomyces' spores. As spores are survival structure, they possess a minimal metabolism for conserving their energetic reserves. A weak but constant energy gain would be so a main advantage for their persistence, permitting a faster awakening or a longer survival. Mature spores of Streptomyces avermitilis, the model high affinity H2-oxidizing bacterium, were shown to express the structural and auxiliary genes of group 5 hydrogenase. As S. avermitilis has been isolated and its genome sequenced, we inactivated hhyS and hhyL, the structural genes of this hypothetical hydrogenase in the bacterium, using a PCR-targeting mutagenesis technique. As expected, this mutation abolished the high affinity H2 uptake activity in the bacterium. Surprisingly, the developmental stage of the mutant strain was interrupted at the onset of sporulation, resulting to a significant loss of survival capabilities. This work highlights a new physiological role for hydrogenase and opens the window to biochemical and genetic characterization of the atypical enzyme of significant biotechnological interest.

Poster Session

BAM-PT1076 - New biological method for desalinization of secondary saline soils

Irina Smirnova[1], Amankeldi Sadanov[1], Svetlana Aitkeldyeva[1]
[1]*Institute of Microbiology and Virology, Almaty, Kazakhstan*

New biological method for desalinization of secondary saline soils by applying bioameliorants - cellulosic wastes of plant was developed. To prepare bioameliorants, a solid-state fermentation was used. For the first time, specifically selected cellulolytic bacteria were applied for the solid-state fermentation. Previously, from silage and crop wastes 89 strains of facultative anaerobic acidotolerant cellulolytic bacteria were isolated. As a result of bacterial screening, the strains with high cellulase and acid-forming activity were selected. These strains were used as biostarter for the solid-state fermentation of wastes. Introduction of bioameliorants to the plough-layer increases the number of cellulolytic bacteria in soil and has a favorable effect on soil processes and saline soil microflora. It also reduces salinization of secondary saline soils due to increased saltiness of the soil solution, which promotes the transit removal of salts of soluble compounds, exercising toxic effect on the plants. Thus, the total medium alkalinity in HCO^{3-} increased relative to control by 1.6 times, total amount of salts grew up as compared with control by 2 times. This indicates a shift in soil pH to neutral side and leads to the creation of favorable conditions for plant. Introduction of bioameliorants to the soil improves rice survival by 28%,the rice yield is increased by 12.6 % against control. Thus, a new biological method for desalinization of secondary saline soils was developed on the basis of introducing bioameliorants, prepared due to cellulolytic bacteria. Application of the method leads to the activation of microbial soil processes and creates the conditions for transit removal of salts of soluble compounds, exercising toxic effect on growth and development of rice plants. This method is ecologically safe, helps to protect the surrounding environment. Cellulolytic bacteria themselves are useful representatives of soil microflora. A patent was received for the method of biological melioration of soil.

Poster Session

BAM-PT1078 - Biodegradation of crude oil and kerosene by *Pseudomonas aeruginosa* and *Aspergillus niger*

Amina Hassaine[1,2], Ouahiba Bordjiba[1]

[1]Laboratoire de Biologie Végétale et Environnement, Département de Biologie, Faculté des Sciences, Université Badji Mokhtar, Annaba, Algeria, [2]Département de Biologie, Faculté des Sciences, Université Kasdi Merbah, Ouargla, Algeria

Hydrocarbons are molecules that pose serious environmental problem because of their toxic, carcinogenic or teratogenic properties The fate of these pollutants in the environment is mainly governed by the biodegradation process. The existence of these phenomena depends on the inherent biodegradability of the pollutant but also the presence of microflora-degrading competent. In this work, we purpose to study the bioremediation capability of *Pseudomonas aeruginosa* and *Aspergillus niger* towards crude oil and kerosene The microbial strains were isolated and identified from industrial wastewater samples from Sonatrach Skikda and Hassi Messaoud, we selected them for their ability to grow in the presence of hydrocarbons. To test they ability to biodegrade the two molecules selected, the study of the evolution of such parameters as the microbial kinetics, pH, temperature and rate of biodegradation were conducted. Key words: hydrocarbons, Biodegradation, bioremediation, microbial strains

Poster Session

BAM-PT1080 - Characterization of Enterococcus species occuring on terrestrial vegetation

Pavel Svec[1], Lucie Zatopkova[1], Hana Vecerkova[1], Ivo Sedlacek[1]
[1]*Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic*

A group of 173 presumptive plant-associated enterococcal strains was isolated during the vegetation periods in 2009 and 2013 in the frame of two studies dealing with the investigation of Enterococcus spp. populations occurring on plants. Individual strains of Gram-positive cocci were isolated on Kanamycin esculin azide agar and characterized using rep-PCR fingerprinting with the (GTG)5 primer. Obtained fingerprints were compared with an in-house CCM reference database containing more that 7000 fingerprints representing multiple Gram-positive species including representatives of all hitherto described Enterococcus spp. In total, 102 isolates matched reference database entries and were assigned as Enterococcus faecalis (23 strains), Enterococcus haemoperoxidus (20), Enterococcus plantarum (14), Enterococcus casseliflavus (12), Enterococcus faecium (9), Enterococcus ureilyticus (8), Enterococcus moraviensis (6), Enterococcus rotai (6), Enterococcus mundtii (2), and Enterococcus durans (2). Remaining 71 strains were identified as members of other genera (e.g. Lactococcus, Leuconostoc) or were not clustered with any fingerprints included in the database. Overall results obtained in the frame of the present work revealed that plants are inhabited by diverse and taxonomically rich Enterococcus spp. populations. This work was supported by the project CZ.1.07/2.3.00/20.0183.

Poster Session

BAM-PT1082 - A functionally active methanotrophic microbial community in the Canadian High Arctic ice-wedge polygon soil

Ianina Altshuler[1], Jennifer Ronholm[1], Lyle Whyte[1]
[1]McGill University, Montreal, Canada

Arctic permafrost is thought to harbour ~50% of total stored soil organic carbon. Climate has an effect on the Arctic that is twice as strong as that experienced at lower latitudes. These carbon stores, if metabolized by microbial communities into volatile greenhouse gases (GHGs), may contribute to a positive feedback loop of climatic change. One of the most potent GHGs is methane and its flux from soils to the atmosphere is partially governed by microbial methanogenic and methanotrophic organisms. How these microbes respond to climatic change, and subsequent permafrost thaw, will effect if Arctic ice-wedge polygon soils will be a source or a sink of methane. The objective of this study is to explore the functional microbial community in Arctic ice-wedge polygon soil, during the summer, and measure the potential methane absorption. We performed metatranscriptome sequencing to gauge the functional microbes currently present in three locations at an ice-wedge polygon site: the polygon interior, the wedge, and the polygon interior under extensive moss growth. Preliminary metatrascriptome results show the presence of mRNA involved in methanotrophy at each of the ice-wedge polygon sites. Methane monooxygenase was found in both soluble (sMMO) and particulate (pMMO) forms. In the future, we will quantify the transcripts of genes involved in methanotrophy at each of the ice-wedge polygon sites. Furthermore, we have set up microcosms of two active Arctic soil types to monitor methane oxidation at high and low methane concentrations. Ongoing monthly gas chromatography readings of the microcosms indicate that methane oxidation is present in the high methane treatment, but not at atmospheric methane concentrations. This research will characterize active microbes in the Arctic soil and help model soil methane flux.

Poster Session

BAM-PT1084 - Microbial community dynamics during the phytoremediation of gold mine residues at Val d'Or, QC using Frankia-inoculated alders.

Katrina Callender[1,2], Sebastien Roy[3], Damase Khasa[4,5], Charles W. Greer[2], Lyle Whyte[1]
[1]McGill University, [2]National Research Council Canada - EME Montreal, [3]Université de Sherbrooke, [4]Université Laval, [5]Centre d'étude de la Forêt

Phytoremediation, the use of plants and associated microbes to degrade or stabilize contaminants, has gained popularity as an efficient, cost-effective alternative to ex-situ techniques as it also restores aesthetic value, important for reclaiming large expanses of contaminated or degraded land. Alders, pioneer trees and shrubs, can grow in contaminated, nutrient poor environments, partially due to their symbiotic root associations with nitrogen-fixing bacteria, such as Frankia, and ECM fungi. This study, consisting of both field and greenhouse trials, assessed the performance of Frankia-inoculated alders in gold mine residues from Val d'Or, Quebec and investigated effects on microbial community dynamics. Alders were able to establish and grow on moderately alkaline mine residues and positively impacted soil physico-chemical properties in addition to soil microbial community structure and functions, demonstrating their successful establishment. After 2 years of growth, soil quality improved as soil pH decreased to 7.5, and total extractable metal concentrations for aluminum, manganese, sodium, copper, etc. decreased up to 2-fold to within Natural Resources Canada recommendations. Microbial enumeration and mineralization assays of C-14 labelled acetate demonstrated increases in soil bacterial density and activity and rhizosphere soils contained up to 2 orders of magnitude more bacterial cells per gram than bulk soils. Metagenomic and high-throughput sequencing of 16S rRNA genes (bacteria) and the ITS region (fungi) were performed for soil, root nodule and root tip samples using the Ion Torrent sequencing platform, revealing a bacterial-dominated (≥ 93.7% sequences) soil microbial community. Changes in microbial diversity and community composition were observed as plants established, including an increase in the abundance of the bacterial class Alphaproteobacteria complemented by a decrease in Betaproteobacteria, suggesting this group may be a useful indicator species. Genomics techniques can be applied to further investigate interactions of alders with other phytoremediating plants, fungi and bacteria and develop more efficient phytoremediation techniques.

Poster Session

BAM-PT1086 – WITHDRAWN - Acetate cycling between methanogenic archea and acetogenic bacteria in nutrient-limited environments

Lindsay Rollick[1], Gerrit Voordouw[1]
[1]*Petroleum Microbiology Research Group, University of Calgary, Calgary, Canada*

Methane-producing archea (methanogens) and acetic acid producing bacteria (acetogens) exist in many low nutrient environments where electron acceptors are absent. Methanogens often outcompete acetogens due to the greater energy yield in the methanogenic over the acetogenic reaction (Equations 1 and 2). 1. $4H2+HCO3^{-}+H^{+}\rightarrow CH4+3H2O$ $\Delta G° = -135.6$ kJ. 2. $4H2+2HCO3^{-}+H^{+}\rightarrow CH3COO^{-}+4H2O$ $\Delta G° = -104.6$ kJ. Nevertheless, acetogens play a significant role in carbon cycling in the low nutrient deep biosphere of marine and terrestrial environments. Production of acetic acid from H_2 and CO_2 in the subsurface may contribute as a microbially-enhanced oil recovery, because acetic acid dissolves carbonate rock and H_2 and CO_2 diffuse readily through water- and oil-containing regions of carbonate reservoirs. Acetogens were enriched from oil field produced waters with excess H_2 and CO_2. in high and low nutrient media. The latter lacked added nitrogen, phosphorus and trace elements and may be more representative of subsurface conditions. In high nutrient medium, a maximum of 8.6 mM of acetic acid was produced. However, this disappeared within 24 days. As only 1.9 mM methane was produced during this period, the loss is not attributable to acetotrophic methanogenesis. DNA pyrosequencing of 16S rRNA genes indicated Ralstonia as the main community component. Ralstonia can convert acetate to polyhydroxybutyrate (PHB) granules (Sutoh et al 2003), explaining the removal of acetic acid. In low nutrient medium 155% more acetic acid and 46% less methane were produced without acetic acid loss, suggesting predominance of acetogenesis. In the absence of added bicarbonate acetogenesis was limited, but still predominated over methanogenesis. In conclusion it appears that production of acetic acid from H_2 and CO_2 may be competitive with methanogenesis under the low nutrient, excess substrate conditions. With higher nutrients (but still limited), acetic acid may be converted to PHB by Ralstonia in the subsurface preferentially over methanogenesis.

Poster Session

BAM-PT1088 - Screening of Bacillus sp. strain with Cu2+ adsorption capacity from lotus mud and study on its biological characteristics

Donglan He[1], Gui Qin[1]

[1]*College of Life Science,South-Central University for Nationalties, Wuhan, China*

4 bacteria strains with Cu2+ adsorption capacity were isolated from lotus mud with broth medium containing Cu2+ and the biological characteristics of the strain which having the strongest copper adsorbability were studied. The results showed that there were many bacteria which could adsorb copper and resistant to salt (NaCl), acid (HCl) and alkali (NaOH) in lotus mud. 4 of the bacteria strains had high adsorption to copper tested by atomic adsorption spectrometry. The adsorption efficiency to Cu2+ was 30.81% to 67.33%. The strain with the highest adsorbability to Cu2+ was identified as Bacillus subtilis according to its morphologic, physiological and biochemical character, which including individual morphological feature. Colonial morphology, Voges—Prokauer test(V.P test), methyl red test(MR), indole test(IND) and citrate test(CIT). It could decompose and exploit glucose to produce organic acids as well as to decompose tryptophan to generate indole. And glucose and citric acid are advantage carbon among glucose, lactose, sucrose, methanol, ethanol, potassium sodium tartrate, mannite and citric acid. Urea, yeast extract and peptone are advantage nitrogen to the strain which compared with (NH4)2SO4, KNO3. NH4NO3. But it couldn't use methanol, ethanol and KNO3 Meanwhile the optical growth temperature and pH was 37 ℃ and 7.01 by Single factor experiment and orthogonal experiments respectively.

Poster Session

BAM-PT1090 - Effects of low-shear modeled microgravity on a microbial community and its potential application

Hideki Aoyagi[1]

[1]*Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan*

[INTRODUCTION]: About 100 years ago, a technique for isolating microorganisms and growing them in pure culture was developed. Since then, many microorganisms have been isolated and utilized in useful metabolites production. However, it becomes difficult to isolate and maintain pure cultures of new microorganisms. This has limited progress in industrial microbiology. Using independent molecular techniques, it has been estimated that more than 99% of microorganisms existing in nature cannot be isolated and cultured. To overcome this problem, development of novel cell cultivation system is necessary. In this study, the effects of low-shear modeled microgravity on a microbial community were investigated, and the potential application of low-shear modeled microgravity in the screening of microorganisms was evaluated. [RESULTS AND DISCUSSION]: Pond water was inoculated into some different media, and microbial cell growth rates under low-shear modeled microgravity and normal gravity were compared. Compared with normal-gravity culture, cell growth and sugar consumption rates were higher in low-shear modeled microgravity culture. Furthermore, when the microbial communities obtained under the various culture conditions were subjected to denaturing gradient gel electrophoresis (DGGE), three different groups of microorganisms were observed: a) microorganisms whose growth rates were increased by low-shear modeled microgravity; b) microorganisms whose growth rates were suppressed or inhibited by low-shear modeled microgravity; and c) microorganisms whose growth rates were not affected by low-shear modeled microgravity. Sequence analysis of the microorganisms whose growth rates were increased by low-shear modeled microgravity showed that some had high similarity with microorganisms that have not yet been successfully isolated. Some DGGE bands present in the low-shear modeled microgravity culture were also present when those were cultivated on agar plates. I show that it is possible to isolate new microorganisms by using combinations of low-shear modeled microgravity, normal gravity, and agar plate culturing techniques.

Poster Session

BAM-PT1092 - Recovery from VBNC, a suggested state in environmental water, to culturable state in Vibrio cholerae by co-cultivation with human cells

Daisuke Imamura[1], Tamaki Mizuno[1], Shin-ichi Miyoshi[2], Sumio Shinoda[1]
[1]Collaborative Research Center of Okayama University for Infectious Diseases in India, Kolkata, India, [2]Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan

Cholera has plagued people of Bengal area including West Bengal state in India and Bangladesh for long time. It still remains to be a serious problem with regular outbreaks in this region. We have carried out to isolate Vibrio cholerae O1 and O139 serogroups from the environmental water such as ponds and rivers in Kolkata, a capital city of the West Bengal state. However, these serogroups were rarely isolated in spite of occurrence of cholera in Kolkata based on the hospital-based surveillance. One of possible reasons is the viable but nonculturable (VBNC) state of V. cholerae. If these serogroups persist as a VBNC state in the environmental water and resume growth only when it senses the signal of entrance into a human intestine, it would not be isolated by traditional methods with incubation on a media plate. Therefore, our group has tried to identify factors that are able to convert VBNC state cells of V. cholerae into the culturable state. We recently reported that mammalian cell extract could convert VBNC state cells into the culturable state and this conversion factor was catalase. In the present study, it was found that the state convertible by mammalian cell extract and catalase was transient and the bacterial cells eventually entered into an inconvertible state by the addition of these factors. Then reconversion of the inconvertible sate cells to culturable state was investigated and co-culture with HT-29 human colon carcinoma cells was shown to be effective for the reconversion. This behavior was observed in both O1 and O139 serogroups. These results are consistent with the idea that VBNC state cells of V. cholerae resumes growth when it enters human intestine.

Poster Session

BAM-PT1094 - Evaluation of denaturing gradient gel electrophoresis in combination with enrichment culture techniques to identify bacteria in commercial microbial based consortial products

Renuka Subasinghe[1], Ajith Dias Samarajeewa[1], Rick Scroggins[1], Lee Beaudette[1]
[1]Biological Assessment and Standardization Section, Environment Canada, Ottawa, Canada

The ability to assess the microbial composition in new or existing commercial microbial based consortia products is important for compliance of government regulations and for human and environmental risk assessment. Complete identification of the bacterial species in commercial microbial based consortia products is challenging due to high diversity and complexity of these products. In previous studies, genomic methods explored included denaturing gradient gel electrophoresis (DGGE), clonal restriction fragment length polymorphism (C/RFLP) and clonal-sequencing methods on non-enriched products. Current research was performed to further enhance our ability to directly identify bacterial species from commercial consortia products. To enhance the growth of potential pathogenic bacterial strains, a commercial microbial based consortium product was incubated under three different temperatures 22°C, 28°C and 37°C on MacConkey broth, Azide Dextrose broth and Gram Negative (GN) media. Genomic DNA was then extracted from each enrichment culture. Using the V3 and V6 hyper-variable regions of the 16S ribosomal DNA (rDNA), unique primers were developed. The resultant PCR amplicons were separated by DGGE, cloned and sequenced for each enrichment condition. DGGE data analysis indicated that the MacConkey broth enrichment resulted in DNA sequences consistent with *Escherichia* or *Shigella* and *Morganella spp*. *Proteus* and *Morganella spp.* were found in the GN medium at both 28°C and 37°C. The sequences consistent with *Vagococcus* and *Enterococcus spp*. were detected with the Azide Dextrose broth at both 28°C and 37°C. This study indicates that enrichment of commercial consortia products at different temperatures and in different media would be a promising approach in identification of complex commercial microbial based consortia products.

Poster Session

BAM-PT1096 - Antibacterial properties of a novel silver-stabilized hydrogen peroxide and mechanism of cell assciation and enhanced activity

Nancy Martin[1], Paul Bass[2], Steven Liss[2]
[1]Biomedical and Molecular Sciences, Queen's University, Kingston, Canada, [2]Environmental Studies and Chemical Engineering, Queen's University, Kingston, Canada

A new generation peroxide stabilized with ionic silver (Huwa San peroxide (HSP)) is being considered for secondary disinfection of potable water. Recent pilot studies demonstrated that HSP contributes to the reduction of trihalomethanes (80%), and remains residual through the distribution system. The efficacy of HSP in suspended cultures of indicator bacteria (14) was determined, and contact time (CT) relationships were established for exposures to HSP, laboratory grade hydrogen peroxide (HP), and sodium hypochlorite at concentrations of 20 ppm and at pH 7 and 8.5. HSP was more effective than hypochlorite at the higher pH but equally effective at pH 7. HSP was more effective than HP. CT assays were performed at two concentrations of E. coli (K12) (10^3 and 10^6 CFU.mL-1), and with and without inactivation (catalase). HSP was less effective at the lower microbial concentration with neutralization. At 10^6 CFU.mL^{-1} there were no differences between experiments with and without neutralization. It was postulated that HSP was protected from degradation through cell association at the higher cell concentration, and that catalase is ineffective against cell associated HSP. At the lower cell density most of the HSP was likely in solution, was neutralized, and was not carried over with cells to the recovery medium. For HP there were no differences. Silver may facilitate this association through electrostatic interactions at the cell surface. Addition of mono (K^+) and divalent (Ca^{2+}) cations (0.005-0.05M) reduced the efficacy of HSP but not HP. Ca^{2+} was found to be slightly more effective. A CO_2 measurement system distinguished the effect of silver and peroxide in HSP on metabolic activity of a P. aeruginosa PAO1 biofilm. Inhibition was primarily associated with HSP or HP whereas HSP-silver and silver nitrate were found to have no, or a negligible, effect on metabolic activity over two hours of continuous exposure.

Poster Session

BAM-PT1098 - Analysis of the interaction between cellulosomal mannanases and the scaffolding protein from Clostridium cellulovorans

Gaku Ishikawa[1], Daichi Nakajima[1], Reiji Tanaka[1], Toshiyuki Shibata[1], Kouichi Kuroda[3], Mitsuyoshi Ueda[3], Yutaka Tamaru[1,2], Hideo Miyake[1,2]

[1]Graduate School of Bioresources, Mie University, Mie, Japan, [2]Department of Bioinformatics, Mie University Life Science Research Center, Mie, Japan, [3]Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Clostridium cellulovorans produces an enzyme complex known as the cellulosome, which efficiently degrades cellulose and hemicellulose. We have reported the whole genome sequence of C. cellulovorans. Among genome sequences, two and four cellulosomal mannanases belonging to GH family 5 and 26, respectively, were identified. Both mannanase GH5A (ManGH5A) and mannanase GH26A (ManGH26A) were detected by transcriptome and proteome analysis when C. cellulovorans was grown on various carbon sources. They have a unique domain structure, in which the dockerin domain of ManGH5A is located at its N-terminus and ManGH26A contains four CBMs. On the other hand, CbpB, a novel scaffolding protein comprises a 429-residue polypeptide that includes a CBM, an S-layer homology module and a cohesin domain. However, it is not clear whether the cohesin domain of CbpB functions as well as that reported for CbpA and other scaffolding proteins from Clostridia. In this study, we characterized ManGH5A and ManGH26A, and analyzed the interaction between the cellulosomal mannanases and CbpB. The recombinant proteins of ManGH5A, ManGH26A and CbpB were expressed in E. coli cells. The kinetic parameters of ManGH5A and ManGH26A were determined. ManGH26A showed the highest activity among previously reported mannanases. Analysis of the interaction between either ManGH5A or ManGH26A and CbpB was demonstrated by isothermal titration calorimetry. Titrations of mannanase (ManGH5A or ManGH26A) with CbpB for the dissociation constant Kd were 4.79 × 10^-10 M or 1.40 × 10^-6 M, respectively. The results indicated that ManGH5A binds tightly to CbpB, with an approximately 3,000-fold higher affinity than ManGH26A. The highly conserved region of the dockerin domain of ManGH5A differs from those of other cellulosomal enzymes from C. cellulovorans. These results suggest that the difference in the affinity between cellulosomal mannanases and CbpB was accounted for by a difference in the highly conserved amino acid residues.

Poster Session

BAM-PT1100 - Spatial variation in microbial diversity in waste disposed paper industrial sites in North East India

Gauri Dutt Sharma[1]
[1]*Bilaspur University, Bilaspur. India*

World wide paper consumption is estimated 13.5 million tons, which produces organic and inorganic wastes disposed off as landfills causing environmental pollution. The incomplete treated sludge pollution lead to change in microbial community. The present study was carried out for two years in different stages of Paper Industrial wastes disposed degraded sites in North East India. The seasonal variation in relation to physico-chemical characteristics of wastes disposed sites were closely related to bacterial population. The enzyme activity of microbes changed up to 70% due to soil moisture holding capacity. The microbial community and their activity was increased significantly due to decrease in pH and increase in phosphorus contents. A negative correlation was observed between soil organic nitrogen content and microbial population. Fungal and bacterial communities exhibited antagonism, however, under natural condition, seasonal change was an important observation. Climate of the study sity was sub tropical and was found to influence fungal community with less alteration than bacterial diversity. The bacterial species like Chromatium, Chlorobium, Thiocystis and Thiospirillum were dominant in Paper Industry wastes disposed sites. Cellulose degrading bacteria were isolated from organic matter rich polluted sites and were characterized using 16s rRNA molecular technique and phytorelationship was established with other pollution degrading bacterial species.

Poster Session

BAM-PT1102 - Impact of heavy metals in Gold Mine Tailings Ponds on the Actinorhizal symbiosis in alder shrubs

Kathleen Barrette[1,2], Jean-Philippe Bellenger[1,2], Evgeniya Smirnova[3], Sébastien Roy[1,2]
[1]Université de Sherbrooke, Sherbrooke, Canada, [2]Centre d'étude et de valorisation en diversité microbienne, Sherbrooke, Canada, [3]Centre Technologique des résidus industriels, Rouyn-Noranda, Canada

Heavy metals do not decay in the environment and pose a challenge for revegetation of gold mine sites. Alder shrubs *Alnus rugosa* and *Alnus crispa* are studied for their symbiosis with *Frankia*, a nitrogen fixing soil bacterium. This association can assist revegetation on nutrient-poor substrates such as mine tailings. In this project, tailings were sampled on the acidogenic Doyon Gold Mine tailings ponds (Québec) and sterilized via irradiation. An *in vitro* experiment was conducted to measure how the nitrogen-fixing symbiosis could influence alder development in such adverse substrates. Different volumetric ratios of tailings and pristine sand were prepared (0%, 35%, 65% and 100%) for sterile alders to be grown in. Increasing ratios of tailings had an adverse effect on the development of both alder species, and in all plants, whether inoculated, or not, with *Frankia* sp. However, in each substrate, and for both alder species, inoculated alders developed more aerial biomass than non-inoculated alders. As an example, seeding volume index (SVI: an established proxy for aerial biomass) values with increasing tailings ratios (0%, 35%, 65% and 100%) were as follow for *A.rugosa*: 5528 mm^3, 675 mm^3, 124 mm^3 and 2 mm^3. Control alders (not inoculated with *Frankia*) showed lower SVI for each concentration and the same decreasing trend is present. The toxicity of the tailings was measurable even in the 35% and 65% treatments. Foliar chlorophyll content was measured by fluorescence to quantify environmental stress and compare results with alders planted on the tailings ponds. Our results suggest that an organic amendment may be required to attenuate tailings toxicity towards alders outplanted on the mine site. We demonstrated that a functional actinorhizal symbiosis can establish and enhance alder development in these hostile, anthropogenic substrates. Alders are foreseen as key early successional species that could assist the rehabilitation of disturbed ecosystems.

Poster Session

BAM-PT1104 - Physicochemical properties and microbial ecology of aerobic granules and filamentous outgrowth in laboratory scale sequencing batch reactors

Hussain Aqeel[1], Mahendran Basuvaraj[2], Katja Engel[3], Michael Hall[3], Josh Neufeld[3], Steven Liss[1,2]

[1]Department of Chemical Engineering, Queen's University, Kingston, Canada, [2]School of Environmental Studies, Queen's University, Kingston, Canada, [3]Department of Biology, University of Waterloo, Waterloo, Canada

Instability of granular sludge due to filamentous outgrowth leading to bulking, and finally development of granules has been recently observed in laboratory scale conventional activated sludge sequencing batch reactors. Three distinctive stages were observed during granulation. During the initial stage (stage1), the granular structure was compact, spherical and on average 0.2 mm in diameter. Filamentous outgrowth was a dominant feature during stage2 resulting in bulking and partial biomass washout. The filaments eventually integrated and enmeshed within the granulated structure, resulting in gradual biomass accumulation in the bioreactor and improved settling properties. The resulting granular structures in stage3 were spherical in shape, compact, and considerably larger in size (2-5 mm in diameter). Relative hydrophobicity of the stage1 biomass (36%) decreased during development of stage2 and stage3 structures (5% and <1%, respectively). The protein to polysaccharide ratio (PN/PS) in the extracellular polymeric substance extracted from the stage1 and stage3 granules was higher (2.8 and 5.7, respectively) as compared to the stage2 PN/PS (1.5). Imaging of the biomass samples using confocal laser scanning microscopy (CLSM) coupled with molecular specific fluorescent staining, revealed that β-polysaccharides dominated the outer layer of stage1 and stage3 granules. β-polysaccharides in stage3 granules were more distributed throughout the structure and may be contributing to the stability of the granules. 16S rRNA gene sequencing (Illumina), showed a significant shift in the microbial community during granulation. Cyanobacteria dominated stage2 structures, which can cause instability of microbial granules and filamentous bulking. Chitinophaga (a cyanobacterial predator) dominated stage3 structures, which likely accounts for the loss of Cyanobacteria in the resulting granules. Rhodanobacter which, is capable of complete denitrification, was a dominant feature of stage1 and stage3 granules.

Poster Session

BAM-PT1106 - Inoculation of willows growing in organically contaminated soil with beneficial bacterial endophytes

Stacie Tardif[1], Etienne Yergeau[2], Marc St-Arnaud[3], Lyle G. Whyte[1], Charles W. Greer[2]
[1]Microbiology, Natural Resources, McGill University, Montreal, Canada, [2]National Research Council Canada, Energy, Mining and Environment, Montreal, Canada, [3]Institut de recherche en biologie végétale, Université de Montréal, Montreal, Canada

Phytoremediation uses plants and their associated microbial communities to degrade or render organic and inorganic pollutants harmless. Bioremediation of organic pollutants is decidedly more problematic for plants alone as most organic pollutants are degraded by microorganisms in the rhizosphere. As such, knowledge of key microbial players that assist in, or increase the rate of bioremediation of organics is of considerable interest. Endophytic microorganisms are particularly useful as they play a more significant role in protecting plants from the toxic effect of certain pollutants and allow the plants to colonize otherwise harsh environments. This study focused on bridging the gap of knowledge on endophytic species, in particular, those associated with willow cultivars in a phytoremediation study on soil contaminated by polycyclic aromatic hydrocarbons (PAH). Isolation and characterization of endophytic bacterial strains of the willows growing in a highly contaminated environment were performed. Key endophytic bacteria demonstrated the potential for PAH degradation and were found to contain the ACC deaminase, an enzyme present in bacteria that are able to significantly diminish the stress response in plants. Following this, willow cultivars were inoculated with these strains in order to test their ability to increase plant growth, development and survival as well as to analyze their influence on remediation rates. These greenhouse-grown willows were harvested after one growing season in PAH contaminated soil and Ion Torrent sequencing of the 16S rRNA and 18S rRNA genes was used to identify the microbial communities present in the rhizosphere and in the roots and stems of these differently inoculated willows. Contamination levels of individual pots and plant growth parameters were monitored at the beginning and end of the growing season. The pollutant concentration and bacterial inocula have shown to influence the microbial community structures and plant growth rates. In depth statistical analyses will be performed to further explore underlying relationships between these microbial community structures, growth rates and remediation rates.

Poster Session

BAM-PT1108 - Quorum quenching bacteria isolated from wastewater treatment plant, and genes for N-acyl-homoserine lactone degrading enzymes from the isolates

A-Leum Kim[1], Chi-Ho Lee[1], Jung-Kee Lee[1]
[1]*Department of Biomedicinal Science & Biotechnology, Paichai University, Daejeon, Korea*

Quorum sensing (QS) is a regulatory mechanism employed by bacteria to coordinate the behavior of their community in response to population density. Bacteria recognize the changes in population density by sensing the concentration of signal molecules, N-acyl-homoserine lactones (AHLs). As AHL-mediated QS plays a key role in biofilm formation, the interference of QS, referred as quorum quenching (QQ) has received great deal of attention. The concept of QQ can be applied to membrane bioreactors (MBR) for advanced wastewater treatment as a new strategy to control biofouling. The purpose of our study is to isolate quorum quenching bacteria, which could inhibit biofilm formation, and eventually could reduce biofouling in MBR system of wastewater treatment plant. In this study, we isolated diverse AHL degrading bacteria from the lab-scale MBR and sludge from real wastewater treatment plants. To identify the enzyme responsible for AHL degradation in QQ bacteria, AHL-degrading activities were analysed using cell-free lysate, culture supernatant and whole cell. Acinetobacter sp. strain produced the intracellular QQ enzyme, while Pseudomonas sp. produced the extracellular QQ enzyme. In case of Rhodococcus sp. strain, AHL-degrading activity was observed in whole cell as well as cell-free lysate. The aiiA homologue genes were identified by amplification of conserved region of AHL-lactonase gene in Rhodococcus sp.. In addition, three genes encoding putative AHL-acylases were identified in Pseudomonas sp. 1A1. All three genes encoding AHL-acylase from strain 1A1 was cloned, and the deduced amino acid sequence of three acylase genes shared 52%, 63%, and 68% identities with that of pvdQ, quiP, and hacB from P. aeruginosa PAO1, respectively. These three genes were expressed heterologously in E. coli and Bacillus thurigiensis. Finally, biofilm formation by AHL-producing bacteria was reduced by isolated QQ bacteria, when both the strains were co-cultured.

Poster Session

BAM-PT1110 - Identification of crystal structures produced by strain Pseudomonas syringae pv. phaseolicola by using X-ray crystallography and Energy Emission Spectroscopy

Ashutosh Sharma[1], Rosario Carmen Vallejo Flores[1], Karen Rios Gomez Garcia[1], Gabriela Ruiz Colin[1], Liliana Alvarez Chaparro[1], Olivia Tavera Vazquez[1]

[1]*Escuela de Ingeniería en Alimentos, Biotecnología y Agronomía (ESIABA), Tecnológico de Monterrey, Querétaro, México*

It has been reported that certain bacteria produce crystal structures as a result of mineral precipitation due to specific biological processes. In the present work, production of crystals in a strain of Pseudomonas syringae pv. phaseolicola were identified as a specific mineral called Struvite [NH4MgPO46H2O], that is commonly found in waste water treatment plants as a sediment result of the anaerobic digestion of solids material. Struvite components have been reported as important plant fertilizers but its use has been limited to high value crops because of the additional cost of manufacture. The identification process was performed, by culturing the strain in BD-King solid media, where crystals were observed as colorless, transparent vitreous lusters and hexagonal structures with 3 symmetric perpendicular axes. Further analysis were performed by applying the techniques of Energy Emission Spectroscopy and X-ray diffraction analysis with different purified crystal samples in order to identify the crystal components produced by the bacteria. As per our knowledge, it is the first report of crystal production and identification in this strain.

Poster Session

BAM-PT1112 - Identification of Metagenome cosmids involved in cobalt transport and cobalt-independent Ribonucleotide reductase

Ye Zhang[1], Mary Fernandes[1], Trevor Charles[2], Jiujun Cheng[2], Turlough Finan[1]
[1]McMaster University, Hamilton, Canada, [2]Waterloo University, Waterloo, Canada

The primary route for the transport of cobalt into the α-proteobacterium Sinorhizobium meliloti is through an ABC-type transporter encoded by the cbtJKL genes. ΔcbtJ mutants are defective in cobalt transport and fail to grow when plated on LB medium containing the metal chelator, ethylenediaminetetraacetic acid (EDTA). Here we have used this media to screen a metagenome cosmid library prepared from wheat-field soil DNA. The unique cosmids that complemented the cobalt uptake mutants were isolated and verified. The analysis revealed four clones carried gene clusters that appeared to directly complement the cobalt transport defect as they carried genes annotated as cobalt transporters or in one case the nickel transport genes from E. coli. Five metagenome plasmids, including pTH2835, carried genes that encoded B12-independent ribonucleotide reductase alpha and beta subunits. Complementation by these plasmids appeared to be indirect as the endogenous S. meliloti B12-dependent RNR gene nrdJ, and the cobT gene that is required for cobalamin biosynthesis could be disrupted in cells carrying the RNR α and β genes in trans on pTH2835. Hence, in complex media, the synthesis of a B12-independent RNR protein eliminated the nutritional requirement of S. meliloti for cobalamin and cobalt.

Poster Session

BAM-PT1114 - Introducing *Candidatus* Nitrosofontus exaquare and *Candidatus* Nitrosopurus aquariensis, novel ammonia-oxidizing archaea cultivated from engineered freshwater environments

Laura A. Sauder[1], Josh D. Neufeld[1]
[1]*University of Waterloo, Waterloo, Canada*

Ammonia is a metabolic waste product that threatens aquatic ecosystems with toxicity and oxygen depletion. Ammonia can be oxidized by microorganisms that carry out nitrification, a process that is harnessed by biofilters in engineered environments receiving high ammonia loads. Although ammonia-oxidizing bacteria (AOB) have been studied for decades, the recently discovered ammonia-oxidizing archaea (AOA) are ubiquitous and outnumber AOB in most sampled environments. However, little is known about AOA ecology and metabolism, largely because few laboratory cultures exist. Here, we report the enrichment of two novel AOA, *Candidatus* Nitrosofontus exaquare, which originated from a municipal wastewater treatment plant, and *Candidatus* Nitrosopurus aquariensis, which originated from an aquarium biofilter. These enrichment cultures have been growing stably for over one year with ammonia as a sole energy source and bicarbonate as a sole source of carbon. Phylogenetic analysis of *amoA* and 16S rRNA genes reveal that *Can.* N. exaquare and *Can.* N. aquariensis each belong to novel thaumarchaeal lineages that are distinct from cultured AOA representatives. *Can.* N. exaquare belongs to the soil group I.1b thaumarchaeal lineage, and clusters closely with organisms from high nutrient environments. *Can.* N. aquariensis belongs to the I.1a thaumarchaeal lineage, and clusters with AOA from oligotrophic freshwater and saline environments. Catalyzed reporter deposition—fluorescence *in situ* hybridization (CARD-FISH) visualization revealed that both *Can.* N. exaquare and *Can.* N. aquariensis are highly enriched, grow in biomass clumps, and possess coccoid morphology. *Can.* N. aquariensis is extremely small, with a diameter of ~500 µm, while *Can*. N. exaquare is ~1 µm in diameter. Microautoradiographic labelling of *Can*. N. exaquare cells demonstrated incorporation of ^{13}C into biomass, revealing autotrophic carbon fixation. *Can*. N. exaquare and *Can.* N. aquariensis represent the first cultured AOA representatives originating from engineered freshwater environments, and provide novel insight into AOA ecology and metabolism.

Poster Session

BAM-PT1116 - Suppression of methicillin resistant *Staphylococcus aureus* (MRSA) and *Salmonella enteric* growth by blue light

Violet Bumah[1], Daniela Masson-Meyers[1], Chukuka Enwemeka[1]
[1]*University of Wisconsin-Milwaukee, Milwaukee, USA*

The increase in antibiotic resistant bacteria is of grave concern necessitating alternative prevention and treatment strategies. One such strategy gaining interest is the use of monochromatic radiations. Ongoing research indicates that blue light has the potential to photo-inactivate MRSA and other bacteria. To investigate the growth suppression of two bacteria of medical importance (MRSA and *Salmonella*) using 470 nm blue light. We cultured and plated $5x10^6$ and $7x10^6$ CFU/mL MRSA USA-300 and $1x10^6$ CFU/mL of *Salmonella enteric* serovars Typhimurium and Heidelberg. Plates were irradiated with 55, 110, 165 and 220 J/cm² of blue light, incubated at 37°C for 24 hours and colony counts determined. Blue light irradiation produced a statistically significant dose-dependent reduction in the number of bacterial colonies formed ($p<0.001$) in all strains tested when compared with controls. Irradiation of $5x10^6$ and $7x10^6$ CFU/mL MRSA with 55 J/cm² of blue light produced 92% and 86% inactivation of bacteria, while 110 and 220 J/cm² produced 100% inactivation respectively. Irradiation of *Salmonella* with same energy densities had a commensurate effect as was observed with MRSA, with 55 and 110 J/cm² in *Salmonella* Typhimurium producing 31% and 93% suppression and 165 and 220 J/cm² completely inhibiting growth of colonies. *Salmonella Heidelberg* colonies treated with 55 and 110 J/cm² showed 11% and 84% suppression, while 165 and 220 J/cm² completely inhibited colony growth. Such growth inhibition observed in a gram-positive (MRSA) and gram-negative (*Salmonella*) bacteria indicates the versatile application of blue light in bacteria eradication and could be a viable intervention strategy for decontamination of environments that harbor them.

Poster Session

BAM-PT1118 - Recovery and detection of *Bacillus cereus* strains, *B. megaterium*, *B. thuringiensis* and *B. subtilis* in L-Eye cream

Irene Nadine Tchagou[1], Carolina Arce[1], Thomas Hammack[2]
[1]*Oak Ridge Institute for Science and Education, Maryland, USA,* [2]*Food and Drug Administration, College Park, USA*

Bacillus cereus has been associated to several clinical infections, and is also the cause of posttraumatic endophthalmitis or endogenous infections of the eye, which frequently result in blindness. Eye area cosmetics although preserved can be contaminated during manufacture or use and cause serious health issue. The recovery of Bacillus strains in L-eye cream preserved with methyl and propyl parabens, the effect of the nonionic active surfactants (Tween 80, Tween 20, Tween 60 and Span 20), the efficacy of the preservatives used in L-cream, and the selective agar for Bacillus detection were investigated. L-eye creams (20g) were first thoroughly mixed with 20 ml of Tween 80 (50%), or Tween 20 and with a blend of 20ml of Tween 60 at 20% and Span20 (TS), then the cream-samples were individually inoculated with an aliquot of 7 log cfu/ml of B. cereus 4227A, 6006, and ATCC 14579, B. megaterium ATCC 6458, B. mycoides ATCC 6264, B. subtilis ATCC 15563 and B. thuriengiensis ATCC 35866. The inoculated cream-samples with surfactants were diluted in modified letheen broth (MLB) and analyzed after 30min and during 14-days storage at room temperature. The cream-samples without surfactants were plated on MLA. Presumptive colonies of Bacillus were enumerated on Bacara, Brilliance Bacillus cereus, MYP, and R&F agar by MPN and spiral plating techniques. Tween 80 had the highest inactivating effect on parabens throughout 14 days followed by TS, and then Tween 20. The recovery of all the strains of Bacillus were ~ 4.5 cfu/g in cream-samples neutralized with Tween 80 and TS after 30 min. Dilution broth MLB alone did not neutralize the preservatives in the creams; the population of B. cereus subtilis ATCC 15565 and B. cereus 4227A were 1.83 and 1.49 log cfu/g after 30 min, respectively. Recovery of Bacillus strains depended on neutralizers, time and strains.

Poster Session

BAM-PT1120 - Autotrophic lifestyle at hypersaline environments: abundance and diversity of the biotin dependent acetyl CoA carboxylase using culture dependent and culture independent approaches

Eduardo L. Tosado-Rodriguez[1], Rafael Montalvo-Rodriguez[1]
[1]*University of Puerto Rico, Mayagüez, Puerto Rico*

Autotrophy in microorganisms has been studied over the years and several enzymes involved in this type of metabolism have been identified. Among these proteins, the acetyl CoA carboxylase (ACC) has been reported as one of the key enzymes in the process. However, little is known about carbon fixation at hypersaline environments especially if this process is performed by the Archaea domain. Recently, a study revealed the presence of key enzymes related to the Calvin Benson, Acetyl CoA, and TCA reductive pathways in a Mediterranean deep-sea hypersaline anoxic lake. Since some conclusions of this study directly link the Acetyl CoA reductive pathway to Archaea, we started to investigate the presence of this pathway at the solar salterns of Cabo Rojo, Puerto Rico using an ACC sub-unit (accC) as marker. Water samples were used to extract metagenomic DNA and for the isolation of putative carbon-fixating microorganisms. For isolation, the modified Synthetic Crenarchaeota media with 25.0% NaCl (w/v) and sodium bicarbonate as the only carbon source was used. In both samples the presence of the accC gene was detected by using specific primers in PCR reactions. An amplicon of 466bp corresponding to accC was observed in both samples. Isolates that contained the accC gene were partially characterized using the 16S rRNA gene. All isolates belonged to the haloarchaea. As a result of this, a comprehensive "in silico" analysis was performed looking for the presence of the accC gene in all available genomes of haloarchaea from several databases. It was found that this gene is present in 24 of the 26 available genomes. Also, the other sub-units related to the ACC were present. Phylogenetic analysis of accC genes from databases and solar saltern samples are currently being performed. Preliminary results might suggest a bigger role of halophilic Archaea in autotrophic metabolism at hypersaline environments.

Poster Session

BAM-PT1122 - Characterization of microbial populations responsible for nitrification at the solar salterns of Cabo Rojo, Puerto Rico

Mara F. Cuebas-Irizarry[1], Eduardo L. Tosado-Rodríguez[1], Miguel X. Oyola-Rivera[1], Robertson Thane Papke[2], Rafael Montalvo-Rodríguez[1]
[1]University of Puerto Rico-Mayagüez, Mayagüez, Puerto Rico, [2]University of Connecticut, Connecticut, USA

The Nitrogen cycle has been widely studied for its important role in life. One of the limiting steps is nitrification, which consists in the oxidation of ammonia to nitrite. Evidence suggests that this critical step is performed by members of the Bacteria and Archaea. Aerobic oxidation of ammonia has been found occurring in multiple environments such as soils, marine, and fresh water bodies. Few studies have been performed dealing with nitrification at hypersaline and moderate saline environments. The objective of this research project is the isolation and characterization of ammonia oxidizing microorganisms in marine (3.5% - 5.0%), saline (10.0% - 15%), and hypersaline (20.0%-25.0%) NaCl (w/v) environments from Puerto Rico. A total of 124 isolates were obtained using a minimum medium containing bicarbonate and ammonia as sole carbon and nitrogen sources respectively. The isolates are being characterized using physiological, morphological and molecular properties. Cells were mostly Gram-negative and pleomorphic forming red to pink colonies depending on the isolated strain. For the molecular approach, genomic DNA was extracted from pure cultures and amplification of the 16SrRNA gene was performed with domain-specific primers. Sequence analysis of an amplicon of approximately 650bp revealed that a total of 28 strains belong to the Bacteria domain while 30 were members of the Archaea. Other isolated strains are still under analysis. The archaeal strains were members of the genera: Halogeometricum, Haloarcula, Halobiforma, Haloferax, and Halomicrobium. Strains MC2A, MC4, and MC2B were used to amplify the rpoB, atpB, Ef-2, and GlnA genes for MLSA analysis. Phylogenetic trees using these concatenated genes revealed the possibility of new species of the genus Haloarcula. Full characterization using polyphasic taxonomy is currently on the way. The isolation of halophilic archaea belonging to Euryarchaeota in this minimal medium might suggest a possible role of these microorganisms in ammonia-oxidation at hypersaline environments.

Poster Session

BAM-PT1124 - Diversity and distribution of phototrophs at the central mine site, Nopiming provincial park, Canada

Elizabeth Hughes[1], Chris Maltman[1], Breanne Head[1], Vladimir Yurkov[1]
[1]University of Manitoba, Winnipeg, Canada

A September 2011 sampling trip to the deserted Central Gold Mine, Nopiming Provincial Park, Canada focused on abundance, distribution, and physiological/metabolic capabilities of aerobic anoxygenic phototrophs (AAP) isolated from tailings. Four sites were chosen: Blue Pond, Green Pond, an area of runoff and a mine drainage site. Enumeration revealed 14.6% of the culturable microbial population to be AAP. Five isolates, NM4.16, NM4.18, C4, C9 and C11, were chosen as representative strains for analysis. All grew preferentially on complex carbon sources without vitamin requirements and with an optimal pH of 7.0 to 8.0, with C4 preferring pH 6.0. NM4.18 tolerated the highest pH at 11.0. Optimal temperature for all strains was 28oC (range of 2 to 37oC aside from NM4.16 which survived up to 45oC). C9, C11, and NM4.18 could tolerate 1, 2, and 5% NaCl, respectively. NM4.16 and C4 grew in fresh water medium only. Gelatinase was present in NM4.16 and C4; lipase in NM4.16 and NM4.18; and catalase in C11, NM4.18 and NM4.16. All strains were amylase negative, oxidase positive, Gram negative and non-motile. Of note, isolates were highly resistant to the toxic metal(oid) oxides tellurite, selenite, and metavanadate up to 1000µg/ml except for C11, which was resistant up to only 500µg/ml of metavanadate. All were capable of reducing tellurite to elemental tellurium. In addition to AAP, a physiologically/taxonomically unknown microorganism was found, strain C20. Currently, it is a mystery whether it is a bacterium or yeast. It produces black colonies, reproduces through budding, and is 5µm by 2µm, which is larger than the average bacterium yet smaller than most yeast. 16SrRNA sequencing of this organism as well as the phototrophs will help to solve this puzzle. This study expands our knowledge of diversity in extreme habitats and the potential of microbes for future bioremediation and biometallurgy.

Poster Session

BAM-PT1126 - Behavior of microorganisms in a new state of water under high voltage

Astrid H. Paulitsch-Fuchs[1], Andrea Zsohar[1], Adam D. Wexler[1], Elmar C. Fuchs[1]
[1]*Wetsus, Leeuwarden, The Netherlands*

When a high-voltage direct-current is applied to two beakers filled with water, a horizontal electrohydrodynamic (EHD) bridge forms between the two beakers. This bridge consists of a new state of water in between liquid and solid. It has been shown that E. coli shows increased activity after being transported through this bridge, a surprising result considering that exposure to high voltage is normally used for sterilization. In this work we test the generality of the above mentioned finding3 and study the transport and behavior of other bacteria, algae and yeast cells in this special environment. Organisms were added to one or to both beakers, and the transport of the cells through the bridge was investigated using optical and microbiological techniques. We show that Bacillus subtilis subtilis, Neochloris oleoabundans and Saccharomyces cerevisiae survive the exposure to this hazardous environment, but their behavior is individual. Bacterial and algae cells are drawn toward the anode due to their negative surface charge, whereas yeast cells do not show a preferred flow direction. The activity of Bacillus sub. sub. is dependent on the beaker: cells from the anodic beaker show increased, cells from the cathodic beaker show decreased activity. For algae and yeast, the transport through the bridge is the criterion that decides their activity: N. oleoabundands is weakened by the transport independent from the beaker; and S. cerevisiae reveal, similar to the previously studied E. coli, increased activity after being transported through the bridge. We thus conclusively show that the behavior found for E. coli is highly specific for this bacteria and cannot be generalized to other microorganisms, not even other bacteria.

Poster Session

BAM-PT1128 - Characterization of sulfur metabolizing microbes in cold, saline springs of the Canadian High Arctic

Raven Comery[1], Guillaume Lamarche-Gagnon[1], André Pellerin[1], Boswell Wing[1], Lyle Whyte[1]
[1]*McGill University, Montreal, Canada*

The goal of my research is to characterize sulfur metabolizing microorganisms living in cold saline springs of the Canadian High Arctic. Specifically, my research focuses on microbial communities present in Lost Hammer (LH) and Gypsum Hill (GH) springs, which are the only known cold saline springs in thick permafrost on Earth. GH and LH springs are sulfide/sulfate-rich, and this combined with their saline, cold, and anoxic characteristics make them excellent astrobiological analogue sites, especially for exploring the possibility of microbial life in sulfate-rich deposits on Mars. Previous work has found by 16S rRNA analysis that sulfur metabolism likely plays a major role for the growth and survival of GH spring's microbial community. Preliminary work with LH enrichment cultures has shown that there may be activity by hydrogen-oxidizing, sulfate-reducing bacteria at as low as -20˚C. Further study of these springs is necessary to clarify the extent of the S-metabolizing activity of these organisms. Anaerobically stored samples of sediment and water from LH spring were collected and used to identify S-metabolizing microbes' biosignatures through the analysis of sulfur isotope fractionation patterns. Sulfur isotope fractionation experiments were also performed with hydrogen-enriched LH sediments to determine how the enrichment changes the population's effect on S-isotope composition. To further study this community, it was attempted to isolate psychrophilic sulfide oxidizing, thiosulfate oxidizing, and sulfate reducing bacteria from LH and GH springs. Cultures will be assessed for their ability to metabolize sulfur compounds at low temperatures. Key players of the community will also be characterized through DNA/RNA extraction/sequencing from enrichment cultures and untreated sediments. Overall, LH and GH springs are exciting locations for study due to their extreme conditions.

Poster Session

BAM-PT1132 - The CtrA and GtaI-regulated DprA recombination mediator protein is essential for Rhodobacter capsulatus gene transfer agent (RcGTA) mediated gene acquisition

Cedric Brimacombe[1], Hao Ding[1], Thomas Beatty[1]
[1]University of British Columbia Deptartment of Microbiology and Immunology, Vancouver, Canada

Gene transfer agents are genetic exchange elements that resemble small DNA bacteriophages, however unlike transducing phages, GTAs package and transfer random pieces of the producing cell chromosome to recipient cells. The canonical GTA is found in Rhodobacter capsulatus, termed RcGTA. Many studies have focused on factors affecting production of RcGTA, however the capability of a cell to receive an RcGTA-carried genetic marker has received less attention. We discovered that the knockout of a cell cycle regulator, ctrA, abolished RcGTA-mediated gene acquisition. We also found that recA is required for RcGTA recipient capability, confirming the findings of a prior study. Through a variety of analyses, we discovered that the dprA gene of R. capsulatus is essential for RcGTA-mediated gene acquisition, and that dprA expression is regulated by ctrA and the gtaI quorum-sensing protein. DprA homologues play a key role in the natural transformation of many species, and interact with RecA to facilitate recombination of incoming ssDNA into the chromosome of Streptoccus pneumoniae and Bacillus subtilus. It has also been proposed that DprA alleviates the restriction barrier between naturally transformable Helicobacter pylori strains by binding and protecting incoming dsDNA from nuclease digestion. We purified a 6His-tagged RcDprA protein, and found that it binds both ssDNA and dsDNA, with a preference for ssDNA. We found that 6HisRcDprA can protect dsDNA from endonuclease digestion, and interacts with RecAEc to increase nucleation of RecAEc onto ssDNA. We propose that loss of these activities accounts for the abolition of RcGTA recipient capability in the ΔdprA mutant, and in part the ΔgtaI and ΔctrA mutants. Additionally, we found that, using single-cell analysis, all cells within a population express dprA, indicating that there may be an additional function(s) for this protein. Overall, our results suggest a natural transformation-like pathway of RcGTA-mediated gene recombination into the recipient cell genome.

Poster Session

BAM-PT1134 - Analysis of botulinum neurotoxin type G gene-encoding plasmid in Clostridium argentinense strain 2740

Yoshihiko Sakaguchi[1], Jumpei Uchiyama[2], Tomonori Suzuki[3], Yumiko Yamamoto[3], Koji Hosomi[4], Tomoko Kohda[4], Shigenobu Matsuzaki[2], Tetsuya Hayashi[5], Masafumi Mukamoto[4], Keiji Oguma[3]

[1]Interdisciplinary Research Organization, University of Miyazaki, Miyazaki, Japan, [2]Research and Education Faculty of Medicine, Kochi University, Kochi, Japan, [3]Department of Bacteriology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, [4]Department of Veterinary Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan, [5]Division of Microbiology, Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

Background and aims: Botulinum neurotoxins (BoNTXs) produced by Clostridium botulinum are the most poisonous substances known. Of the eight types (types A to H) of BoNTX, BoNTX type G (BoNTX/G) is produced by C. botulinum type G, which has recently been classified as C. argentinense. Strain 2740 is one representative strain of C. argentinense, and harbors the bontx/G gene possibly on the extrachromosomal genetic element, either bacteriophage or plasmid. We studied the evolutionary features of the extrachromosomal genetic element harboring the bontx/G gene using strain 2740. Methods: (I) The location of the bontx/G gene was confirmed by comparing of the non-toxigenic clone 2740ΔpCAG with the toxigenic clone 2740. (II) The entire extrachromosomal genetic element (pCAG) was sequenced, and its evolutionary traits were examined. Results: (I) Western blotting, Southern blotting, and mouse experiment showed that the pCAG was responsible for the production of BoNTX/G in strain 2740. (II) The complete sequence of pCAG was 140,070 bp with 26.7% G + C content. One hundred thirty-three potential open reading frames, including BoNTX/G, DNA polymerase subunits, and proteins involved in plasmid partition and conjugation, were predicted, suggesting that the pCAG is a large plasmid. Phylogenetic analysis based on the 16S rRNA gene of the host and that of the BoNTX and DNA polymerase III subunits of the pCAG suggested that the progenitor of the bontx/B and bontx/G gene had transferred to the ancestral plasmid of the pCAG and had then evolved in strain 2740. Conclusion: The bontx/B and bontx/G genes may have a common bontx origin, and the pCAG may have evolved in C. argentinense strain 2740.

Poster Session

BAM-PT1136 - High levels of expression of the ctRNA in rhizobial repABC plasmids are necessary for regulation of incompatibility

Cynthia Yip[1], Michael Hynes[1]
[1]University of Calgary, Calgary, Canada

The six plasmids of Rhizobium leguminosarum VF39SM comprise nearly 35% of the bacterium's genome and are all repABC replicons, which are defined by the presence of an operon containing both replication (repC) and partitioning functions (repA and repB) under the control of a single promoter. The repABC operons of the three largest plasmids of VF39SM were found to have strong incompatibility determinants in the non-protein coding regions. However, in all three repABC operons, the intergenic region between repB and repC was the strongest incompatibility factor; this intergenic region has been shown, for most repABC plasmids, to encode a counter-transcribed RNA (ctRNA) that regulates RepC abundance and therefore also the rate of replication initiation. To understand the way in which the ctRNA regulates replication and incompatibility, we carried out mutagenesis on this region from the three largest plasmids of VF39SM using error-prone PCR. Mutants with altered incompatibility were detected by screening for their ability to co-exist in the same cell as the parent plasmid. Mutations that abolished the strong incompatibility phenotype were all localized to the predicted ctRNA promoter regions. Reverse Transcription Polymerase Chain Reaction analysis confirmed that ctRNA was still produced in these promoter mutants, but transcriptional fusions of these mutated promoters to a gusA reporter gene showed a 10- to 50-fold decrease in activity when compared to the wild type promoter. Additionally, deletion of the intergenic region in repABC minimal replicons belonging to the two largest plasmids showed an increase in stability in a rhizobial background, which was found to be due to a significant increase in copy number. For the repABC operons in this study, the intergenic region is critical in establishing incompatibility, stability, and copy number of rhizobial repABC plasmids, and this appears to require a high level of transcription of the ctRNA.

Poster Session

BAM-PT1138 - Functional analyses of *Streptomyces* conjugation system: the molecular function and harmonized expression of the DNA channel, TraB.

Masakazu Kataoka[1], Tetsu Miyatake[1], Mai Kobe[1]
[1]Graduate School of Science and Technology, Shinshu University. Nagano Japan

The *Streptomyces* conjugation system is a very unique system in which the conjugative plasmid is transferred as double stranded DNA (dsDNA). The main molecule for the conjugation is TraB, the DNA channel of FtsK/SpoIIIE family. In this IUMS2014, we show the new aspect of Streptomyces conjugation system by focusing the biochemical, genetic, expression, localization, and interaction properties of the TraB protein from *Streptomyces nigrifaciens* plasmid pSN22. The TraB protein showed ATPase activity in vitro, and the FtsK domain was essential and enough for the activity. Walker type A (270-GMTGSGKT-277) and type B (378-YLLTFEEA-386) domains of TraB are critical to the transfer function of pSN22 derivatives. Point mutations within the Walker type A and B reduced the ATPase activity to half level of the wild type except T277S mutant that keep both transfer capability and ATPase activity. Cellular localization of TraB has been studied. Kosono et al showed membrane localization of TraB of pSN22 (Mol. Microbiol. 1996) by Western blotting and Reuther et al showed that the TraB protein of pSG5 was found in hyphae tip (Mol. Microbiol. 2006). The imaging analysis using fluorescent protein showed the TraB protein of pSN22 was expressed broad membrane region of aerial mycelium, not only hyphae tips. The TraB protein was also found in spores in the early stage. In addition, to confirm that transfer phenomena occurred in aearial mycelia, we introduced disorder of the TraB expression by using TTA codon for *bldA* regulation. The expression of the TraB protein was delayed, although the transfer efficiency was two orders of magnitude lower than that of the wild type, indicating that conjugative transfer of pSN22 took place at aerial mycelium. The decrease of transfer might be caused by small number of DNA channel since expression lag.

Poster Session

BAM-PT1140 - Enhanced production of organic solvent-tolerant extracellular protease by Bacillus subtilis strain KK101 grown on renewable resources

Bartho Okolo[1], Tochukwu Nwagu[1], Stella Maris Obiakor[2], Loveth Nwanze[2], Chinedu Ilorah[2], Evelyn Okperin[2], Rachel Ugwueze[2]

[1]*Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nigeria,* [2]*Department of Microbiology and Biotechnology, Caritas University, Amorji Nike, Nigeria*

Proteases are used in different biotechnological applications and dominate over 60% of the industrial enzyme market. There is a continued search to isolate microorganisms capable of producing copious proteolytic enzymes with unique properties on renewable resources. Several protease-producing bacteria strains were screened for extracellular protease production and the highest producer was selected for further studies. Culture condition and nutrient source studies including ability to tolerate different organic-solvents, nitrogen/carbon sources, and utilization of solid or liquid environmental wastes were carried out. Highest protease producer, Bacillus subtilis strain kk101 grew and elicited protease yield in the presence of petroleum ether, amyl alcohol, diethyl ether, glycerol, iso-octane and chloroform, while methanol led to 50% reduction in extracellular protease yield. Protease production was induced using cocoyam (18.8 U/ml), yam (9.0 U/ml), plantain (11.8 U/ml), guinea corn (12.2 U/ml), maize (9.94 U/ml) as carbon sources and soybean meal (21.1 U/ml), cowpea meal (17.4 U/ml) and blood meal (17.4 U/ml) as nitrogen sources. Plantain peel, bambara nut (Voandzeia subterennea) and cowpea chaff all inhibited protease yield but breadfruit (Artocarpus altilis) chaff elicited protease yield of 17.7 U/ml. Of great interest was the ability of the Bacillus sp to favorably utilize waste waters from food processing for protease production. 100% cassava waste water (from cassava processing mill) supplemented with 0.5% starch gave the protease yield of 27.3 U/ml. Cassava waste water without supplement elicited 24.9 U/ml. Water from corn mill supplemented with 1.6% peptone and 1.5% starch gave 28.1 U/ml protease. Palm oil mill effluent (POME) induced the highest protease production compared to other waste waters. Introduction of 0.06% concentration of isoleucine led to 14.6% increase in protease production and methionine led to 52.1% loss.

Poster Session

BAM-PT1142 - Zombies in bacterial genomes: identification and analysis of defective phage

Scott Mitchell[1], Gabriel Moreno-Hagelsieb[1]
[1]*Wilfrid Laurier University, Waterloo, Canada*

This project is investigating the presence and nature of 'zombies' in bacterial genomes: defective bacteriophage that have become dormant through mutational decay or loss of critical genes, yet have recently been found to carry out important biological functions. Bacteriophage are viruses that infect and reproduce within their bacterial hosts. Phage can either be described as "virulent" or "temperate"; the distinguishing feature between the two is their method of replication. Virulent phage replicate via the lytic cycle, in which phage DNA replicates separately from that of the host's and infected cells are lysed, releasing virions that continue the cycle by infecting other cells. Temperate phage enter a lysogenic cycle; their DNA is integrated with the bacterial genome, becoming a 'prophage' that replicates alongside the host DNA. These prophage can be detected within bacterial genomes using various techniques, including prophage identification tools such as PHAge Search Tool (PHAST) that use database comparisons, tRNA analysis, and hidden Markov scanning to identify prophage sequences and features. A particular sequence may be classified as 'intact,' 'questionable,' or 'incomplete' depending on the region's size and number of known phage genes. The 'questionable' and 'incomplete' sequences are defective phage that have lost critical genes, and these dormant phage often carry genes that are beneficial to the host. It is possible that many of these defective phage are in fact previously virulent phage that have become inserted within the host genome. This study seeks to identify prophage within the genomes of bacterial host species and will examine whether there is a connection between 'defective' phage and previously virulent phage; further, it will characterize how these phage are defective by comparing their sequences to "prototype" phage and identifying which genes have been altered or deleted.

Poster Session

BAM-PT1144 - Soil Carbon Content and Relative Proportion of High Affinity H2-Oxidizing Bacteria Better Predict Atmospheric H2 Soil Uptake Activity than Soil Microbial Community Composition

Mondher Khdhiri[1], Laura Hesse[1], Liliana Quiza[1], Isabelle Lalonde[1], Elena Popa[2], Laura K.Meredith[3], Thomas Röckman[2], Philippe Constant[1]

[1]Institut National de la Recherche Scientifique-Institut Armand-Frappier, Laval, Canada, [2]Institute for Marine and Atmospheric Research Utrecht, Utrecht, Netherlands, [3]Earth Environmental System Science, California, USA

The soil uptake of H2 is responsible for 80% of the global sink of atmospheric H2. The exchange of H2 between the soil and the atmosphere are mainly controlled by the diffusion of this gas combined to microbial production and consumption in soil. H2 oxidation rate (u) is the most difficult variable to parameterize to predict these exchanges. Here, we present a model which combine microbiological and physicochemical variables to parameterize u in soil. Replicated soil samples, showing different H2 oxidation potential, were collected to analyze the composition of microbial community structure, the relative abundance of high affinity oxidizing bacteria (HOB) as well as nutrients, water content and pH. Linear regression models based on carbon content or carbon content and HOB relative abundance in soil succeeded to predict 66 and 92% of u variance, respectively. A second soil survey was undertaken to challenge the model with 14 soil samples collected in agricultural and forest sites. Only the single linear regression fitted with total carbon content predicted u with confidence. This model represents a potential approach to improve resolution where parameterization of u is based on H2 soil uptake rates derived from averaged field observations modulated by soil temperature.

Poster Session

BAM-PT1146 - Nutritional and Microbiological Properties of Cymbium glans from Lower Qua Iboe River (QIR) Estuary, Nigeria

Ime Udotong[1], Justina Udotong[2], Charity Ukot[1]
[1]Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria, [2]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Nutritional, trace elements and microbiological properties of *Cymbium glans* from lower Qua Iboe River (QIR) estuary was investigated. Proximate analysis revealed that the muscle tissue of the shellfish comprises 76.05 - 77.79% moisture, 6.06 - 7.84% ash, 59.15 - 61.25% protein, 4.25 - 4.75% lipid, 6.74 - 22.46% carbohydrate, 5.80 - 6.90% fiber with calorific values ranging from 310.21 to 369.19 Kcal. *C. glans* samples from lower QIR estuary were also found to contain trace elements such as calcium, zinc, copper, magnesium and iron where calcium, zinc and iron levels exceeded the recommended limits set by USDA. Though highly nutritional, *C. glans* exhibited great propensity to bio-accumulate trace elements and microbial contaminants from the environment. The results of the microbiological analysis have revealed that densities of heterotrophic bacteria in freshly collected samples varied between 1.6×10^6 and 1.8×10^6 cfu/g for 330.5 and 594.4 g wet weight of the samples, respectively. On the other hand, the total coliform counts varied between 3.5×10^5 and 2.0×10^6 cfu/g while the range of 6.5×10^5 cfu/g to 5.5×10^6 cfu/g were observed for 330.5g and 594.4g wet weight of *C. glans* from lower QIR estuary. Diverse species of microorganisms were isolated including potential pathogenic species of *Salmonella, Streptococcus, Listeria* and *Escherichia* which may be of serious public health significance.

Poster Session

MEM-PT3001 - A bioinformatics – based characterization of β-glucosidases (GH1 and GH3) in the genus Aspergillus

Richard Auta[1], Paul Hooley[1], Iza Radecka[1]
[1]*University of Wolverhampton, Wolverhampton, UK*

The conversion of cellulose to fermentable sugars is highly dependent on the use a range of efficient enzymes. β-glucosidase is generally responsible for the regulation of the whole cellulolytic process and is a rate limiting factor during enzymatic hydrolysis of cellulose, as both endoglucanase and exoglucanase activities are often inhibited by cellobiose. A bioinformatics based approach to characterize β-glucosidase (GH1 and GH3) encoding enzymes in the genus Aspergillus is described. Each species encodes between 7 and 27 β-glucosidases. Multiple sequence alignments and Phylogenetic tree analysis highlight examples of candidate encoding genes horizontally transferred from bacteria. The application of bioinformatics in the selection and expression of target genes is explained.

Poster Session

MEM-PT3003 - Gas1-based survival screening system for the selection of yeasts with improved protein secretion

Hoon Seo[1,2], Dong-Hyeon Hwang[2], Ohsuk Kwon[2], Doo-Byeong Oh[2], Sang-Ki Rhee[1]
[1]*Soon Chun Hyang University, Asan, Korea,* [2]*Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea*

Gas1 protein is a beta(1,3)-glucanosyltransglycosylase playing an essential role in the assembly of cell wall as localized on the yeast surface through a glycosylphosphatidylinositol (GPI) anchor. When GAS1 gene was disrupted in several yeasts including Saccharomyces cerevisiae, the resulting mutant strains were reported to exhibit hypersensitivity to cell wall-perturbing reagents and temperature-sensitive phenotype together with improved capability of protein secretion due to the loosened cell wall structure. In the present study, functional complementation of cell wall-defective phenotype of GAS1-deletion mutant using recombinant expression of Gas1 protein was employed to generate a screening system for a strain with improved capability of protein secretion. We constructed the expression vectors encoding fusion proteins with N-terminal secretory protein of interest linked to Gas1 proteins without signal sequence. After these vectors were transformed into GAS1-deletion mutant, the growth of the resulting transformants were tested on the agar plates containing cell wall-perturbing reagents. Only the strains expressing Gas1 proteins fused to well secreted proteins showed restored growth phenotype under the cell-wall stress condition. This system can be used to enrich yeasts with improved secretion capability after genome-wide random mutagenesis, which would contribute to the development of super secretory yeasts.

Poster Session

MEM-PT3005 - Determination of lipid-producing capacities of potential oleaginous yeasts

Derya Berikten[1], Ayse Gökdal[1], Merih Kıvanc[1]
[1]Anadolu University, Faculty of Science, Department of Biology, Eskisehir, Turkey

In this century, the environmental problems and limited petroleum reserve on Earth have become more severe. Biodiesel is a useful alternative energy resource and may be used as a substitute for petroleum-based diesel. Biodiesel is made from various vegetable oils and animal fats through transesterification. However, the cost of biodiesel produced with this method is very high. Microbial lipid is considered as a promising feedstock for biodiesel production since it requires short production period, cheaper labor and easiness to scale up. If biodiesel could be produced from lignocellulosics and agro industrial residues, both environment and economy would benefit. There is a small number of microorganisms in nature that can convert carbohydrates into oils. Lipid-producing (oleaginous) microorganisms such as bacteria, yeast, moulds and algae have been known for many years. Some oleaginous yeasts such as Rhodotorula sp., Rhodosporidium sp., Yarrowia lipolytica were reported to accumulate intracellular lipids to as high as 50 % of their cell dry weight. This was typically achieved during growth on nitrogen-limited media with various carbon sources such as sugars, organic acids, hydrocarbons, fats and vegetable oils, and glycerol or crude glycerol. In this study, the lipid-producing capability of oleaginous yeasts is investigated. Ten yeast strains previously isolated from boza and identified by Biolog System as one of Candida rugosa A, one of C. rugosa B, two of Rhodotorula gluntinis, one of R. aurantiaca and five of Yarrowia lipolytica were used in the study. These isolates were grown in nitrogen-limited media and screened for lipid production by the Sudan Black B staining, after which their lipid-producing capacities were studied. The results suggest that Y. lipolytica, C. rugosa A, C. rugosa B and R. gluntinis have the potential for producing fatty acids which can in turn be utilized as substrate for biodiesel production.

Poster Session

MEM-PT3007 - Two-stage maturation of extreme heat-resistant ascospores of *Neosartorya fischeri (Aspergillus fischeri)* involves reduction of bulk water and accumulation of trehalose and trehalose-based oligosaccharides

T.T. Wyatt[1], E.A. Golovina[2], M.R. van Leeuwen[1], H.A.B. Wösten[3], J. Dijksterhuis[1]
[1]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, [2]Laboratory of Biophysics, Wageningen University, and Wageningen NMR Centre, Wageningen, The Netherlands, [3]Microbiology and Kluyver Centre for Genomics of Industrial Fermentation, Institute of Biomembranes, Utrecht University, Utrecht, The Netherlands

Neosartorya fischeri ascospores survive stresses such as high temperature (85 °C) and drought (<0.5 % RH). In this study, acquisition of stress resistance during maturation of *N. fischeri* ascospores was related to accumulation of compatible solutes, the presence of bulk water, and redox stability. Ascospores of 11-day-old cultures were killed by a 2 min treatment at 85 °C, while spores of 15-50 day-old cultures survived this treatment. Spores of 50-day-old cultures even resisted a 50 min treatment at 85 °C. Individual ascospores isolated from 11- and 15-day-old cultures contained 3.9 pg (454 mM) and 12.1 pg (1027 mM) compatible solutes, respectively. This amount increased to 15.4 pg (1051 mM) in ascospores of 50-day-old cultures. The composition of the compatible solutes in the ascospores changed during growth of the culture. Glycerol levels had disappeared in ascospores of 15-day-old-cultures, while mannitol levels decreased after day 20. In contrast, the relative amount of trehalose and trehalose-based oligosaccharides increased until 50 days of culturing. Bulk water, as measured by electron spin resonance (ESR) spectroscopy, was much higher in spores of 11-day-old cultures when compared to spores of 15- to 50-day-old cultures. Ascospore maturation also coincided with increased redox stability. This stability gradually increased during maturation. Dry heat storage of 3 days at 60 °C didn't affect the spin probe immobility or the redox stability of the polar cytoplasmic environment of dried ascospores. However, the redox stability of the more hydrophobic cytoplasmic environment (possibly in the proximity of lipid membranes) did decrease due to dry heat storage. Taken together, this study distinguishes two maturation stages of ascospores. The first stage is accompanied by a reduction of bulk water in the spores, the second stage is characterized by an increase of trehalose and TOS. Redox stability build up was observed during both stages.

Poster Session

MEM-PT3009 - New insights into Penicillium diversity

Cobus Visagie[1], Jos Houbraken[1], Karin Jacobs[2], Keith Seifert[3], Rob Samson[1]
[1]CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands, [2]University of Stellenbosch, Stellenbosch, South Africa,
[3]Eastern Cereal And Oilseed Research Centre, Ottawa, Canada

Penicillium is an important group of moulds. Members are common in indoor environments acting as spoilage organisms and air allergens, as well as in nature where it breaks down dead organic matter. It is a diverse genus with 317 species described from a diverse range on habitats. With the exploration of Penicillium communities from new habitats, this number is fast increasing. In a survey of Penicillium communities from three Fynbos biome sampling sites in South Africa discovered 61 species, of which 25 are new. In a survey of fungal communities in house dust collected from around the world, 50 species were found and 12 that are previously unknown. Sequence data have become crucial for uncovering the true diversity in fungi. It also provides for accurate identifications that is possible also to non-experts in the field. This is, however, only true when you have a complete sequence dataset to compare your strains with. To achieve this, strains from the CBS-KNAW culture collection and private collection of the Applied and Industrial Mycology group (DTO) housed at the same institute in the Netherlands, are extensively being sequenced. And similar to surveys from unique habitats, we are finding a large number of new species that was sitting in these collections. The recent Houbraken et al. (2012) study on the 6 new species related to the commonly found P. chrysogenum, as well as the 9 new species in the P. lagena complex are examples of this. In this presentation we provide insights into the diversity of the genus Penicillium. We provide information on the distribution of species or communities based on the two surveys we have completed and provide notes on species identification in such a diverse genus.

Poster Session

MEM-PT3011 - Diversity of endophytic yeasts in sugarcane leaves in Thailand

Savitree Limtong[1], Pannida Khunnamwong[1]
[1]*Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand*

Endophytic microorganism have been defined as the microorganism that reside the whole or part of their life cycle inside plant tissues without causing any damage or showing negative effect on host plant. This work aimed to study the diversity of endophytic yeasts in tissues of sugarcane leaf in Thailand. One hundred and two samples of sugarcane leaf were collected from thirty-two sampling sites in 12 provinces during January to April 2012. These 12 provinces were in the central and north-eastern parts of Thailand, where the average maximum temperature was 32-36°C and average minimum temperature was 22-25°C throughout the year. Endophytic yeasts were isolated from surface-sterilized sugarcane leaves and 130 isolates were obtained from 83 sugarcane leaf samples. On the basis of the D1/D2 region of the large subunit rRNA gene sequence analysis, 114 strains were identified to 27 known species in 12 genera belonging to Basidiomycota *viz. Bullera coprosmaensis, Bullera japonica, Bullera pseudoalba, Bullera sinensis, Cryptococcus cellulolyticus, Cryptococcus flavescens, Cryptococcus flavus, Cryptococcus laurentii, Cryptococcus nemorosus, Cryptococcus rajasthanensis, Dioszegia takashimae, Jaminaea angkoriensis, Occultifur externus, Pseudozyma antarctica, Pseudozyma aphidis, Pseudozyma hubeiensis, Pseudozyma prolifica, Pseudozyma rugulosa, Pseudozyma tsukubaensis, Rhodosporidium toruloides, Rhodotorula marina, Rhodotorula minuta, Rhodotorula mucilaginosa, Sporidiobolus pararoseus, Sporobolomyces vermiculatus, Tremella globispora* and *Trichosporon asahii* and six species in four genera of Ascomycota *viz. Candida tropicalis, Cyberlindnera rhodanensis, Meyerozyma caribbica, Meyerozyma guilliermondii, Pichia kudriavzevii and Pichia rhodanensis*. Eight strains had similar or identical D1/D2 region sequences with the five undescribed species in the genus *seudozyma, Cryptococcus* and *Sporobolomyces*. Eight strains represented three new yeast species in the genus *Occultifur, Wickerhamiella and Tremella*. The most prevalent species was *Meyerozyma guilliermondii* with a 16% frequency of occurrence followed by *Cryptococcus flavescens* (10%) and then *Pichia kudriavzevii* and *Pseudozyma aphidis*, each with a frequency of occurrence of 6%.

Poster Session

MEM-PT3013 - Diversity of yeasts in corn phylloplanes and their antagonistic activities against pathogenic fungi

Rungluk Kaewwichian[1,2], Wichien Yongmanitchai[1], Savitree Limtong[1]
[1]Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand, [2]National Center for Genetic Engineering and Biotechnology, PathumThani, Thailand

One hundred and twenty-six yeast strains were isolated by the enrichment technique from the phylloplane of 65 corn leaf samples collected from eight districts in four provinces (Lop Buri, Nakhon Ratchasima, Nakhon Sawan and Phetchabun) of Thailand. On the basis of the D1/D2 domain of the large subunit rRNA gene sequences analysis and phylogeny 121 strains were identified as 38 known species in 17 genera belonging to Ascomycota i.e. Candida catenulate, Candida conglobate, Candida glabrata, Candida mesorugosa, Candida orthopsilosis, Candida pseudointermedia, Candida quercitrusa, Candida rugosa, Candida sojae, Candida sorboxylosa, Candida tropicalis, Cyberlindnera rhodanensis, Geotrichum phurueaensis, Hanseniaspora guilliermondii, Hanseniaspora opuntiae, Hanseniaspora thailandica, Kluyveromyces marxianus, Kodamaea ohmeri, Meyerozyma caribbica, Meyerozyma guilliermondii, Millerozyma acaciae, Millerozyma koratensis, Pichia galeiformis, Pichia kluyveri, Pichia kudriavzevii, Torulaspora globosa, Wickerhamomyces edaphicus and Yamadazyma philogaea and Basidiomycota i.e. Cryptococcus flavescens, Cryptococcus heveanensis, Cryptococcus laurentii, Rhodosporidium fluviale, Rhodosporidium paludigenum, Rhodosporidium toruloides, Rhodotorula taiwanensis, Sporidiobolus pararoseus, Sporidiobolus ruineniae and Sporobolomyces nylandii. Two strains had identical sequences with an undescribed Candida species the other two strains were identical with Cryptococcus aff. laurentii. One strain represented a novels species in the genus Yamadazyma. The prevalent species were Kodamaea ohmeri, Meyerozyma caribbica and Rhodotorula taiwanensis, each of which having a frequency of occurrence of 7.9%, followed by Candida tropicalis, Hanseniaspora opuntiae, Pichia kudriavzevii and Rhodosporidium fluvial, each with a frequency of occurrence of 6.4%, and then Cryptococcuslaurentii (5.6%). Only one strain namely Torulaspora globosa DMKU-RK547 significantly inhibited Fusarium moniliforme, Rhizoctonia solani and Helminthosporium oryzae, which were pathogenic fungi of rice and its biocontrol activity seem to be based on the production of siderophore and volatile compounds. The nutrient concentrations had significant different effect on fungal growth inhibition by T. globosa DMKU-RK547 and its living cells are necessary for the biological control activity.

Poster Session

MEM-PT3015 - Analysis of fungal communities in the nasal cavities of patients with allergic rhinitis

Yang Won Lee[1], Daniel Croll[2], Jae Hoon Cho[3], Yu Ri Kim[1], Won Hee Jung[4]
[1]Department of Dermatology, School of Medicine, Konkuk University, Seoul, Korea, [2]Michael Smith Laboratories, University of British Columbia, Vancouver, Canada, [3]Department of Otorhinolaryngology-Head and Neck Surgery, School of Medicine, Konkuk University, Seoul, Korea, [4]Department of Systems Biotechnology, Chung-Ang University, Anseong, Korea

Recent advances in sequencing technologies have enabled the determination of the microbial diversity of various sites of the human body. These culture-independent sequencing methods could eliminate bias toward microorganisms that are able to grow in the standard laboratory medium; they have been used in a number of studies to identify microbial communities on the human skin. The characterization of microbial diversity provides insight into the differences between healthy and diseased skin, such as that seen in psoriasis and atopic dermatitis. Allergic rhinitis (AR) is an inflammatory condition of the nasal mucosa caused by environmental allergens, such as pollen, animal dander and mold, and it affects approximately 14% of the adult population. In this study, we investigated how nasal fungal communities are influenced by AR. The diversity of fungal communities on the nasal vestibule skin surface, which is the most anterior part of the nasal cavity, of patients with AR was analyzed by the culture-independent pyrosequencing method and compared with that of healthy individuals. We identified a total of 66 genera from both AR samples and healthy controls, and found that Malassezia predominated in the nasal vestibule skin surface. Species-level analysis identified a total of eight different Malassezia species and found that M. restricta is the most abundant species in the site. The diversity of fungal communities from the AR samples and that of healthy controls were compared using the Shannon diversity index (SDI). Although high interpersonal variation was observed, some of the AR samples displayed significantly higher SDI than healthy controls at genus-and species-level suggesting the potential influence of AR on fungal communities in the nasal vestibule. Overall, this study successfully compared the diversity of fungal communities in the nasal cavities of AR patients and that of healthy individuals.

Poster Session

MEM-PT3017 - Speciation in motion in Saccharomyces paradoxus

Guillaume Charron[1], Jean-Baptiste Leducq[1], Christian R. Landry[1]
[1]*Université Laval, Quebec City, Canada*

Speciation implies the development of a reproductive barrier between populations that originated from a common ancestor. Speciation has been studied for more than a hundred years and even with the tremendous progress genetics made in the last decades, many fundamental questions remains on the molecular basis of this process lying at the heart of planetary biodiversity. As most speciation studies constitute a retrospective look at the speciation process, models at early or intermediate stages of divergence would give us new insights about the unfolding of molecular steps required in order to reach the full-fledged species status. Compared to Saccharomyces cerevisiae, which lineages distribution was greatly influenced by anthropic activities and domestication, S. paradoxus distribution seems rather untouched by human influence and could be linked to ecological factors. North-American S. paradoxus population is known to be composed of two principal lineages, an American and a European lineage that was introduced more recently in a transoceanic migratory event. A third lineage was identified as a singleton isolate in Pennsylvania. We recently isolated more strains from this third lineage in eastern Québec province and later determined that the two American lineages were distributed and phenotypically divergent in a manner that suggested the influence of ecological factors. Here we report experimental evidence for partial reproductive isolation between and within the two North-American populations of S. paradoxus. These yeasts might be a great model for the molecular study of speciation as they give us access to a continuum of reproductive isolation with which it will experimentally be possible to isolate the molecular factors of speciation.

Poster Session

MEM-PT3000 - Effect of Trichoderma harzianum on strawberry plant growth and fruit yield

Carlos Manuel Bucio-Villalobos[1], María Luz Espinosa Carmona[1], Fidel René Díaz Serrano[1], Oscar Alejandro Martínez Jaime[1], Juan José Torres Morales[1]
[1]*Guanajuato University, Irapuato, Mexico*

An experiment was performed under greenhouse conditions where strawberry plants of the variety "Camino Real" were grown on coconut fiber or volcanic rock (tezontle) substrates. A working suspension containing T. harzianum (strain C4) concentrated at 10-7 conidia/ml was used to watering using 50 ml/per plant/per month. ANOVA and Tukey tests were used for statistical analysis. T. harzianum did not show statistically significant effect on the number of leaves per plant (19.8 versus 20.1 in the control group), as compared to the coconut fiber substrate for which the number increased up to 23.9 leaves per plant when compared to the tezontle substrate (16.0 leaves per plant). Similar results were obtained on the use of the fungus as it did not increase significantly the fruit weight when compared to the control (9.0 and 8.5 g/fruit, respectively). The increase in the number of leaves shown by the coconut fiber substrate yielded fruits with higher weight (9.7 g/fruit) compared to the fruits produced on the tezontle substrate (7.8 g/fruit). There was no a high variation between treatments on the fruit diameter. Brix values had a pattern opposite to that found for plant growth and fruit yield; 7.6° Bx was obtained on the T. harzianum group, and 7.0° Bx value on the control, while the stress of the plants developed on tezontle substrate favored fruit sugar concentration (8.3° Bx) as compared to fruits obtained on the coconut fiber substrate (6.3° Bx). T. harzianum was recovered from 50 % of roots inoculated with that fungus, while none (0.0 %) recovered from the control group. On this work we concluded that the organic coconut fiber substrate had a positive effect on plant growth and fruit yield, so it performed better than the mineral tezontle substrate; also, the fungus T. harzianum had no effect on those parameters.

Poster Session

MEM-PT3002 - Fruiting-inducing activity of glycolipids and their analogous compounds against Pleurotus ostreatus

Takeshi Nishimura[1], Yumi Magae[1], Seiji Ohara[1]
[1]Forestry and Forest Products Research Institute, Tsukuba, Japan

Fruit body differentiation is an important process for mushroom cultivation. External factors like light, temperature, nutrition conditions are known to be effective on the fruit body differentiation. However, the molecular mechanism underlying fruit body differentiation is barely understood; the hormones or external signal molecules have been not identified. In our trials to find chemical substances that effectively stimulate the fruiting of Pleurotus ostreatus (oyster mushroom) under laboratory conditions, we found that the sucrose ester of fatty acids (SE), triterpenoid glycoside (saponin) could induce the fruit bodies in P. ostreatus. In addition, 3-O-alkyl-D-glucoses were also effective where the activities correlated with the alkyl chain group. 1),2),3) From the common structure of these substances that a sugar linked to a hydrophobic component, glycoconjugates, generally known to be involved in a variety of biological events like cell signaling and differentiation, were possibly suggested . In this conference, our further trial to find chemical substances which stimulate fruiting at the significantly low concentrations will be presented. For this purpose, the chemical library consisting of various glycoconjugates and their analogous compounds is now being constructed. The paper disc method is basically applied to the test of fruiting-inducing activity.4) Since now, a glucosylceramide (GlcCer) from a mushroom and glyceroglycolipid analogs having a glucose (GlcDAG) or a cellobiose moiety (Glc2DAG) were found to be effective when the carbon numbers of acyl chain lengths were appropriate. In addition to these, further novel substances and the structure-activity relationship will be discussed.
Reference: 1) Nishimura T., Magae Y., Ohara S. (2004) Glyocobiol.14, 1085, 2) Magae Y., Nishimura T., Ohara S. (2005) Mycol. Res.109, 374-376, 3) idem (2009) Curr. Chem. Biol. 3, 231-237, 4) Kawai G. and Ikeda Y. (1983) BBA, 719, 612-618. Acknowledgment: This work was supported by JSPS KAKENHI Grant Numbers 24658160

Poster Session

MEM-PT3004 - Controlling the growth of Dekkera bruxellensis by using potassium metabisulphite in recycled fermentations for fuel alcohol production

Sandra Regina Ceccato-Antonini[1], Anna Livia Paraluppi[1], Ana Paula Guarnieri Bassi[1], Vanda Renata Reis[1]
[1]Universidade Federal de São Carlos, Dept Tecnologia Agroindustrial, Araras - Sao Paulo - Brazil

Sulphite treatment is the most common way to prevent grape must spoilage in winemaking because the yeast Saccharomyces cerevisiae is particularly resistant to this chemical whereas for non-Saccharomyces it is highly toxic. The use of potassium metabisulphite (PMB) in alcoholic fermentation for fuel alcohol production to control yeast contaminants was not reported earlier. In this work, we evaluated the effect of adding PMB in the concentration of 250 mg/L (which was optimized in previous work) to control the growth of Dekkera bruxellensis, one of the most important contaminant yeasts of the alcoholic process, utilizing recycled fermentation. Pure and co-cultures of S. cerevisiae (industrial strain PE-2) and D. bruxellensis (strain CCA155) were utilized in flask fermentations with sugar cane juice as substrate along six fermentation cycles lasting 12 h each. Cells were centrifuged after each fermentative cycle and inoculated in a new fermentation medium. PMB was added at the start of each fermentation cycle. Growth was monitored by plating the samples in YPD without and with actidione to estimate the colony numbers of S. cerevisiae and D. bruxellensis, respectively. Analysis of alcohol production, pH and residual sugars were also taken. In the initial fermentation cycles, the colony number of S. cerevisiae decreased but at the end of all cycles, the colony number was higher than the initial value when PMB was added. However, the colony number of D. bruxellensis was reduced by half. In co-culture without addition of PMB, the colony number of D. bruxellensis increased 100 times along the fermentative cycles. Although the growth of the contaminant yeast may be controlled by the usage of PMB, without great impact on the growth of S. cerevisiae, the alcohol production decreased significantly in fermentation with PMB, around 30% when compared to pure fermentation of S. cerevisiae without PMB. Support: Fapesp (2011/17928-0; 2012/03401-3).

Poster Session

MEM-PT3006 - Novel trehalose-based oligosaccharides from extreme stress-tolerant ascospores of *Neosartorya fischeri (Aspergillus fischeri)*

T.T. Wyatt[1], M.R. van Leeuwen[1], G.J. Gerwig[2], E.A. Golovina[3,4], F.A. Hoekstra[3,4], E.J. Kuenstner[5], E.A. Palumbo[5], N.L. Snyder[5], C. Visagie[1], Jan Dijksterhuis[1], A. Verkennis[1], J.P. Kamerling[2], H.A.B. Wösten[6]
[1]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, [2]NMR Spectroscopy, Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, The Netherlands, [3]Laboratory of Biophysics, Wageningen University, and Wageningen NMR Centre, Wageningen, The Netherlands, [4]Laboratory of Plant Physiology, Wageningen University, Wageningen, The Netherlands, [5]Davidson College, Department of Chemistry, Davidson, USA, [6]Microbiology and Kluyver Centre for Genomics of Industrial Fermentation, Institute of Biomembranes, Utrecht University, Utrecht, The Netherlands

Ascospores of *Neosartorya, Byssochlamys* and *Talaromyces* can be regarded as the most stress-resistant eukaryotic cells. For example, they can survive exposure at temperatures as high as 85 °C for 100 min or more. Here we describe the identification and characterization of novel trehalose-based oligosaccharides (TOS) as compatible solutes that are accumulated to high levels in ascospores of the fungus *Neosartorya fischeri*. These compounds are also found in other members belonging to the genus *Neosartorya* and occur in other genera within the order Eurotiales that also include *Byssochlamys* and *Talaromyces*. These oligosaccharides consist of a trehalose backbone with one, two or three glucose molecules attached via an α-1,6 linkage. The tetra- and pentasaccharide, dubbed neosartose and fischerose, respectively, have not been reported in nature before. *Neosartorya fischeri* ascospores that contain TOS and trehalose are more viscous and more resistant to the combined stress of heat and desiccation than the ascospores of *T. macrosporus* that contain predominantly trehalose. TOS glasses have a higher glass transition temperature (Tg) than trehalose, and they form a more stable glass with crystallizing molecules, such as mannitol. Our data indicate that TOS are important for prolonged stabilization of cells against stress.

Poster Session

MEM-PT3008 - ITS region based diversity of Penicillium spp. from Indian Himalaya

Kusum Dhakar[1], Anita Pandey[1]
[1]G B Pant Institute of Himalayan Environment & Development, Kosi-Katarmal, Almora, India

25 species of Penicillium (designated as GBPI_P numbers), isolated from soil samples from high altitudes (1800-3610 m above mean sea level) in Indian Himalaya, have been identified following ITS region (ITS1-5.8S-ITS2) analysis (1). Two of the species (GBPI_P216 and GBPI_P31) were seen closely related to Penicillium pinophilum showing 100 and 99% similarity to AB606412 and AB455516, respectively. While GBPI_P66 and GBPI_P101 showed 99% similarity to P. simile and P. raistrickii, respectively, the species were seen closely related in the phylogenetic tree. Species designated as GBPI_P79 and GBPI_P5 appeared somewhat distant, exhibiting 95 and 97% similarity to Penicillium sp. (EU142904 and HQ738282), respectively. GBPI_P86 showed 99% similarity with two fungal spp., Hypocrea rufa (AB374532) and Penicillium sp. (EU076970). The fungal isolate GBPI_P53 showed 99% similarity to P. citreonigrum. In this case, it was interesting to note that GBPI_ P5 that showed 97% similarity to Penicillium sp. (HQ738282) also appeared close to this species (GBPI_P53). Rest of the species exhibited their similarity (98-100%) to different species of Penicillium namely P. virgatum, P. janthinellum, P. thomii, P. commune, P. jensenii, P. spinulosum, P. chrysogenum, P. ochro-chloron, P. canescens, P. glabrum and P. restrictum. The temperature and pH requirements of these species were found to be between 4 to 37ºC (opt. 24 ºC) and 1.5 to 14 pH (opt. 5 to 9), respectively. Occurrence of cold and pH tolerant species of Penicillium in high altitude soils is attributed to the environmental specificities prevailed under Indian Himalaya. The isolation and characterization of 25 Penicillium species from low temperature environment under mountain ecosystem will have ecological, biotechnological and taxonomic implications in future research. (1) Dhakar K, Sharma A, Pandey A. Cold, pH and salt tolerant Penicillium spp. inhabit the high altitude soils in Himalaya, India. World J Microbiol Biotechnol DOI: 10.1007/s11274-013-1545-4.

Poster Session

MEM-PT3010 - Diversity of the genus Talaromyces based on a polyphasic taxonomy

Neriman Yilmaz[1], Jos Houbraken[1], Rob Samson[1]
[1]*CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands*

The genus Talaromyces was described by Benjamin in 1955 as a sexual state of Penicillium that produces soft walled ascomata that are covered with interwoven hyphae. Pitt (1980) accepted 23 subgenus Biverticillium and 16 Talaromyces species were accepted. During the 1990's, the use of molecular techniques in fungal taxonomy revealed the close phylogenetic relationship of Penicillium subgenus Biverticillium and Talaromyces. Recently, the one fungus one name concept was adopted in the International Code of Nomenclature for algae, fungi and plants. Subsequently, Samson et al. (2011) transferred all Penicillium subgenus Biverticillium species into Talaromyces, accepting 69 species. In our study of the genus Talaromyces, we applied a polyphasic species concept and included morphological, molecular and physiological characters. With this, we observed that the diversity of Talaromyces is much higher than first generally thought, and our data shows that there are close to 90 species in the genus. This does not only include species from unexplored habitats, but also species synonymized previously based on morphology that we are now able to show were in fact distinct. Examples of recent studies showed that Talaromyces purpurogenus actually represents four distinct species. Additionally, of the eight synonyms of T. rugulosus, two of them were shown to be unique, two were synonyms of other species and four that were confirmed as synonyms of T. rugulosus. The main aim of our study is to provide a polyphasic taxonomy of Talaromyces.

Poster Session

MEM-PT3012 - Comparative Genomics and Evolutionary Analysis Reveal Gene Duplication in Hydrophobins of Three Species of Wood-Degrading Fungi

Anthony C Mgbeahuruike[1], Andriy Kovalchuk[2], Fred Asiegbu[2]
[1]University of Nigeria, Nsukka, Nigeria, [2]University of Helsinki, Helsinki, Finland

Hydrophobins are small, secreted proteins playing important roles at different stages of fungal life cycles. Hydrophobins are characterized by the presence of eight cysteine residues arranged in a strictly conserved motif. The inventory and evolutionary analysis of hydrophobin genes from three wood-degrading basidiomycetes, Phlebia brevispora, Ganoderma sp. and Bjerkandera adusta were carried out. Numerous copies of hydrophobin genes were found in the genomes of the three analyzed fungal species. Results of the phylogenetic analysis of the identified proteins showed a high degree of sequence similarity, an indication that the sequences may have arisen from a series of duplication events. The presence of several clusters of adjacent copies of the hydrophobin gene in a particular location in the genome further supports the hypothesis that gene duplication has played a role in the evolution of hydrophobins in the analyzed species. Key words: basidiomycota, gene duplication, gene cluster, phylogeny, evolution, proteins

Poster Session

MEM-PT3014 - Species of genus Aspergillus in wheat grains of Slovak origin

Dana Tančinová[1], Mária Dovičičová[1], Zuzana Mašková[1], Soňa Felšöciova[1]
[1]*Slovak University of Agriculture, Nitra, Slovakia*

The aims of the study were: to determine the actual spectrum of the aspergilli inhabiting the wheat grains of Slovak origin; to bring the information about the mycotoxigenic potency of isolated strains; to record the findings of new species for Slovakia. The exogenous mycobiota was determined by the plate dilution method, the endogenous mycobiota was determined by direct placing of superficially sterilized grains on agar plates. More than 100 pieces of undamaged grains from each sample were superficially sterilized and 100 grains from each sample were placed on plates. Out of 73 investigated samples, 60 samples were inhabited by at least one species of the genus Aspergillus and/or associated perfect genera, namely Eurotium and Emericella. Totally 20 species and 12 species groups were detected. Eurotium amstelodami (45.21%), Aspergillus flavus (42.47%) and E. repens (34.25%) were encountered with the highest frequency (given in brackets). Altogether 68 various extrolites (using thin layer chromatography and LC-MS/MS) were detected in the pure cultures of representative isolates of the species spectrum. Detected mycotoxins were aflatoxins, fumigaclavine, fumigaclavine C, fumitremorgin A&B, cyclopiazonic acid, penicillic acid, ochratoxin A, patulin, sterigmatocystin, verruculogen, viomellein, vioxanthin and xanthomegnins. The isolations of Eurotium appendiculatum, E. cristatum, Aspergillus tubingensis, A. tritici and A. westerdijkiae are the first findings of the species in Slovakia. The endogenous isolation of aflatoxigenic strain A. parasiticus from the wheat grain is the first finding of the species in food commodity of Slovak origin. The aflatoxigenic potency was not found in any of 41 Aspergillus flavus isolates from 28 samples by using the thin layer chromatography. The Paper was supported by the project: Development of International Cooperation for the Purpose of the Transfer and Implementation of Research and Development in Educational Programs conducted by the Operational Program: Education, ITMS code: 26110230085.

Poster Session

MEM-PT3016 - Diversity of Epiphytic Yeasts on Rice Leaves in Thailand

Sasitorn Jindamorakot[1], Somjit Am-In[1], Savitree Limtong[2]
[1]National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand, [2]Department of Microbiology, Facculty of Science, Kasetsart University, Bangkok, Thailand

Three hundred and seventy nine isolates of epiphytic yeasts were isolated from 112 rice leaves from 12 provinces in Thailand. Among them, 354 isolates were identified based on the sequences of D1/D2 domain of 26S rDNA. Two hundred and ninety eight isolates (78.6%) were identified as 50 known species in 18 genera of Bullera (18 isolates), Candida (24 isolates), Cryptococcus (22 isolates), Debaryomyces (2 isolates), Hyphopichia (1 isolates), Jaminaea (1 isolates), Kodamaea (8 isolates), Lodderomyces (1 isolates), Meyerozyma (10 isolates), Pichia (3 isolates), Pseudozyma (125 isolates), Rhodosporidium (10 isolates), Rhodotorula (30 isolates), Sporidiobolus (5 isolates), Sporobolomyces (27 isolates). Torulaspora (2 isolates), Trichosporon (4 isolates) and Wickerhamomyces (5 isolates). Twenty one isolates (5.5%) were identified as undescribed species in Candida (1 isolate), Cryptococcus (9 isolates), Pseudozyma (4 isolates) and Rhodotorula (7 isolates). Furthermore, thirty five isolates (9.2%) differed from the nearest species in 4 nucleotides or more in the D1/D2 domain and were considered to represent 20 new species of Blastobotrys (1 isolate), Bullera (2 isolates), Candida (1 isolate), Cryptococcus (13 isolates), Occultifur (2 isolates), Pseudozyma (8 isolates), Rhodosporidium (4 isolates), Rhodotorula (3 isolates) and Sakaguchia (1 isolates) and the remaining 25 isolates (6.6%) are wating for identify. Yeast-like fungi in the genera Aureobasidium, Acremonium, Cephalosporium, Hyponectria, Melanopsichium, Moesziomyces, Sarocladium, Sporisorium, Syncephalastrum and Ustilago also found on the rice leaves. These epiphytic yeasts will be screened for promising auxins producing strains emphasize on indole-3-acetic acid auxin.

Poster Session

MEM-PT3018 - A study of Botrytis Virus X transmission and vegetative incompatibility in *Botrytis cinerea*

Gregor Kolbe[1], Matt Templeton[2], Mike Pearson[1]
[1]School of Biological Sciences, University of Auckland, Auckland, New Zealand, [2]The New Zealand Institute for Plant & Food Research ltd, Auckland, New Zealand

Botrytis cinerea, commonly known as grey mould is a necrotrophic ascomycete which infects over 200 crops and horticultural species. Currently, control of *B. cinerea* relies heavily on fungicides since there are few host crops where resistant cultivars are available. There are several concerns with the use of fungicides including the emergence of fungicide resistant strains and fungicide residue on the edible part of the crop which is often consumed without further processing. These factors make biological control agents an attractive candidate to add to the *B. cinerea* control tool-box. *Botrytis virus X* (BVX) has some potential as a biocontrol agent for *B. cinerea* as it has been shown to reduce host pathogenicity. However, the only known mode of horizontal transmission of mycoviruses is via hyphal anastomosis and *B. cinerea* has many genetically determined vegetative incompatibility (VI) groups, making it unlikely that two given isolates are able to successfully complete hyphal anastomosis. VI is a potential barrier to virus transmission, although it is suppressed in some fungi during early stages of germination, so called conidial anastomosis. In order to determine whether conidial anastomosis can provide a mechanism for the transmission of BVX in *B. cinerea* a conidial anastomosis assay was optimized for imaging with fluorescence microscopy, in order to study anastomosis of incompatible strains expressing different fluorescent proteins. Six strains from four different compatibility groups and differing BVX status were all able to perform conidial anastomosis at similar rates. An expression vector system optimized for *B. cinerea* with multiple antibiotic resistances and fluorescent protein markers was modified for nuclear localization by engineering a histone-GFP fusion protein using bacterial in-vivo cloning. We demonstrated that, unlike has been reported in other fungi, conidial anastomosis is subject to some degree of VI in *B. cinerea* albeit less than would be expected in vegetative fusion.

Poster Session

VIR-PT2001 - Genetic analysis for Astrovirus gastroenteritis in Saudi children

Mahmoud Aly[1], Aisha Alkhairy[1], Sameera Aljohani[2,3], Hanan Balkhy[1,2,3]
[1]KAIMRC, Riyadh, Kingdom of Saudi Arabia, [2]KFMC, Riyadh, Kingdom of Saudi Arabia, [3]KSAU, Riyadh, Kingdom of Saudi Arabia

Background: Human Astroviruses (HAstV) are a frequent cause of viral gastroenteritis in infants. Many children die annually of viral gastroenteritis. These diseases indirectly put a severe burden on the healthcare system and economy of Saudi Arabia and worldwide. The occurrence of Astrovirus infections and the prevalent Astroviral genotypes that circulate in the Saudi children population so far remain unclear. Objective: The aim of this study was to determine the prevalence of astrovirus gastroenteritis in Riyadh, Saudi Arabia as well as to characterize the Astrovirus genotypic diversity in Saudi Arabia. Methods: Stool samples (570) were collected from King Abdulaziz Medical City hospital. Enzyme-linked immunosorbent assay (ELISA) was used to screen samples for the occurrence of Astroviral infection in both hospitalized and outpatient populations. Viral isolation and RNA extraction from 200 fecal specimens were done by Magnapure. RT-PCR and sequencing techniques were used to detect the prevalence of astrovirus infection. The data collected were analyzed using SPSS 20 software. A lineage tree was using unweighted pair group method with arithmetic mean. Results: The preliminary findings from patients <6 years of age show that 25 fecal samples were positive for Astrovirus infection among tested 200 isolates. The sequencing analysis identified the presence of HAstV1 whereas the sequencing alignment and phylogenetic analysis of type-1 capsid protein gene showed the similarity of Riyadh (Saudi) isolate clustering with isolates from other countries such as Japan, USA, China and Brazil. Conclusion: Twenty five positive cases of Astrovirus infection among children less than 6 years old were detected. Furthermore, HAstV1 was identified to be present in a number of samples. More studies will be required to further explore the Astrovirus gastroenteritis incidence and genetic diversity in Saudi Arabia.

Poster Session

VIR-PT2003 - Sequence-independent amplification coupled with DNA microarray analysis for detection and genotyping of noroviruses in fecal specimens

Haifeng Chen[1], Yuan Hu[2], Huijun Yan[3], Mark Mammel[1], Sunee Himathongkham[4]
[1]Food and Drug Administration,CFSAN, Laurel, USA, [2]Food and Drug Administration, ORA, Jamaica, USA, [3]Sun Yat-sen University, Guangzhou, China, [4]Food and Drug Administration,ORA, Rockville, USA

Noroviruses (NoVs) have high levels of genetic sequence diversities, which lead to difficulties in designing robust universal primers to efficiently amplify specific viral genomes for molecular analysis. We here described the practicality of sequence-independent amplification in conjunction with DNA microarray analysis for simultaneous detection and genotyping of human NoVs in fecal specimens. We showed that single primer isothermal linear amplification (Ribo-SPIA) of genogroup I (GI) and genogroup II (GII) NoVs could be run through the same amplification protocol without using virus-specific primers. Related virus could be subtyped by the unique pattern of hybridization with the amplified product to the microarray. Of the 22 clinical fecal specimens as blinded samples, two were tested GI positive and 18 were GII positive as well as 2 negative for NoVs. A NoV GII positive specimen was also identified as having co-occurrence of hepatitis A virus. The study showed that there was 100% concordance for positive NoV detection at genogroup level between the results of Ribo-SPIA/microarray and the phylogenetic analysis of viral sequences of the capsid gene. In addition, 85% genotype agreement was observed for the new assay compared to the results of phylogenetic analysis.

Poster Session

VIR-PT2005 - The establishment of a rabbit model to elucidate the mechanism of neuroinvasion by an emergent Australian West Nile virus

Willy Suen[1], Natalie Prow[2,3], Wenqi Wang[1], Nicole Broad[4], Roy Hall[2,3], Peter Kirkland[5], Helle Bielefeldt-Ohmann[1,2]
[1]School of Veterinary Science, University of Queensland, Gatton, Australia, [2]Australian Infectious Diseases Research Centre, University of Queensland, St. Lucia, Australia, [3]School of Chemistry and Molecular Bioscience, University of Queensland, St Lucia, Australia., [4]University of Queensland Biological Resources, University of Queensland, St Lucia, Australia, [5]Elizabeth Macarthur Agriculture Institute, Menangle, Australia

In 2011, south-east Australia observed an outbreak of encephalitis involving almost 1000 horses. Amongst the causative arboviruses, a novel strain of virulent West Nile virus, WNV(NSW2011), was identified. Its ability to invade the central nervous system (neuroinvasion) was a common feature of the equine epizootic, resulting in encephalitis or encephalomyelitis with a mortality rate of ~10%. The neuroinvasive potential of WNV(NSW2011) was unusually high for an Australian WNV subtype. But the neuropathogenesis of WNV as a whole group, let alone WNV(NSW2011), has not been well defined. Furthermore, WNV infection in the current animal models, mice and hamsters, does not produce a similar disease profile to that of the equine infection. The current project sets out to explore and establish a New Zealand White (NZW) rabbit (Oryctolagus cuniculus) model to study the neuropathogenesis of this novel Australian WNV subtype. Virus kinetics is assessed by quantifying the viral load (using quantitative RT-PCR and virus titration), identifying the distribution of viral antigen (using immunohistochemistry), and assessing the pathology in neural and extra-neural tissues at various termination time points post-inoculation. Cytokine expression and characterization of virus-induced cell death in the central nervous system (CNS) are also assessed in order to study the host response associated with viral neuroinvasion. We have observed from an initial pilot study that NZW rabbits could be a suitable animal model. Histopathological evidence of encephalomyelitis and a low-grade viremia were observed associated with peripheral WNV(NSW2011) infection, similar to that in the equine host. A complete characterization of the clinical, pathological, virological and immunological outcomes of WNV(NSW2011) infection is underway, via a series of comprehensive challenge trials. Data to be generated from this project will ultimately lay the foundation for future therapeutic/vaccine efficacy trials, as well as flavivirus pathogenesis studies, using the rabbit model.

Poster Session

VIR-PT2007 - Antiviral activity evaluation of the natural plant extract Larrea tridentata in dengue virus infection.

Irais Castillo Maldonado[1,2], Maria Maximina Bertha Moreno Altamirano[2], Javier Morán Martínez[1], Luis Benjamín Serrano Gallardo[1]

[1]*Universidad Autònoma de Coahuila, Torreón, Coahila., México,* [2]*Instituto Politècnico Nacional, México, D. F.*

Dengue is the illness transmitted by Aedes aegypti and albopictus vectors and there is not a vaccine or treatment against this virus. The continuous antiviral search against Dengue is an effort that is winning interest due to the increase of their incidence in the world. Therefore, investigation on new drugs is a necessity and, the screening of plants as a possible source of antiviral drugs is an alternative to be explored. The aim of this study was to evaluate the antiviral activity in vitro of Larrea tridentata for dengue virus serotype 2 (DENV2). The Larrea tridentate plants were collected in the Chihuahuanses´s Desert, to the North of Mexico, dried, pulverized and marinated in methanol. The toxicity of the methanolic extracts of this plant was determined, with the DL50 by in vitro of Artemia saline Assay in plate of 96 wells. The studies to evaluate the antiviral effect of the Larrea tridentata were carried out by infecting VERO and BHK-21 cells with DENV2, and by determining first the Maximum not Toxic Dose that permitted a percentage of the 80% of viability of the cells by the alamarBlue® assay. The antiviral effect was evaluated by infecting the VERO/BHK-21 cells with DENV and the methanolic extracts of the plant under different conditions and observing if a citophatic effect was presented. At the same time the envelope (E) and the nonstructural protein 1 (NS1) synthesis, as indicative of virus replication, were detected by an immunofluorescence and an ELISA assays. The results obtained for the non-Toxic Maximum Dose (DMNT) obtained in Artemia saline for Larrea tridentate were of 60 µg/ml. The Vero and BHK 21 cells viability exposed to the methanolics extracts permits more than 85%, all the antiviral assays indicated that Larrea tridentata decreases the dengue virus replication in VERO and BHK-21 cells.

Poster Session

VIR-PT2009 - Infection rates and phylogenetic analysis of hepatitis G virus (HGV)/GB virus C (GBV-C) among Qatari blood donors

Aisha Khan[1], Nadima Ali[1], Makiyeh Khalili[2], Abdelfatah Nasrallah[1], Tameem Hadwan[1], Naema Al Molawi[2], Raed AbuOdeh[3], Gheyath Nasrallah[1]

[1]Qatar University, Doha, Qatar, [2]Hamad Medical Corporation, Doha, Qatar, [3]University of Sharjah, Sharjah, UAE

Background: GBV-C/HGV (belongs to Flaviviridae family) shares several similarities to HCV including genome structure and rout of transmission. However, its association with human diseases is unknown. The incidence of GBV-C infection has been studied worldwide, however, to our knowledge, no previous studies were conducted in Qatar. Objectives: the goal of this pilot study is to answer preliminary questions about GBV-C in Qatar; this includes studying the GBV-C infection rate in Qatar and how genetically similar tested strains are to previously reported GBV-C isolates in other regions of the world. Methods: 580 blood donor samples were collected and RNA was extracted from plasma, reverse transcribed, then subjected for GBV-C detection by nested PCR using specific primers targeting a 205 bp located in the hypervariable 5'-UTR of the GBV-C. To determine the predominant GBV-C genotype(s), the 5-UTR amplicons (randomly picked from 25 positive samples) were cloned and sequenced. The resulted sequences were assembled and analyzed using CLC and MEGA5 softwares. Results: GBV-C infection rate among all blood donors was 17.1 %. Interestingly, no significant difference was noted in the infection rates among Qatari (17.8 %) and all other non-Qatari nationality groups' lives in Qatar (17.0%). For instance, the infection rate among Indians was 16.2%, Egyptians 18.7%, and Jordanians 21.1%. Sequence analysis of the 25 5'-UTR amplicons yielded the European genotype 2 as the most predominant. Conclusion: although our data showed that GBV-C infection rate in Qatar is relatively higher than many other published reports around the word, yet, it is still close to other neighboring countries. Interestingly, genotype two was reported to be dominant in several countries surrounding Qatar such as UAE, and Kuwait. Finally, these results will broaden our knowledge about GBV-C in Qatar. This should benefit epidemiologists in the region and may have an impact on blood banks screening policy

Poster Session

VIR-PT2011 - Tick-borne encephalitis virus alters membrane structure and replicates in dendrites of primary mouse neuronal cultures

Minato Hirano[1], Kentaro Yoshii[1], Mizuki Sakai[1], Rie Hasebe[1], Osamu Ichii[1], Hiroaki Kariwa[1]
[1]Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Neurological diseases caused by encephalitic flaviviruses are severe and associated with high levels of mortality. However, detailed mechanisms of viral replication in the brain and features of viral pathogenesis remain poorly understood. We carried out a comparative analysis of replication of neurotropic flaviviruses: West Nile virus, Japanese encephalitis virus and tick-borne encephalitis virus (TBEV), in primary cultures of mouse brain neurons. All the flaviviruses multiplied well in primary neuronal cultures from the hippocampus, cerebral cortex and cerebellum. The distribution of viral-specific antigen in the neurons varied: TBEV infection induced accumulation of viral antigen in the neuronal dendrites to a greater extent than infection with other viruses. Viral structural proteins, non-structural proteins and dsRNA were detected in regions in which viral antigens accumulated in dendrites after TBEV replication. Replication of a TBEV replicon after infection with virus-like particles of TBEV also induced antigen accumulation, indicating that accumulated viral antigen was the result of viral RNA replication. Furthermore, electron microscopy confirmed that TBEV replication induced characteristic ultrastructural membrane alterations in the neurites: newly formed laminal membrane structures containing virion-like structures. This is the first report describing viral replication in and ultrastructural alterations of neuronal dendrites, which may cause neuronal dysfunction. These findings encourage further work aimed at understanding the molecular mechanisms of viral replication in the brain and the pathogenicity of neurotropic flaviviruses.

Poster Session

VIR-PT2013 - Abietane diterpenoids suppress replication of influenza virus by blocking the phosphatidylinositol-3-kinase (PI3K)-Akt, extracellular signal-regulated kinase (ERK), and NF-κB signaling pathway

Su-Jin Park[1], Hyung-Jun Kwon[1], Young Min Kim[1], Cho Kyoung-Oh[2], Young Bae Ryu[1], Woo Song Lee[1]
[1]Korea Research Institute of Bioscience and Biotechnology, Jeonbuk , Republic of Korea, [2]Chonnam National University, Gwangju, Republic of Korea

Despite the availability of vaccines and antiviral drugs, influenza remains a major worldwide plague. In this study, we evaluated anti-influenza activity and its underlying mechanisms (such as PI3K-Akt, ERK, and NF-κB signaling pathways) of abietane diterpenoids isolated from Torreya nucifera. As a result, 18-hydroxyferruginol (1) and 18-oxoferruginol (2) exhibited strong anti-influenza activity in post-treatment assay. During the virus replication steps, two compounds inhibited stronger viral RNA in late stages (12-18 h) than in early stages (3-6 h). Moreover, two compounds inhibited the PI3K-Akt signaling involved in the virus replication step at late stage. ERK and NF-κB signaling pathways related to viral replication were also notably inhibited by two compounds. In particular, blockade of PI3K-Akt signaling by these compounds inhibited viral replication via sabotage of influenza ribonucleoprotein nucleus-to-cytoplasm export. These results suggest that abietane diterpenoids may be potent antiviral agents that act by inhibiting the cellular signaling related in viral replication.

Poster Session

VIR-PT2015 - Study of Yellow Fever infection in Gallus gallus domesticus used to 17DD vaccine production, a histopathological approach

Pedro Paulo de Abreu Manso[1], Isabella Carneiro Gonçalves[1], Yuli Rodrigues Maia[1], Yan Salema de Medeiros[1,2], Jessica Maria dos Santos Ferro[1], Igor José da Silva[1], Barbara C E P Dias de Oliveira[1,2], Marcelo Pelajo-Machado[1]
[1]Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, [2]IFRJ, Rio de Janeiro, Brazil

Yellow fever is a viral disease that affects populations on tropical region of South America and Sub Saharan Africa. The yellow fever vaccine is the only way to prevent this viral infection. However, despite being produced for over seventy years, there are no available pathological studies of this virosis in Gallus gallus embryos. This biological system is used to produce the Yellow Fever Vaccine. The goal of this work is to elucidate the histopathological basis of infection in Gallus gallus by 17DD yellow fever virus. To this end, chicken embryos with 9 days of development were infected with the vaccine strain and collected 24, 48, 72 and 96 hours post infection, and processed according to standard paraffin embedding protocols. Five micrometer-thick sections were stained with HE or PAS and observed by brightfield microscopy. Histologically, some embryos appeared mild reaction to viral infection, manifested only by some embryonary development changes. It can be observed by extensive areas of hematopoiesis and ossification in bone marrow of the infected animals, which are not observed in non infected controls. Extensive areas of perivascular hematopoietic activity were observed in the vitellin membrane of infected animals with a quantitative increase of erythrocytic and granulocytic populations that normally populate this region. Few animals present characteristic hepatocyte apoptosis. In addition to these findings, we observed lung and gut epithelial cells budding in some of the animals analyzed, accompanied by apparent vacuolation of these cells. Heart, spleen and kidneys showed no changes, suggesting that these organs may be not involved in this infection. We conclude that: a) the embryos present a variable spectrum of illness, like in humans; b) apparently the bone marrow of infected animals starts before control animals; c) there is an increase of hematopoiesis in bone marrow and in the yolk sac of infected animals.

Poster Session

VIR-PT2017 - The impact of the emergence and re-emergence of different Dengue Virus serotypes in the State of Rio de Janeiro, Brazil

Manoela Heringer[1], Rita Maria Nogueira[1], Ana Maria de Filippis[1], Monique Lima[1], Jaqueline Simões[1], Priscila Nunes[1], Bianca De Santis[1], Simone Sampaio[1], Flavia dos Santos[1]

[1]*Flavivirus Laboratory, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil*

Rio de Janeiro (RJ) has been important for the introduction and spread of dengue viruses (DENV) and over the last 27 years was marked by extensive epidemics. The existence of a continuous surveillance program aims to detect and monitor the circulation of DENV serotypes in the state, where the four serotypes co-circulate. Given the limited options for prevention and control, it has been shown that laboratory diagnosis plays an important role in the Epidemiological Surveillance System, by monitoring infections and confirming new cases. The goal of this study was to describe the epidemiological, laboratory and clinical dengue cases occurred in RJ, from January 2010 to December 2012. A total of 2,833 dengue suspected cases were analyzed, and 1,323 cases (47.5%) were confirmed. The MAC-ELISA confirmed 32.6% of the cases, the RT-PCR, 56.3%, and 33.1% of the cases were confirmed by virus isolation. NS1 capture test confirmed 27.5% of the cases. DENV-2 prevalent in 2010, while during 2011 the prevalent serotype was DENV-1. In 2011 the introduction of DENV-4 was detected, and an outbreak caused by this serotype was reported in 2012. Our analysis has shown that patients with secondary infection had higher risk of presenting severe forms of the disease (OR = 7,87 / 95%IC = 2,15-30,56 / p <0,001). Moreover, the severe forms were more frequent on children <15 years old, and infected by DENV-2 (OR = 1,8 / 95%CI = 0,10-1,22 / p <0,05). From the total of the fatal cases confirmed (n = 67), 60% were due to secondary infections. Fatal cases were more frequent in children <15 years old in 2011 in comparison to other years. The DENV-2 was responsible for 42.8% of deaths in 2010, DENV-1 was identified in 71.47% of deaths in 2011 and in 2012, DENV-4 was responsible for 25% of deaths. Financial support: FAPERJ, CNPq, PAPES VI/CNPQ, CAPES and FIOCRUZ

Poster Session

VIR-PT2019 - Antigenic mutants of the chlorovirus PBCV-1: Evaluation of glycan biosynthesis by sequence analysis of the gene a064r

Garry Duncan[1], David Dunigan[2], James Gurnon[2], James Eudy[3], James Van Etten[2]
[1]Biology Department, Nebraska Wesleyan University, Lincoln, USA, [2]Plant Pathology Department and Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, USA, [3]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, USA

Paramecium bursaria chlorella virus (PBCV-1) is a member of the Phycodnaviridae family (genus Chlorovirus). This large, dsDNA virus (330,611 bp) encodes many proteins not commonly found in viruses. While other viruses use the host machinery for protein glycosylation of capsid proteins, PBCV-1 appears to encode at least some of the machinery to glycosylate its capsid proteins. Five groups of PBCV-1 antigenic mutants have been isolated; the differences in the antigenic phenotypes are correlated with altered glycan structure of the major capsid protein. We sequenced the genomes of eleven antigenic mutant strains of PBCV-1 to a depth of >40X coverage using Roche 454 pyrosequencing technology and discovered that 10 of 11 antigenic mutants had mutations in CDS a064r. Surprisingly, none of the 11 antigenic mutants contained mutations in any of the 20 viral genes suspected of encoding proteins involved in some biochemical event related to glycosylation. Graves et al. (Virology 285:332-345 (2001)) had previously sequenced the a064r gene, which encodes a protein of at least three domains, from four antigenic mutants. All four contained mutations in domain 1 of A064R, which is the glycosyltransferase domain. We have subsequently sequenced the genomes of nine additional antigenic mutants using Illumina's Next Generation Sequencing, and we have sequenced the a064r gene of other antigenic mutants. A pattern for the five classes of antigenic mutants has emerged that shows a relationship between antigenic mutant type and the domain, or region of the domain, in which the mutation occurs.

Poster Session

VIR-PT2021 - Analysis of the Herpes Simplex Virus 1 tegument by flow cytometry

Nabil El Bilali[1], Kerstin Radtke[1], Johanne Duron[1], Roger Lippé[1]
[1]University of Montreal, Montreal, Canada

Herpes simplex virus type 1 (HSV-1) is a multilayered structure particle with a DNA genome surrounded by a capsid, tegument and an envelope. While the composition and assembly of the capsid is well understood, the details about tegument acquisition are still unclear. Proteomics analysis suggests that 23 viral proteins and possibly up to 49 different host proteins are incorporated in the tegument layer of mature virus. These proteins appear to be acquired sequentially from the site of assembly in the nucleus to the site of final envelopment. Studies further suggest that the composition vary among individual viral particles. We hypothesised that this variability affects the infectivity of individual viral particles. We recently developed a flow cytometry approach to individually analyze and enrich nuclear HSV-1 capsids. We extend this powerful technology to assess tegument variability in individual viral particles and probe the infectivity of sorted viral subpopulations. The data reveal that while some HSV-1 tegument proteins have stable stoichiometries among viral particles, others vary significantly. Most interestingly, virions selected for their high content in some tegument proteins appear more infectious than their counterpart with low amount of the same tegument proteins. A detailed analysis of GFP tagged viruses detected significant impact of the label on the incorporation of multiple tegument proteins, an important parameter that should be considered when using tagged viruses. These findings are useful to better understand the composition of viral particles, the interplay among tegument proteins and may ultimately address the difference in infectivity of different strains.

Poster Session

VIR-PT2023 - Emergence of acyclovir-resistant herpes simplex virus 1 affects the outcome of hematopoietic stem cell transplantation

Satsuki Kakiuchi[1,4], Masanori Tsuji[2], Hidekazu NIshimura[3], Mutsuyo Ito-Takayama[1], Chang-Kweng Lim[1], Yukie Yamaguhi[1], Shuichi Taniguchi[2], Masashi Mizuguchi[4], Akira Oka[4], Masayuki Saijo[1,4]
[1]National Institute of Ifectious Dieases, Tokyo, Japan, [2]Toranomon Hospital, Tokyo, Japan, [3]Sendai Medical Center, Sendai, Japan, [4]The University of Tokyo, Tokyo, Japan

Background: Reactivation of herpes simplex virus 1 (HSV-1) frequently occurs and often causes intractable lesions in patients with hematopoietic stem cell transplantation (HSCT) despite the prophylactic acyclovir (ACV) administration. Although high prevalence rate of ACV-resistant (ACVr) HSV-1 in HSCT patients has been suggested, there has been no prospective cohort study on this issue. Objective: The objectives are to unveil the incidence of ACVr HSV-1 emergence in HSCT patients prospectively and to analyze its impact on the prognosis of HSCT. Patients and methods: HSCT patients in Toranomon Hospital, Tokyo, Japan, were enrolled in the study from June 2010 to May 2012. Oropharyngeal (OP) swab samples were collected weekly regardless of symptoms during the period of -6 and 100 days of transplants. Vero and HEL cells were used for isolation. Plaque reduction assay (PRA) was used to assess the sensitivity of HSV-1 isolates to ACV. For the ACVr HSV-1, nucleotide sequence of the viral thymidine kinase (TK) and/or DNA polymerase (DNA-pol) genes was determined to identify the mutations responsible for the resistance. Sensitivity of ACVr HSV-1 to other anti-herpes compounds was also evaluated by PRA. Results: 2800 OP samples were collected from 268 HSCT patients. Of 101 HSV-1 isolates from 40 patients, 34 from 11 patients showed ACV-resistance. Patients with ACVr HSV-1 were significantly less likely to survive. ACV-resistance was attributable to TK and DNA-pol mutations in 6 and 5 patients, respectively. Furthermore, most of the DNA-pol mutants showed cross-resistance to foscarnet, but higher sensitivity to ganciclovir. Conclusion: 15% of HSCT patients shed HSV-1 during the HSCT treatment through reactivation mechanism until day 100 from HSCT. Emergence of ACVr HSV-1 was not uncommon and resulted in a significantly higher mortality. Treatment with foscarnet of DNA-pol-associated ACVr HSV-1 may fail due to high frequency of cross-resistance to foscarnet.

Poster Session

VIR-PT2025 - Equine herpesvirus 1 specific antibody seronegativity is a significant risk factor for developing myeloencephalalitis

Michael Studdert[1], Kemperly Dynon[1], Charles El-Hage[2], Gary Anderson[2], Carol Hartley[1]
[1]The University of Melbourne, Parkville, Australia, [2]Veterinary Clinical Centre, The University of Melbourne, Australia

Most laboratories investigating outbreaks of EHV1 and EHV4 disease including myeloencephalitis rely on serum neutralization and complement fixation tests. SN and CF tests do not discriminate between EHV1 and EHV4 antibodies. EHV1 and EHV4 are major causes of acute upper respiratory tract infections. EHV1 also causes abortion, perinatal foal deaths and myeloencephalitis. In recent years there has been an increase in the incidence of EHV1 myeloencephalitis particularly in the United States. In this presentation we update our interpretation of data from an outbreak of EHV1 myeloencephalitis in which five of 10 pregnant mares developed myeloencephalitis. Three of the five mares became recumbent, developed complications and were euthanized. The diagnosis of EHV1 myeloencephalitis was supported by necropsy findings, polymerase chain reaction identification of the virus and by serology in which an EHV1 specific antibody detection ELISA (Svanovir® EHV1/4 Ab discriminating ELISA; United States Patent 5,922,327) was used. The status of EHV1 infection in the five in contact mares was similarly monitored. 3 of 3 (100%) affected mares for which appropriate sera were available had low or borderline EHV1 antibody titers when first tested on either day 6 or 7 and 0/5 (0%) unaffected mares had low or borderline titers when first tested on either day 7 (4 mares) or day 13 (one mare). The P-value (Fisher's Exact test) for this comparison is P=0.018. All 10 mares were EHV4 antibody positive when first tested and these titers remained more of less stable during the episode.

Poster Session

VIR-PT2027 - Identification of cellular factors that interact with the C-terminal domain of herpes simplex virus 1 UL24 protein

Miharisoa Rakotomanga[1], Angela Pearson[2]
[1]Institut National de la Recherche Scientifique, Montreal, Canada

The herpes simplex virus type 1 (HSV-1) gene UL24 is conserved among all Herpesviridae family members. The multifunctional UL24 protein is important for viral replication in vitro as well as for pathogenesis in a mouse model of ocular infection. The N-terminal domain of UL24 is highly conserved between orthologs of the protein. This domain is located in the nucleus when expressed alone, and is sufficient to induce nucleolar modifications. Little is known about the role of the C-terminal domain of UL24 which is poorly conserved. When expressed alone, this part of the protein is found in the cytoplasm and the Golgi apparatus. In this study, we tested the hypothesis that the C-terminal domain of UL24 can interact with cellular factors involved in cytoplasmic functions of the protein by performing a yeast two-hybrid screen. We screened a HeLa cell library fused to the GAL4 activation domain using UL24 aa 190-269 as bait. From a total of 9x10^7 transformants screened, four clones fulfilled the criteria for interaction of gene products. The gene sequences of these clones all corresponded to the coding sequence of the mature form of C1QBP. The C1QBP protein is a multifunctional protein mainly located in the cytoplasm and mitochondria, and has been shown to play a role in different viral infections. Studies are ongoing to characterize the UL24-C1QBP interaction and to investigate its role in HSV-1 infection.

Poster Session

VIR-PT2029 - Molecular partners of the Herpes simplex virus type-1 gM glycoprotein

Imane EL Kasmi[1], Kerstin Radtke[1], Roger Lippé[1]
[1]*University of Montreal, Montreal, Canada*

Herpes simplex virus type 1 (HSV-1) affects 70-80 % of the world population and causes facial mucosal lesions commonly referred to as cold sores. The virus replicates in the nucleus, then crosses the two nuclear envelopes by budding and fusion by a poorly characterized process that likely involves many regulatory proteins. Recently it has been shown that the viral glycoprotein M (gM), a protein known to modulate membrane fusion, is targeted specifically to the both nuclear membranes early in the infection but is later redirected to the TGN. The glycoprotein N (gN), a viral protein conserved among related viruses interacts with gM and dictates its localization in these other viruses. To better understand the mechanisms that govern gM localization, we examined the putative gM/gN interaction in HSV-1. We confirm that the two proteins form a complex but that gM is dominant over gN in HSV-1. This interaction is functionally relevant as overexpression of gN strongly induces the formation of syncytia in infected cells. We are now screening partners that may interact with gM and modulate its location and function. These approaches help us clarify the targeting mechanism of gM and the importance of this targeting in the virus.

Poster Session

VIR-PT2031 - Involvement of human herpesvirus-6 infection in the development of myalgic encephalomyelitis/chronic fatigue syndrome, fibromyalgia and encephalopathy

Modra Murovska[1], Santa Rasa[1], Maksims Cistjakovs[1], Silvija Roga[2], Angelika Krumina[3], Inara Logina[4], Svetlana Chapenko[1]

[1]A.Kirchenstein Institute of Microbiology and Virology, Riga Stradins University, Riga, Latvia, [2]Department of Pathology, Riga Stradins University, Riga, Latvia, [3]Department of Infectology and Dermatology, Riga Stradins University, Riga, Latvia, [4]Department of Neurology and Neurosurgery, Riga Stradins University, Riga, Latvia

Human herpesvirus-6 (HHV-6) is a neurotropic virus associated with a wide variety of neurologic disorders. Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), fibromyalgia (FM) and encephalopathy (EP) are serious diseases of the central nervous system that often are caused by a viral infection, but in most cases their etiology remains unknown. The aim of this study was to determine frequency of HHV-6 infection and to evaluate its possible significance in the development of ME/CFS, FS and EP. Peripheral blood samples of 50 patients with ME/CFS, 15 - with FM and 90 apparently healthy blood donors, as well as 15 blood and brain tissues' samples from 15 autopsies with unspecified encephalopathy were analysed for the presence of HHV-6 infection. Nested PCR was used to detect viral genomic sequence in peripheral blood, cell-free blood plasma and tissue DNA, real-time PCR – to determine HHV-6 load in peripheral blood leukocytes (PBL) and brain tissues. HHV-6 genomic sequence was detected in 19/50 (38%), 9/15 (60%), 23/90 (25.5%) and in 11/15 (73.3%) DNA samples isolated from peripheral blood of patients with ME/CFS, FM, and apparently healthy donors and from encephalopathy autopsies peripheral blood and brain tissue, respectively. HHV-6 genomic sequence was also detected in 3/19 (15.8%) and 4/9 (44.4%) cell-free blood plasma DNA samples isolated from ME/CFS and FM patients, respectively, versus in none of healthy blood donors. The highest HHV-6 load was detected in EP brain tissues (9431.8 copies/10^6 cells) and in ME/CFS and FM patients PBL (3795.8 copies/10^6 cells and 2230.3 copies/10^6 cells versus 87 copies/10^6 cells in healthy persons). In all HHV-6 positive cases HHV-6B was identified. The higher detection rate of HHV-6 infection and HHV-6 load in EP autopsy materials, ME/CFS and FM patients' blood samples allow suggesting this infection as a potential risk factor for the diseases development.

Poster Session

VIR-PT2033 - Middle east respiratory syndrome Coronavirus in dromedary camel herd, Saudi Arabia

Maged G Hemida[1,2], Ranawaka Perera[4,6], Mohamed AL-hammadi[1], Lewis Siu[5,6], Ming-yuan Li[5,6], Leo LLM Poon[4,6], Linnda Saif[8], Abdelmohsen Alnaem[9], Malik Peiris[4,5,6]

[1]Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, Saudi Arabia, [2]Department of Virology, Faculty of Veterinary Medicine, Kaferelsheik University, Egypt, [3]Centre of Influenza Research, The University of Hong Kong, Hong Kong SAR, China, [4]HKU-Pasteur Pole, The University of Hong Kong, Hong Kong SAR, China, [5]School of Public Health, The University of Hong Kong, Hong Kong SAR, China, [6]Key Lab. of Protein and Peptide Pharmaceuticals, Chinese Academy of Sciences - University of Tokyo Joint Laboratory of Structural Virology and Immunology, Beijing, China, [7]Food Animal Health Research Program, Veterinary Preventive Medicine, [8]Department, OARDC/The Ohio State University, Wooster, USA, [9]Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, Saudi Arabia.

Ongoing transmission of MERS-CoV to humans underscores the need to understand the animal sources of zoonotic infection. MERS-CoV RNA has been detected in dromedary camels and dromedary infection was shown to precede human infection. We carried out a prospective study in a dromedary camel herd (70 animals) in Al-Hasa through the peak calving season (December-February) to document the virological features of MERS-CoV infection in these animals. Virus isolation in Vero-E6 was attempted from 7 positive nasal swab and faecal specimens that had copy number >104 per µl in the original sample in the UpE PCR assay. The full genome of MERS-CoV was obtained directly from the clinical specimens and the phylogenetic trees were constructed. MERS-CoV viruses circulating in dromedaries over a period of 1 month were genetically identical over the full 30100 nucleotide genome. The full genome sequence of MERS-CoV from camels in this study is 99.9% similar to other clade B human MERS-CoV genomes. The mutation rate of 0 (95% credible interval 0 – 2.7x10-6) nucleotide substitutions per site per day. This revealed a lower mutation rates compared with that observed in humans. Genome organization of the MERS-CoV from camels was identical to the virus in humans. The dromedary viruses phlyogenetically clustered within clade B of MERS-CoV and were most closely related to the strain MERS-CoV_FRA/UAE and with MERS-CoV detected in Buraidah. We found 6 nucleotide mutations in the S gene unique to these dromedary viruses. Of these, 3 were non-synonymous (S457G, L773F and V810I). In conclusion, the genetic stability of MERS-CoV within this dromedary herd over a one-month period and the high MERS-CoV sero-prevalence previously reported in dromedaries suggest that dromedaries may be the natural reservoir of the virus, rather than being a spill-over or intermediate host.

Poster Session

VIR-PT2035 - Recombinant coronavirus nucleocapsid protein as a diagnostic antigen

Rebecca Willmot[1], Ian Jones[1], Robin Gopal[2], Maria Zambon[2]
[1]School of Biological Sciences, University of Reading, UK, [2]Public Health England, Colindale, UK

Coronaviruses (CoV) primarily infect the upper respiratory and gastrointestinal tract of birds and mammals making them an important class of infections for agriculture, industry and human health. In 2003 an endemic of severe acute respiratory syndrome (SARS) resulted in approximately 8000 infections with a 10% mortality rate. This discovery added to four coronaviruses previously documented as being able to infect and cause disease in humans; OC43, HKU1, 229E and NL63. Recently a new coronavirus infecting humans has been described, the Middle Eastern respiratory syndrome coronavirus (MERS-CoV). Both SARS and MERS are thought to have originated from coronaviruses found in bats which were transmitted to man through different intermediate vectors. The coronavirus N protein is a ~60kDa protein found associated with the genome of the virus. Despite a common function in RNA binding CoV N proteins are antigenically distinct making them useful as diagnostic antigens for tests of seroconversion. To assess the MERS-CoV N protein as a diagnostic antigen we have expressed a soluble full length His-tagged N protein in E.coli. Recombinant N protein was purified to homogeneity by IMAC chromatography and was observed as a single species of the predicted molecular weight with minimum breakdown. When used as a capture antigen in ELISA tests with a number of human CoV positive sera, recombinant MERS-CoV N protein was shown to react strongly with MERS positive sera but not with sera from other CoV infections. Similar data was obtained by western blot. These data suggest recombinant MERS-CoV N protein is a suitable antigen for sero-surveillance. The expression of a number of other CoV N proteins to provide a mini array of N proteins for tests of a variety of human and animal sera for their history of coronavirus infection will be described.

Poster Session

VIR-PT2037 - Characterisation of the severe acute respiratory syndrome - Coronavirus polymerase complex

Lorenzo Subissi[1], Mickael Bouvet[1], Bruno Canard[1], Isabelle Imbert[1], Etienne Decroly[1]
[1]*CNRS/AMU, Marseille, France*

SARS-Coronavirus (CoV) and Middle East respiratory syndrome- CoV (MERS-CoV) are emerging viruses causing severe pneumonia associated with high mortality rates. The replication of SARS-CoV is driven by a replication/transcription complex (RTC) made of 16 viral nonstructural proteins (nsp). The RNA-dependent RNA polymerase (RdRp) nsp12 is a key enzyme of the SARS-CoV RTC machinery. In this work, we show that the RNA polymerase activity of SARS-CoV nsp12 is strongly enhanced by a protein complex formed by nsp7 and nsp8, allowing primer-dependent processive RNA synthesis. In addition, this ternary complex can also catalyse de novo RNA synthesis, making this tripartite complex theoretically able to replicate the whole coronavirus genome. The active polymerase complex was also shown to interact with nsp14, a bi-functional protein containing an mRNA cap-specific (guanine-N7-)-methyltransferase and a 3’-5’ exoribonuclease (ExoN) domain. We demonstrate that nsp14 hydrolyses double-stranded RNA (dsRNA) with a 3' to 5' directionality. Interestingly, a single mismatched nucleotide at the 3’-end of a dsRNA molecule mimicking an erroneous replication product acts as a substrate for nsp14 ExoN. Using primer/template combinations mimicking errors during RNA replication, we demonstrate that the addition of nsp14 to the active polymerase complex allows mismatch correction. This proofreading mechanism, unique in RNA virus world, might be involved in both correcting replication errors and may might resistance to polymerase inhibitors such as nucleoside analogues. These results help understanding how coronaviruses ensure their genome stability.

Poster Session

VIR-PT2039 - PRD1-BF1 drives VEGF-mediated innate immune suppression in tumor vasculature

Rozanne Arulanandam[1], Cory Batenchuk[1], Fernando A. Angarita Celis[2], Sophie Cousineau[1], Carolina Ilkow[1], Jean-Simon Diallo[1], Brian Lichty[3], Christina Addison[1], J. Andrea McCart[2], John C. Bell[1]
[1]CICR - OHRI, Ottawa, Canada, [2]Mount Sinai Hospital, Toronto, Canada, [3]McMaster University, Hamilton, Canada

Wound healing is a complex process that requires the co-regulation of angiogenic and immunosuppressive mechanisms. During oncogenesis, these processes are exploited to promote tumor vascularization and the evasion of anti-tumor immune responses. While Vascular Endothelial Growth Factor (VEGF) has shown to impact the success of cancer immunotherapies such as oncolytic viruses, direct evidence of VEGF-mediated immunosuppression in tumor vasculature has yet to be characterized. We establish that upon loss of contact inhibition, VEGF/VEGFR2 signaling through Erk1/2 and Stat3 leads to an up-regulation, nuclear localization and activation of the master repressor of the immune response, PRD1-BF1/Blimp-1, in human endothelial cells. While PRD1-BF1 does not contribute to the mitotic effects of VEGF, it represses ~60% of the genes targeted by VEGF to suppress innate immunity. In vivo characterization of this response demonstrates that PRD1-BF1 is specifically up-regulated in tumor vasculature, where it functions as a key determinant of immunosuppression.

Poster Session

VIR-PT2041 - Oncolytic effect of Newcastle Disease Virus in a human cell line of diffuse large B-Cell lymphoma

Diana Sánchez[1], Rosa Elena Sarmiento[1], Gabriela Cesarman[2], Rosana Pelayo[3], Luis Núñez[1], Luis Alberto Medina[2]
[1]Faculty of Veterinary Medicine (FMVZ)- National Autonomous University of Mexico (UNAM), Mexico DF, Mexico, [2]National Cancer Institute (INCan), Mexico DF, Mexico, [3]Oncology hospital, National Medical Center S.XXI - Mexican Institute of Social Security (IMSS), Mexico DF, Mexico

Among the treatment options that are currently being investigated for the treatment of cancer include oncolytic Viruses; viruses that naturally or associated with genetic modification possess the ability to kill cancer cells. Newcastle disease virus has been proposed as a promising therapeutic agent because it has shown a selective ability to kill cancer cells, associated with both a direct effect (due to replication) as immunostimulant. Among the types of cancer for which this virus has been tested in vitro include the following human cells: breast cancer, colon carcinoma, anaplastic astrocytoma, cervical adenocarcinoma. In relation to hematologic malignancies, it has been observed that the virus has oncolytic potential of malignant T cell lines. The aim of this study was to evaluate the oncolytic potential of LaSota strain in a human cell line of Diffuse Large B Cell lymphoma (DHL4). A vaccine strain LaSota was propagated in embryonated specific pathogen free chicken 9 days old. The viral titer was obtained in medium infective dose in chicken embryos . The DHL4 cell line was reproduced and maintained in RPMI -1640 medium supplemented with fetal bovine serum , glutamine and antibiotics. These cells were infected at a multiplicity of infection of 100. Necrosis and apoptosis was assessed by flow cytometry using Annexin V - fluorescein isothiocyanate / propidium iodide. The experiments were carried out at 48 and 72 hours. Data analyzes were performed using the FlowJo 9 and Prisma 6 softwares. It was observed that the virus was able to induce apoptosis in cells, with more evident effect at 72 hours post- infection. We conclude that this strain of virus could also be considered as an alternative therapy for Diffuse Large B Cell Lymphoma.

Poster Session

VIR-PT2043 - Regulation of PKR activation by orf virus E3 proteins

Yeu Yang Tseng[1], Fong Yuan Lin[1], Shun Fang Cheng[1], Wei Li Hsu[1]
[1]Graduate Institute of Microbiology and Public Health College of Veterinary Medicine National Chung Hsing University, Taichung, Taiwan

Orf virus belongs to parapoxvirus in the family of Poxviridae. E3 proteins encoded by E3L gene is one of the orf viral proteins contribute to evasion of host immunity. E3L gene is conserved in viruses of Poxviridae; vaccinia virus (VV) is the most studied virus in the family. Amino acid sequence alignment indicated that OV E3 shares 31% identity with the VV E3, which acts as double-stranded RNA (dsRNA) binding protein that helps virus evades interferon (IFN) response and promotes viral growth. Our preliminary results indicated that in addition to the full length protein, E3L gene also encoded an N-terminal truncated isoform (shE3) using the second ATG codon downstream of the initiation ATG. While the function of VVE3 has been extensively studied, little is known about OVE3. In this study we demonstrated full-length E3 expresses throughout the cell, whereas shE3, without deduced nuclear localization signal (NLS), predominantly remains in cytoplasm. As evidenced in the in vitro and in vivo assay systems, despite the difference in cellular distribution, both E3 isofoms harbor binding ability with double-stranded RNA (dsRNA)-activated protein kinase (PKR) and dsRNA that in turns inhibits PKR activation leading to decrease of the translation initiation factor eIF2-α subunit phosphorylation. This study demonstrated for the first time that the OV E3 isofoms physically interacts with PKR and its substrate, the presence of shE3 isoform in the course of orf virus infection, and also their potential impact on PKR activation. Since shE3 shares similar abilities to OVE3 in the respects of dsRNA binding and PKR interaction, considering the difference in cellular distribution and expression kinetics, whether OV shE3 acts in concert with or serves as a competitor of full-length E3 in evading cellular defense system requires further investigation.

Poster Session

VIR-PT2045 - Identification and classification of Torque teno mini virus in Taiwan

Kuang-Liang Hsiao[1], Yan-Jun Lin[1], Chiung-Ling Lin[2], Li-Yu Wang[3], Hsin-Fu Liu[1,2]
[1]Institute of Bioscience and Biotechnology, National Taiwan Ocean University, Taiwan, [2]Department of Medical Research, Mackay Memorial Hospital, Taiwan, [3]Department of Medicine, Mackay Medical College, Taiwan

Torque teno mini virus (TTMV) is a member of human Anellovirus with a closed circular genome of single-stranded DNA. The first Anellovirus, Torque teno viruses (TTVs), spread worldwide with extremely high prevalence. There is no information concerning about TTMV in Taiwan. In this study, TTMV DNA was detected from two different populations; 131 plasma and 120 serum samples were collected from Han Chinese in Taipei and from Eastern Taiwanese indigenes in Hualien respectively. Conserved untranslated region of TTMV genome was detected by using semi-nested PCR. TTMV DNA presented in 88.5% (116/131) and 95.8% (115/120) in Han Chinese and eastern Taiwanese indigenes. It is interesting to note that this difference is statistically significant ($p = 0.033$). The coding region sequence of TTMV was obtained by semi-nested inverted long-range PCR. PCR products were cloned and sequenced for phylogenetic analysis. A total of 10 TTMV isolates were obtained of which 3 isolates obtained from Han Chinese and 7 from eastern Taiwanese indigenes. Phylogenetic analysis was performed with 22 known TTMV in the GenBank. The Maximum-Likelihood tree was constructed based on aligned nucleotide sequences of viral open reading frame 1 by using MEGA5 package with best fitting GTR+G+I model and statistically evaluated by 1,000 replications of bootstrap. In our result, TTMV can be classified into 5 major clades; 2 of our isolates are closely related to Japanese strains, whereas others represent eight novel genotypes. Three isolates from the same individual belong to 2 different groups indicating that TTMV infected host with intra- and inter-genotypes mix-infection. In conclusion, eight novel genotypes were identified, and human TTMV can be further classified into at least 5 phylogenetic groups in current state.

Poster Session

VIR-PT2047 - Full genome analysis of four circoviruses newly detected in lower vertebrates (fishes, frogs and a turtle)

Zoltán László Tarján[1], Judit Pénzes[1], Andor Doszpoly[1], Mária Benkő[1]
[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences , Budapest, Hungary

In the past couple of years, members of the Circoviridae family have been demonstrated in representatives of a sharply increasing number of animal species worldwide. Moreover, the metagenomic approaches led to the discovery of numerous novel circoviral sequences in different environmental samples, such as sewage waters, fecal samples, raw meat products, as well as in novel vertebrate and invertebrate hosts. The first circoviruses found in fish have been described in Hungary recently. By screening of lower vertebrates, we detected several novel circovirus-like sequences in samples from fish, amphibians and a reptile. Here we report the full genomic sequence of the putative circoviruses, found in a common bream (Abramis brama), in a European eel (Anguilla anguilla), in two captive-bred green tree frogs (Litoria caerulea) and in a red-eared slider (Trachemys scripta elegans). Initial detection of the viruses was by a consensus nested PCR targeting the most conserved (~ 350-bp) fragment of the replication-associated protein gene (rep). For the acquisition of the entire genome, we used inverse nested PCRs with specific primers designed from the respective rep sequences. The full rep sequence of each of the four viruses showed clear homology to its counterparts in previously described circoviruses, however the putative capsid protein genes were more divergent. Accordingly, the size of the full genomes also showed considerable differences. In phylogenetic analyses, the two piscine and the frog circoviruses clustered into the Circovirus genus. However, the genome arrangement of the slider virus resembled that of cycloviruses, recently proposed as a novel genus of the Circoviridae. The eventual pathogenic role of these newly characterized viruses is unclear. The bream and the frogs seemed to be healthy. The eel showed typical signs of the so-called cauliflower disease. The red-eared slider had a concurrent adenovirus infection and one-sided bacterial otitis. Support: OTKA K100163.

Poster Session

VIR-PT2049 - Screening of human papillomavirus types in a local geographic region: can we consider the HPV types variability important?

Ledy Oliveira[1], Silvia Cavalcanti[1]
[1]*Universidade Federal Fluminenese, Niterói, Rio de Janeiro, Brasil*

Objective: The goal of the present study was to trace the spectrum of uncommon HPV types in Rio de Janeiro state according to cytological status. A meta-analysis study population included 891 women whose cervical smears have been taken from 2000 to 2010, recorded in five Brazilian studies. MY09/11 consensus primers were used to detect HPV DNA. HPV typing was performed by restriction fragment length polymorphism analysis (RFLP) following polymerase chain reaction (PCR) amplification. Results: The patients aged 14-79 years. Most women presented normal cytology (75.5%). The overall HPV prevalence was 32.8% (292/891). The frequency of HPV infection to each cytological category was: normal (19%), ASCUS or AGUS (35.7%), LSIL (80.4%), HSIL (78.3%), and CA (96.6%). Thirty-four different HPV genotypes have been identified. HPV 16 was found in 27.7% of HPV positive persons. The types 26, 35, 39, 51, s82 (high risk), 40, 42, 44, 54, 70, LX100 (low risk), and 32, 62, 69, 83, and 84 (undetermined), were found in a low frequency and, except HPV 39, were not associated with abnormal cytology. Their predominance in young women, the fact that they have been more easily found in co-infections and the unlikely link with risk for cervical cancer allow labeling them as opportunistic infections of little clinical importance. Conclusion: The establishment of these infections, regardless to be classified in low or high risk, may be useful to focus HPV types that effectively can cause damage to cervical tract and they still are not in the vaccination.

Poster Session

VIR-PT2051 - Viruses and ancient remains: a molecular approach based on anelloviruses

Sandra Bedarida[1], Philippe Biagini[1]
[1]*UMR 7268, ADES _ Aix-Marseille University / EFS / CNRS Viral Emergence and Co-evolution Unit, Marseille, France*

Palaeomicrobiology is a growing area in microbiology, dedicated to the detection and characterization of micro-organisms in archaeological or historical specimens. The study of past infections is essential to improve our understanding of historical epidemics, the origins of current infections and anticipate the possible re-emergence of eradicated pathogens. The first studies focused on bacterial infections, which have marked human history, such as Yersinia pestis, Mycobacterium tuberculosis and leprae, but few took an interest in viruses, except studies on the Spanish flu. Building on the works of paleogenetics and old bacteriosis studies, we developed a methodology targeting non-integrated DNA viruses. Such viruses are a material of choice, because they present better conservation and lower fragility than RNA viruses over time. Due to their ubiquitous status and high prevalence in present human population, we selected viruses of the Anelloviridae family, in order to optimize this approach. Our rigorous methodology respects strict aseptic conditions, implements negative controls and positive controls from the investigators, with the aim of minimizing potential contamination by the investigators or the environment. In this study, we selected dental pulp samples since this particular tissue offers optimal protection for the intrinsic genetic material and is likely to be colonized by pathogens through vascularization. A wide variety of samples have been selected coming from distinct locations, different periods and various environments: specimens of the Roman era (France, 2nd c.) the Middle-Ages (France, 8th-10th c.), victims of the plague buried in mass graves (Venice, 14th-18th c.; Marseille, 1712) and a cemetery of soldiers from Napoleon's Great Army (Kaliningrad, 1812). Following the results obtained with anelloviruses, this sensitive and efficient methodology was exported to other pathogens, like Variola or Hepatitis B viruses. Our molecular approaches and results are exposed.

Poster Session

VIR-PT2053 - The structure of viroids

Tamara Giguère[1], Charith Raj Adkar-Purushothama[1], François Bolduc[1], Jean-Pierre Perreault[1]
[1]*Département de Biochimie, Université de Sherbrooke, Sherbrooke, Canada*

Viroids are small single-stranded RNA pathogens causing significant damage to plants. Since they do not encode for any proteins they are dependant solely on their structure for their infection, replication, and propagation. As many as 30 species of viroids have been discovered and they are classified into two families, viz., Pospiviroidae and Avsunviroidae. Family members of the Pospiviroidae replicate in the nucleus with an asymmetric rolling circle mechanism and are predicted to have a rod-like structure. Where as members of the Avsunviroidae family replicate in the chloroplast in a symmetric rolling circle fashion with the help of an autocatalytic hammerhead motif and are thought to have a branched structure. Thus far, elucidation of the secondary structures of viroids has been limited due to the exhaustive and time consuming classical approaches. Hence, the method of high-throughput selective 2'-hydroxyl acylation analyzed by primer extension (hSHAPE) has been adapted for the probing of viroid structure. The data obtained by this approach were then used as the input for computer-assisted structure prediction. The resolution of the structures of all of the members of the Avsunviroidae family and many viroids from the Pospiviroidae family provided a global view of the complexity of these infectious non-coding RNAs. For instance, for the Avsunviroidae, the structural differences between the two polarities, and any plausible tertiary interactions, were also analyzed. Interestingly, the structures of the (+) and (-) strands were found to be different for each viroid species. In case of Pospiviroidae, many structures from all of the different genera were probed and each had its unique characteristics. Surprisingly, some species had a very different structure then previously predicted. To conclude, this compendium of viroid structure will surely help to better understand its biology and pathology.

Poster Session

VIR-PT2055 - HIV-1 Tat protein induces the production of IDO in human monocyte derived-dendritic cells through a direct mechanism: effect on T cells proliferation

Remi Planes[2], Elmostafa Bahraoui[1,2]
[1]Université Paul SABATIER, Toulouse, France, [2]INSERM U1043, Toulouse, France

During HIV-1 infection, an increase of indoleamine 2,3 dioxygenase (IDO) expression, and dendritic cells (DC) dysfunction were often associated with AIDS disease progression. In this work, we showed that Tat induces IDO protein expression and activity in a dose dependent manner by acting at the cell membrane level. Using different Tat-fragments, we show that the N-Terminal domain, Tat 1-45, but not the central region, Tat 30-72, is sufficient to induce the expression of active IDO. Tat protein is also able to induce several cytokines in MoDCs, including IFN-γ, a strong inducer of IDO. In order to understand whether IDO is induced directly by Tat protein or indirectly following IFN-γ production, complementary experiments were performed and showed that: i) at the kinetic level, Tat induced IDO expression before the production of IFN-γ ii) treatment of MoDCs with Tat-conditioned medium was unable to stimulate IDO expression, iii) coculture of MoDCs in a transwell cell system did not allow IDO expression in MoDCs not previously treated by Tat, iv) direct contact between Tat-treated and untreated MoDCs was not sufficient to induce IDO expression in a Tat-independent manner, and v) treatment of MoDCs in the presence of IFN-γ pathway inhibitors, Jak I and Ly294002, inhibited IFN-γ-induced IDO but had no effect on Tat-induced IDO. At the functional level, our data showed that treatment of MoDCs with Tat led to the inhibition of their capacity to stimulate T cell proliferation. This impairement was totally abolished when the stimulation was performed in the presence of 1MT, an inhibitor of IDO activity, arguing for the implication of the kynurenine pathway. By inducing IDO, Tat protein may be considered, as a viral pathogenic factor, in the dysregulation of the DC functions during HIV-1 infection.

Poster Session

VIR-PT2057 - Role of silent mutations in adaptation of RNA bacteriophage to inhibitory environment

Akiko Kashiwagi[1], Ryu Sugawara[1], Fumie Tsushima-Sano[1], Tomofumi Kumagai[1], Tetsuya Yomo[2,3]
[1]Hirosaki University, Hirosaki, Japan, [2]Osaka University, Osaka, Japan, [3]ERATO JST , Osaka, Japan

To investigate how mutations that do not alter amino acid sequence can be positively selected, we performed a thermal adaptation experiment of single stranded RNA bacteriophage Qβ where the culture temperature was increased from 37.2°C, 41.2°C, to inhibitory temperature of 43.6°C in a stepwise manner in three independent lines. The phage became able to grow at the inhibitory temperature following 2 months of serial daily passage. Whole-genome analysis showed that 8 mutations were commonly observed in all three lines. Reconstruction and fitness analyses of Qβ containing only mutations common to all three lines indicated that five mutations that did not result in amino acid sequence changes but increased the amplification ratio appeared in the course of adaptation to growth at 41.2°C. Moreover, these mutations provided a suitable genetic background for subsequent mutations, altering the fitness contribution from deleterious to beneficial. These results clearly showed that silent mutations play important roles in adaptation of Qβ to inhibitory environments.

Poster Session

VIR-PT2059 - Gut homing and HIV-1 permissiveness are specifically regulated by retinoic acid in CCR6+ T-Cells

Delphine Planas[1,2], Annie Gosselin[1], Patricia Monteiro[1,2], Jean-Philippe Goulet[3], Sandrine DaFonseca[1], Aurélie Cleret-Buhot[1,2], Mohammed-Ali Jenabian[4], Jean-Pierre Routy[5,6], Petronela Ancuta[1,2]

[1]CHUM-Research Center, Montréal, Canada, [2]Department of Microbiology, Infectiology and Immunology, Université de Montréal, Montréal, Canada, [3]CATRaGENE, Montréal, Canada, [4]McGill University Health Center, Montréal, Canada, [5]Division of Hematology, McGill University Health Center, Montréal, Canada, [6]Immunodeficiency Service, McGill University Health Center, Montréal, Canada

The majority of HIV-infected CD4+ T-cells are localized in gut-associated lymphoid tissues (GALT). The integrin β7, CCR6, and CCR9 mediate the gut-homing and their expression is induced by retinoic acid (RA), a metabolite of vitamin A produced by the GALT dendritic cells. We previously identified CCR6 as a marker for memory CD4+ T-cells permissive to HIV and demonstrated that RA (retinoic acid) selectively increased HIV replication in CCR6+ T-cells by interfering with yet uncharacterized mechanism at entry and/or post-entry levels. In search for the identification of new molecular determinants of HIV permissiveness, we performed a genome-wide analysis of gene expression in RA-treated CCR6+ versus CCR6- T-cells. Among 15,303 "present calls", 1,538 and 1,285 probe sets were modulated by RA in CCR6- and CCR6+ T-cells, respectively, with only 466 probe sets being regulated by RA in both subsets (p-value <0.05). Gene classification using Gene Ontolog revealed that RA-treated CCR6+ T-cells expressed a specific transcriptional signature that included known HIV permissiveness factors, while RA-treated CCR6- T-cells expressed genes linked to HIV resistance. A number of transcripts were validated by real-time PCR and flow cytometry as being differentially expressed in RA-treated CCR6+ and CCR6- T-cells. We identified a molecular signature associated with HIV permissiveness in RA-treated CCR6+ T-cells. This signature includes RARESS3 or RA-induced gene 1 (RIG-I); the transcription factor KLF2 that regulates CCR5 transcription; CCR5, a major HIV co-receptor; CXCR6, a minor HIV co-receptor also involved in cell-to-cell transmission of HIV; CCR9, a chemokine receptor essential for the migration in lamina propria. At the opposite, RA-treated CCR6- T-cells upregulated expression of ABCA1, a known HIV restriction factor. Our studies reveal that transcriptional changes induced by RA in CCR6+ T-cells are associated with HIV permissiveness and gut-homing potential thus, providing a molecular mechanism for preferential HIV replication in GALT CD4+ T-cells.

Poster Session

VIR-PT2000 - High throughput sequencing analysis reveals genetic variability and selection pressure in different murine norovirus genomic regions during in vitro replication

Axel Mauroy[1], Bernard Taminiau[2], Carine Nezer[3], Elsa Ghurburrun[1], Denis Baurain[4], Georges Daube[2], Etienne Thiry[1]

[1]Veterinary Virology and Animal Viral Diseases, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, Belgium, [2]Food Microbiology, Department of Food Sciences, Faculty of Veterinary Medicine, University of Liège, Belgium, [3]Quality Partner, Liège, Belgium, [4]Unit of Eukaryotic Phylogenomics, Department of Life Sciences, University of Liège, Belgium

Murine norovirus (MuNoV), a single stranded positive sense RNA virus belonging to the Caliciviridae family, is considered as a representative model for human norovirus infections, one of the most important etiological cause of both epidemic and sporadic gastroenteritis cases worldwide. Four open reading frames are described into its genome: ORF1 codes the non-structural (NS) proteins, including the viral RNA dependent RNA polymerase (RdRp); ORF2 codes the single capsid protein (VP1), wherein two domains are present: a relatively conserved domain (“shell”) and a more variable domain (“protruding”); ORF3 codes a minor structural protein; and ORF4, currently only found in viruses genetically related to MuNoV codes a virulence factor. In this study, we demonstrated by high throughput sequencing that, during serial passages of MuNoV in cell culture, the substitution rates, estimated by Bayesian inferences, did not significantly differ across the five targeted genomic regions except one. These rates were similar in four genomic regions encompassing partial non-structural 1-2 protein (NS1-2)-, NS5-, NS6-, NS7 (RdRp)- and VP1-coding sequences (coding the conserved part of the protein also including the ORF4 region). In the partial minor structural protein-coding region, this substitution rate was however estimated to be at least one log higher when expressed as substitution/site/day. The precise localisation of the detected nucleotide point mutations (substitution, deletion and insertion) were reported as well as the quantitative increase or decrease of the sequences harbouring them along ten cell culture passages. The non-silent amino acid mutations were also depicted in 3D models for four out of the five studied regions. These results have important implications for different norovirus research fields, especially in terms of diagnosis, classification methodology and genetic evolution.

Poster Session

VIR-PT2002 - Emerging viruses associated with gastroenteritis cases in Kolkata, India

Triveni Krishnan[1], Sumio Shinoda[2]
[1]National Institute of Cholera and Enteric Diseases, Kolkata, India, [2]Okayama University of Infectious Diseases, Okayama, Japan

Noroviruses and Astroviruses were associated with acute viral gastroenteritis besides rotaviruses and adenoviruses, among children and adults in Kolkata, India. The deduced amino acid sequence of the 172bp nucleotide region of the RNA polymerase gene showed a clustering of the Kolkata NoV strains NVKOL277 and NVKOLNC14 with strains from Brazil and Djibouti respectively. Strain NVKOL290 showed closest homology with a strain from Italy. Strain NVKOLN21 showed 100% homology with previously reported Indian strain and another Italian strain. Strains NVKOLN26 and NVKOLN34 showed varied identity with Japanese and USA strains respectively. The Kolkata strain NVKOLNC14 shared 100% aa identity with Indian, Japanese and US strains. Phylogenetic analysis of the 282bp region of the capsid gene of Kolkata strains revealed they were divergent and clustered with Bristol/GII.4 [n=8], Fayetteville/GII.13 [n=7], Seacroft/GII.6 [n=5] NoVs. Strain IDH340 clustered with Hawaii/GII.1; IDH1521 clustered with Tiffin/GII.16 and IDH495 and IDH500 clustered with Toronto24/GII.3. Two virulent, recombinant NoV strains were also detected; the strain Hu/NoV/IDH1501/2009/IND was a NoV recombinant strain with genes for GII.1-like polymerase (RdRp) and GII.13-like capsid whereas the strain Hu/NoV/IDH1873/2009/IND was a NoV recombinant strain with GII.5-like RdRp gene and capsid gene being GII.13-like. Partial molecular characterization of Astroviruses detected among infants, children and adults showed that ORF1a (289bp) and ORF2 (449bp) of 12 HAstV positives were potential inter-genotype recombinants. Sequence alignment and comparison of partial ORF1a region of HAstVs detected during the study indicated several were novel variants of genotype HAstV8 while one was a novel variant of genotype HAstV2. Sequence alignment and comparison of partial ORF2 region of HAstVs indicated that they showed close identity to HAstV1 (n=10), HAstV2 (n=1) from USA and HAstV3 (n=1) from Germany. Sequence analysis of complete ORF1a, ORF1b and ORF2 indicated that the astroviruses were novel variants divergent from hitherto reported strains.

Poster Session

VIR-PT2004 - Evaluating the laboratory diagnostic response against severe cases of Dengue infection, 2012-2013, São Paulo State, Brazil.

Mariana Sequetin Cunha[1], Vivian Regina Silveira[1], Adriana Yurika Maeda[1], Sarai Joaquim Santos Silva[1], Renato Souza Pereira[1], Iray Maria Rocco[1], Michele Higa Froes[2], Ana Lucia Rodrigues Oliveira[1], Ivani Bisordi[1], Akemi Suzuki[1]

[1]Instituto Adolfo Lutz, São Paulo, Brazil, [2]Centro de Vigilância Epidemiológica, São Paulo, Brazil

Dengue fever is the most prevalent arthropod-borne viral infection in the world, with an estimated 2.5 billion people living in areas under risk of infection. The virus belongs to the family Flaviviridae, genus Flavivirus, and has four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Due to unplanned and uncontrolled urbanization, sewer and waste management systems, substandard housing and water accumulation, which have created ideal conditions for its primary vector Aedes aegypti, dengue fever incidence has increased in the past years. Albeit it has a low mortality rate, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which are more serious manifestations often associated with secondary dengue virus infections may occur, mainly in areas where more than one serotype circulates simultaneously. DHF is characterized by plasma leakage and a hemorrhagic diathesis that is apparent by the time of defervescence, typically after five days of fever. During the period between June 2012 and July 2013, 16.033 dengue diagnosis requests were sent to the Instituto Adolfo Lutz, São Paulo, Brazil, from different municipalities. A total of 378 were death cases with dengue suspicious, and therefore IgM MAC-ELISA assay in-house, real-time PCR (qRT-PCR) and indirect immunofluorescense assay (IFA) were performed. Samples used for diagnosis were serum, blood, liver, spleen or lungs. 78 cases were confirmed by at least one of these tests, and one tested positive for Hantavirus IgM. MAC-ELISA tested positive in samples varying from day 1 to 46 after onset of symptoms. qRT-PCR was able to amplify dengue virus from day 1 to 12, while IFA from days 1 and 4. qPCR and IFA demonstrated 5 DENV-1 and 15 DENV-4. Adolfo Lutz Institute is a reference laboratory, and therefore plays an important role on dengue diagnosis and surveillance in Brazil and its differential diagnosis.

Poster Session

VIR-PT2006 - Human genetics and flaviviruses infection in Africa

Cheikh Loucoubar[1], Oumar Faye[1], Fatim Ba[1], Olivier Telle[2], Abdourahmane Sow[1], Gamou Fall Drame[1], Richard Paul[2], Ousmane Faye[1], Anavaj Sakuntabhai[2], Amadou Alpha SALL[1]
[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]Institut Pasteur, Paris, France

Dengue is one of the most important vector borne disease distributed worldwide. In Africa, dengue disease was thought to be rare but recent outbreaks suggest that substantial parts of Africa may be at a tipping point in terms of dengue transmission with enhanced activity. However, the difference in severity of dengue in Africa comparatively to other continents may be explained partially by differences in human genetic background. In order to address dengue threat in Africa, it is important to assess the role of genetic and non-genetic factors and their interactions. The objectives of the project are to study epidemiological aspects and human genetic variants conferring susceptibility to dengue infection and severe dengue disease in African populations. We will first work on dengue determination and prevalence before identification of human genetic variants which confer susceptibility to infection and severe dengue disease in African populations using family-based genetic linkage and association methods in a Senegalese family-cohort and case/control from outbreak in rural and urban settings. The genetic markers identified can be used in models predicting risk of dengue epidemic in African populations together with other factors including mosquito, climate and viral factors. Preliminary results indicates suggestive linkage of markers on chromosome 12 around 96 cM for Dengue (LOD score = 1.9, p-value = 0.00167) and 112 cM for Zika (LOD score = 2.2, p-value = 0.000763). Interestingly these markers are close to OAS genes, located on chromosome 12 at 113 cM, known to be implicated in West Nile using mouse model (Brinton, 2002; Guénet & Desprès, 2002 and 2003) and candidate gene for Dengue. These results suggest a role of OAS genes or genes around or in linkage disequilibrium in the susceptibility to flaviviruses infection. However, further investigations have to be done on this chromosome 12 region to validate these preliminary results.

Poster Session

VIR-PT2008 - Sylvatic DENV-2 antagonizes IFN signaling pathway by degradation of STAT2

Ma Gonzalez-Rodriguez[1], Ignacio Mena[1], Adolfo García-Sastre[1]
[1]*Icahn School of Medicine at Mount Sina, New York, USA*

Dengue virus (DENV) belongs to the Flaviviridae family and exists as numerous genetic strains that are grouped into four antigenically distinct serotypes (DENV-1 to DENV-4). Each of the four serotypes is maintained in two ecologically and adaptively distinct transmission cycles: human or endemic cycle and sylvatic cycle. Sylvatic dengue is transmitted most likely among non-human primates and Aedes sp. mosquitoes. Few documented human cases of dengue fever and dengue hemorrhagic fever by sylvatic DENV-2 have been reported in regions where endemic dengue is present. Although sylvatic DENV transmission is mainly confined to the forest, it is clear that these viruses come into regular contact with humans in south Asia and Africa and can cause severe disease as well as transient spillover in urban settings. Antagonism of the type I interferon (IFN) signaling is a crucial mechanism for endemic cycle DENV immune evasion. Dengue protein NS5 antagonizes the interferon signaling pathway by targeting the Signal Transducer and Activator of Transcription 2 (STAT2), an important component of the IFN-alpha signaling pathway, by decreasing its levels of expression. However, it is unknown if this mechanism also occurs in sylvatic DENV strains. Here we characterized the interaction of sylvatic cycle DENV-2 strains DakArA510, DakHD10674 and P8-1407 NS5 (S-NS5) proteins with human STAT2 (hSTAT2). Sylvatic strains S-NS5s in absence of manipulation of the viral genome were able to bind hSTAT2. In addition, when S-NS5 was expressed in its proteolytically–processed form it was able to mediate endogenous hSTAT2 degradation thus inhibiting type I IFN signaling. These results provide new evidence of sylvatic DENV capability to evade the immune response in humans, highlighting the potential threat that these viruses represent for human health.

Poster Session

VIR-PT2010 - Critical determinant of neurological disease in highly pathogenic tick-borne Flavivirus in mice

Kentaro Yoshii[1], Yuji Sunden[1], Kana Yokozawa[1], Manabu Manabu[1], Hiroaki Kariwa[1], Michael Holbrook[2], Ikuo Takashima[1]

[1]Hokkaido University, Sapporo, Japan, [2]NIAID Integrated Research Facility, Frederick, USA

Tick-borne encephalitis virus (TBEV) and Omsk hemorrhagic fever virus (OHFV) are highly pathogenic tick-borne flaviviruses wherein TBEV causes neurological disease in humans while OHFV causes a disease typically identified with hemorrhagic fever. Although TBEV and OHFV are closely related genetically, the viral determinants responsible for these distinct disease phenotypes have not been identified. In this study, chimeric viruses incorporating components of TBEV and OHFV were generated using infectious clone technology and their pathological characteristics were analyzed in a mouse model to identify virus-specific determinants of disease. We found that only four amino acids near the C-terminus of the NS5 protein were primarily responsible for the development of neurological disease. Mutation of these four amino acids had no effect on viral replication or histopathological features, including inflammatory responses, in mice. These findings suggest a critical role for NS5 in stimulating neuronal dysfunction and degeneration following TBEV infection and offer new insights into the molecular mechanisms of tick-borne flavivirus pathogenesis.

Poster Session

VIR-PT2012 - Role of the host nucleolar helicase DDX56 during flavivirus infection

Colleen Reid[1], Zaikun Xu[1], Tom C. Hobman[1]
[1]University of Alberta, Edmonton, Canada

Flaviviruses include clinically significant members West Nile Virus (WNV), Tick-borne Encephalitis Virus (TBEV), and St. Louis Encephalitis Virus (SLEV) for which there are no vaccines or therapeutic options available. These viruses contain small, single-stranded RNA genomes and thus rely on cellular machinery for their replication and assembly of infectious progeny. Previously, our lab identified the host DEAD-box nucleolar helicase DDX56 as an important host factor during WNV infection [1]. DDX56 appears to be critical for infectivity of WNV as titers of virus produced in cells lacking DDX56 are >100-fold lower than virus produced in control cells. Whereas DDX56 is not required for viral replication or protein synthesis, loss of its helicase activity results in virions that contain less genomic RNA [1]. We hypothesize that DDX56 functions in assembly of infectious WNV virions by enhancing packaging of viral RNA. Interestingly, WNV infection results in translocation of DDX56 from the nucleolus to unidentified cytoplasmic elements that colocalize with the viral capsid protein. However, the mechanism by which this occurs is not known. To better understand this process, the distribution of DDX56 in mock and infected cells is being studied using three-dimensional structured illumination super resolution microscopy (3D-SIM). This method allows for an 8-fold increase in 3D resolution of subcellular structures facilitating unparalleled characterization of viral replication and assembly sites by light microscopy. In addition to determining whether DDX56 associates with viral replication and/or assembly sites, I will determine which viral protein(s) induces relocalization of DDX56 from the nucleolus to the cytoplasm. Finally, I will examine whether DDX56 is also important for assembly of other flaviviruses. Preliminary data suggest that nucleolar DDX56 levels decrease during SLEV infection. 1. Xu et al. (2011) J Virol 85, 5571-80

Poster Session

VIR-PT2014 - Dengue virus 2 isolates from Piauí (Brazil) reveal a new route for virus dissemination in Brazil

Leandra Barcelos Figueiredo[1], Tetsu Sakamoto[1], Luiz Felipe Leomil Coelho[2], Eliseu Soares de Oliveira Rocha[1], Marcela Menezes Gomes Cota[1], Gustavo Portela Ferreira[3], Jaquelline Germano de Oliveira[4], Erna Geessien Kroon[1]
[1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Universidade Federal de Alfenas, Alfenas, Brazil, [3]Universidade Federal do Piauí, Parnaíba, Brazil, [4]Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Brazil

Dengue virus (DENV) is the most widespread arthropod-borne virus, and the number and severity of outbreaks worldwide has increased in recent decades. DENV is caused by the viruses DENV-1, 2, 3 and 4. The viruses are genetically distant, and the species has been subdivided into different genotypes based on phylogenetic studies. The study of dengue evolution in endemic regions is important because diagnosis is often achieved through molecular tests. In Brazil, the main route of introduction of DENV-2 is thought to occur in the southeast region and to originate in the Caribbean islands. The molecular epidemiology of DENV-2 was analyzed by sequencing the envelope (E) gene from eight isolates related to dengue fever in patients from Piauí during the 2006/2007 outbreak. The results indicated a high similarity among the isolated viruses, as well as other DENV-2 viruses circulating in Brazil and Central and South America. A phylogenetic analysis based on E genes from DENV-2 revealed that these viruses were grouped together with viruses of the American/Asian genotype in two distinct lineages, showing the co-circulation of two American/Asian lineages in northeast Brazil during 2006/2007. By applying a spatiotemporal dynamics analysis on to DENV samples, we show that most of the samples from Piauí clustered with the more recent lineage of DENV-2 registered in Brazil and predate the first outbreak of this lineage described in Rio de Janeiro (2007/2008). All of these findings indicate that the northeast region of Brazilian seems to be an important route of introduction and dissemination of this virus in the country. These findings can help to further elucidate the complex phylogeographic history of dengue viruses and their evolution in dengue endemic regions in Brazil.

Poster Session

VIR-PT2016 - Recombinant dengue virus type 2 helicase protein interacts with viral RNA and preserves properties of the native protein

Raíza Bizerra[1], Jaime Amorim[1], Andrea Balan[2], Rúbens Alves[1], Sara Pereira[1], Lennon Pereira[1], Naína Garcia[1], Luís Carlos de Souza Ferreira[1]

[1]Department of Microbiology, Biomedical Science Institute, University of São Paulo, Brazil, [2]Brazilian National Laboratory of Biosciences (LNBio), Center of Research in Energy and Materials (CNPEM), Campinas, Brazil.

Dengue fever is endemic in tropical and subtropical regions and the development of an effective vaccine is a worldwide priority. The nonstructural protein 3 (NS3) of dengue virus (DENV) is essential for viral assemblage and replication. The protein encompasses a helicase domain (NS3H) that contains epitopes recognized by cytotoxic T lymphocytes, that probably play an important role in infection control. Accordingly, this study aimed to the generation of a recombinant NS3H protein with properties similar to the native protein. The recombinant protein was generated in E. coli as insoluble protein. Optimization of culture conditions led to the production of a soluble NS3H form that was purified by affinity chromatography. Mice were immunized with the recombinant NS3H protein and the anti-NS3H antibodies recognized the protein expressed in DENV2 infected cells. Moreover, the recombinant protein preserve the ability to interact with the viral RNA and remained stable at in different temperatures. The recombinant NS3H was also recognized by antibodies from mice infected with a DENV2 strains originally isolated from a human subject. In addition, mice immunized with NS3H combined with different adjuvants (LTK63, Alum or CpG) developed NS3H-specific antibody and T cell responses. Altogether, the present results indicate that the recombinant NS3H protein preserves conformational and antigenic determinants of the native viral protein and represents a valuable reagent for the development of experimental vaccines and diagnostic tests. Research supported by FAPESP and CNPq grants.

Poster Session

VIR-PT2018 - Identification of specific epitopes/mimotopes for bovine viral diarrhea virus using phage display

Ana Marina Orozco Rivero[1], Rosa Elena Sarmiento[1], Gohar Gevorkian[2], Jose Alberto Cano[1], Maria Elenena Munguía[2], Gonzalo Acero[2], Elvia Lazo[1]
[1]Facultad de Medicina Veterinaria y Zootecnia, UNAM, México City, México, [2]Instituto de Investigaciones Biomédicas, UNAM, México City, México

Bovine Viral Diarrhea Virus (BVDV) infection in cattle herds is an important economic factor, causing reproductive fails. One diagnosis option is the use of ELISA test. This technique requires growing the virus or the production of recombinant proteins, both options are cumbersome and could exist differences between batches. One alternative is the identification of peptides that can mimic the BVDV epitopes. Filamentous bacteriophage M13 can display copies of foreign peptides on pIII. We use Phage Display technology in our laboratory to search for novel agents that can be use in diagnosis or vaccine. In this study our goal was to identify specific epitopes/mimotopes to hyperimmune rabbit sera against BVDV ATCC (VR-1422) using a commercially available phage display peptide library Ph.D.™-7 (E8100S) and Ph.D.™-C7C (E8120S). 30 phage clones were selected from the 3 – 5th rounds of biopanning, from which we selected more reactive clones (16) and their specificity to rabbit sera and bovine serum samples (ELISA, IDEXX and RT-PCR characterized for BVDV) were evaluated in phage ELISA. The most reactive clones were sequenced to identify the displayed peptides and evaluated in different ELISA diagnostic formats.

Poster Session

VIR-PT2020 - Deep RNA sequencing reveals hidden features of gene transcription in Paramecium bursaria chlorella virus 1

Irina Agarkova[1,2], Guillaume Blanc[3], Michael Mozar[3], David Dunigan[1,2], James Van Etten[1,2]
[1]Department of Plant Pathology, University of Nebraska, Lincoln, USA, [2]Nebraska Center for Virology, University of Nebraska, Lincoln, USA, [3]Laboratoire Information Structurale and Genomique, Aix-Marseille Universite, Marseille, France

Paramecium bursaria chlorella virus 1 (PBCV-1) is the prototype of the genus Chlorovirus (family Phycodnaviridae) that infects the unicellular, eukaryotic green alga Chlorella variabilis NC64A. The 331-kb PBCV-1 genome contains 416 major open reading frames. The virion contains 148 unique virus-encoded proteins and 1 host protein. A mRNA-seq approach was used to analyze PBCV-1 transcriptomes at 6 time points during the first hour of infection. Total of 17 million of reads were generated and mapped to the PBCV-1 genome at single-base resolution. The analysis identified a rapid activation of viral gene transcription at the onset of infection and the rapid takeover of the host by the virus. There was a significant transcription of 50 viral genes as early as 7 min after infection. Sequential initiation of transcription of the remaining virus genes was observed at latter times. Clustering analysis of transcription profiles identified groups of genes that differed by their time of peak expression and by the dynamics of their accumulation. Computer analyses identified potential internal cleavage sites in 50 annotated major ORFs dispersed over the entire genome, 27 of them occurred on the strand opposite a major gene. Fifteen ORFs contained more than one exon junction, suggesting that complex alternative splicing configurations might occur in PBCV-1. Six potential introns were selected for experimental validation using RT-PCR and sequencing. Predicted cleavage of introns was confirmed by electrophoresis on gels and sequencing for 4 of the ORFs. In all six cases, a RT-PCR product corresponding to the un-cleaved transcripts was obtained and the un-cleaved transcript was present in higher concentrations than the processed transcript. This analysis of RNA-seq data revealed for the first time that a substantial number of PBCV- 1 transcripts have the potential to be processed. However, the biological significance of the excision events is unknown.

Poster Session

VIR-PT2022 - A comparison of Cytomegalovirus drug resistance assays from Canada and Singapore

Elizabeth McLachlan[1], Pei Tee Huan[2], Alberto Severini[1], Evelyn Koay[2,3], Julian Tang[4,5]

[1]Public Health Agency of Canada, National Microbiology Laboratory, Winnipeg, Canada, [2]Molecular Diagnosis Centre, National University Hospital, Singapore, [3]Department of Pathology, National University of Singapore, Singapore, [4]Alberta Provincial Laboratory, Edmonton, Canada, [5]Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada

BACKGROUND. Cytomegalovirus (CMV) causes severe post-transplant morbidity and mortality. The use of anti-CMV drugs can lead to drug resistance (DR), typically via mutations in the CMV UL97 and UL54 genes. Although detecting these mutations allows an earlier therapy switch, sequencing these genes is difficult and the interpretation suffers from a lack of comprehensive, correlating phenotypic data. METHODS. Twelve samples already tested for CMV DR in Singapore (Molecular Diagnosis Centre, National University Hospital) were sent to Canada (National Microbiology Laboratory), for comparative testing. The Singapore assay amplifies short UL97 and UL54 sequences at loci associated with known CMV DR mutations (defined using a local CMV reference sequence). The Canadian assay sequences the entire UL97 and UL54 genes then submits them to a German CMV DR interpretation website (http://www.informatik.uni-ulm.de/ni/staff/HKestler/hcmv/). RESULTS. The results were similar within the sequenced regions common to both assays. The Canadian assay detected additional mutations (mostly polymorphisms) outside the regions covered by the more limited Singapore assay. Common polymorphisms identified by both assays included: G678S and A688V in UL54, and D605E in UL97. A mutation at position 685 (N685S) in UL54 was seen in all 12 samples in the Singapore results, but in only 1/12 samples (as S685N) in the Canadian assay. The sensitivity of the Singapore assay was ~2000 copies/mL CMV DNA. The Canadian assay has a much more variable sensitivity, apparently dependent on the level of DNA fragmentation. CONCLUSION. Most differences in the results from the two assays are probably due to the use of different CMV reference sequences: the Singapore assay uses a Japanese CMV reference strain, whereas for the Canadian results, the German interpretation website probably uses one of European origin (TB40-BAC4) A more comprehensive, internationally standardised phenotyping database is required for accurate CMV DR testing, ideally, along the lines of HIV genotyping.

Poster Session

VIR-PT2024 - Immune response to HSV-1 infection is mediated by CD8+-Ifn gamma production and macrophages-iNos expression in mice trigeminal ganglia, after interactions of Toll-like receptors

Guilherme Pimenta Zolini[1], Graciela Kunrath Lima[2], Natalia Lucinda[1], Rosa Maria Arantes[3], Natalia Lima Pessoa[1], Erna Geessien Kroon[4], Marco Antônio Campos[1]

[1]Centro de Pesquisas René Rachou, Belo Horizonte, Brazil, [2]Escola de Veterinária, UFMG, Belo Horizonte, Brazil, [3]Departamento de Patologia, UFMG, Belo Horizonte, Brazil, [4]Departamento de Microbiologia, UFMG, Belo Horizonte, Brazil

Herpes simplex virus type 1 (HSV-1) is a neurotropic DNA virus that causes various human clinical manifestations, ranging from simple cold sores to blindness or encephalitis. In this work, TLR2-/-, TLR9-/-, TLR2/9-/- and wild type mice were intranasally infected with HSV-1, and were euthanized at the fifth day post infection and then trigeminal ganglia and brain were collected. TCD8+ IFN gamma cell producers (cytometry assay) were higher in trigeminal ganglia of infected than in uninfected mice. Infected wild type mice showed increase in the expression of TLR1, TLR2, TLR3, TLR6, TLR7 and TLR9 in trigeminal ganglia (real time PCR), but not in brain, compared to control. The infected knockout mice had increased expression of TLR1, TLR3, TLR6, TLR7 and additionally of TLR2 (for TLR9-/-) and of TLR9 (for TLR2-/-) in trigeminal ganglia, compared to non infected mice, but decreased expression when compared to wild type mice. iNOS expression in trigeminal ganglia of infected wild type was higher than in trigeminal ganglia of knockout mice, what not occurs with the expression in trigeminal ganglia of gp91phox and p22phox (real time PCR), although the three genes had higher expression in trigeminal ganglia of wild type and knockout infected mice in comparison to non infected mice. In addition, intraperitoneal macrophages of wild type mice produced more nitric oxide (Griess reaction) after exposition to HSV-1 than TLR knockout mice. By real time PCR, we showed that RAG-/- mice were not capable to express cytokines, and iNOS-/- mice showed an excessive response to cytokines expression. Finally, in survival assay with wild type, CD8-/-, RAG-/- and iNOS-/- mice, we demonstrated the importance of the CD8 and iNOS, as CD8-/-, RAG-/- and iNOS-/- infected mice had 100% of mortality, compared to 10% in wild type infected mice.

Poster Session

VIR-PT2026 - The survey on equine Herpesvirus 4 in Thoroughbred racehorses and Jeju ponies of Korea

Eunjin Choi[1], Yooni Oh[1], Seungmin Song[1], Jaeyoung Song[1], Seonjoo Yang[2]
[1]Animal and Plant Quarantine Agency, Anyang, Korea, [2]Korea Racing Authority, Gwacheon, Korea

Equine herpesvirus 4 (EHV-4) are a major causative agent of equine respiratory disease known as equine rhinopneumonitis in horse. EHV-4 causes respiratory disease throughout the year among all horse populations. An inactivated vaccine has been commercially available for use as prophylactic aids in reducing the burden of the disease in horses caused by EHV infection in Korea. Only limited cases were reported on EHV infections in Korea. In this study, we investigated to understand serological state and isolate circulating EHV-4 in thoroughbred racehorses and Jeju ponies in Korea. A total of 552 blood samples were collected from thoroughbred racehorses and Jeju ponies in eight provinces Equine sera were tested for the presence of EHV-4 antibodies by virus neutralization test. A total of 110 nasal swabs were collected from horses with respiratory diseases. The samples were tested by PCR and the filtered swab solution was inoculated in monolayered equine dermal cells for virus isolation. The purified EHV-4 DNA was sequenced in both directions. Sequences were aligned and clustered using PHYLIP. A total of 528 among 552 horses tested were positive for EHV-4. Serologically, samples showed 100% positivity in racehorses, stallions and mares, whereas foals, pleasure horses and Jeju ponies showed positivity of 67.8%, 99.0% and 88.9%, respectively. In 2.7% (3) of the 110 nasal swab samples EHV-4 was detected by PCR. One EHV-4 strain was isolated in a swab sample collected from a 2-year-old foal. Inoculated cells were observed with typical cytopathic effects (CPE) for herpesvirus after 4 days of postinoculation by microscopy. The partial glycoprotein B (gB) sequences were analyzed and compared with EHV-4 NS80567 strain. The sequence homologies of gB from the strain were 99.8%~100% in nucleotide and were 99.5%~100% in amino acids, respectively. The results demonstrated that EHV infections are endemic in horse herds in Korea.

Poster Session

VIR-PT2028 - Investigating the role of Dok proteins in the immune response during infection with herpes simplex virus 1

Soumia Lahmidi[1], Mitra Yousefi[1], Pascale Duplay[1], Angela Pearson[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada

Cellular immunity plays an important role in the ability of the host to control herpes simplex virus 1 (HSV-1) infections. HSV-1 uses different strategies to escape the immune system, one of which is to inactivate T cells. Dok-1 and Dok-2 proteins are the two members of the Dok family expressed in T cells. Dok proteins negatively regulate various biological processes including the proliferation and differentiation of T cells. The involvement of Dok in the control of viral infections is not yet known. The purpose of this project is to determine the influence of Dok-1 and Dok-2 in the T-cell response following infection with HSV-1. We hypothesize that Dok-1 and Dok-2 down-regulate the anti-HSV-1 immune response during acute infection. Mice deficient for the Dok-1 gene (KO) and wild type mice (WT) were compared using a model of ocular HSV-1 infection. We found that viral replication during acute infection in the eye was similar in WT and Dok-1 KO mice. All mice showed similar clinical disease with periocular inflammation visible at 5 days post-infection (dpi). The HSV-1-specific CD8+-T-cell response in the spleen and draining lymph nodes was similar in Dok-1 KO and WT mice at 8 dpi; however, the frequency of CD8+CD62Llow T cells was reduced in the spleen in Dok-1 KO as compared to WT mice. Because redundancy of function between Dok-1 and Dok-2 has been described previously, these experiments will be repeated with double knockout Dok1/2 mice. These studies will contribute to a better understanding of the immune response against HSV-1.

Poster Session

VIR-PT2030 - Molecular comparison of sturgeon alloherpesviruses

Andor Doszpoly[1], Igor S. Shchelkunov[2], Thomas B. Waltzek[3]
[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, [2]All Russia Research Institute for Veterinary Virology and Microbiology, Pokrov, Russia, [3]Department of Infectious Disease and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, USA

Alloherpesviruses constitute a significant group of pathogens of cultured fish and therefore are of great veterinary importance worldwide. This study focuses on the molecular characterization of alloherpesviruses of sturgeon species in Eastern Europe and North America. One of the most important viral disease of sturgeon species in aquaculture is caused by the Acipenserid herpesvirus 2 (AciHV-2). The virus belongs to the Ictalurivirus genus within the family Alloherpesviridae, under the order Herpesvirales. In North America, the herpesviral diseases of sturgeons were described decades ago from white sturgeon (Acipenser transmontanus). Later, novel AciHV-2 strains were found in shortnose sturgeon (Acipenser brevirostrum) and lake sturgeon (Acipenser fulvescens). In Russia, alloherpesviruses were isolated from Siberian sturgeon (Acipenser baerii) in the new millennium. An approximately 8000-bp-long fragment, between two well conserved genes, was amplified and sequenced from all genomes. In terms of position, orientation and size of the ORFs, the organization of the sequenced genome fragment was identical or very similar to the corresponding genome part of the firstly sequenced AciHV-2 isolated from white sturgeon. Serological comparison assays also suggest that these viruses belong to the same virus species, the Acipenserid herpesvirus 2. Although, herpesviruses are considered to be host specific viruses, it seems that the AciHV-2 could cause disease in different sturgeon species. Besides the above mentioned species, the Russian sturgeon (Acipenser gueldenstaedti), the sterlet (Acipenser ruthenus) and some sturgeon hybrids seem to be susceptible to the AciHV-2. The closest relatives of AciHV-2 are Ictalurid herpesvirus 1 and 2. These viruses infect catfish species, and their genomes have balanced G+C content. However, the AciHV-2 genome has low G+C content, this fact and the high mortality caused by the virus suggest that the virus has not co-evolved with the sturgeons, but represents a host switch. The financial support was provided by the grant OTKA PD104315.

Poster Session

VIR-PT2032 - Identification of B cells as a major site for koi herpesvirus (KHV) latency

Aimee Reed[1], Satoko Izume[1], Brian Dolan[1], Michael Kent[1], Scott LaPatra[2], Ling Jin[1]
[1]Oregon State University, Corvallis, USA, [2]Clear Spring Foods, Idaho, USA

Koi herpesvirus (KHV) is a new emerging herpesvirus that is highly pathogenic in koi and common carp. Our previous study demonstrated that KHV becomes latent in peripheral white blood cells (WBC). In this study, KHV latency was further investigated in WBC that were separated into IgM+ or IgM- WBC. The majority of the IgM+ cells were also positive by Pax5 antibodies, a marker specific to B cells. The KHV genome presence in IgM+ WBC was about 20-fold greater than in IgM- WBC. To determine if KHV has gene expression during latency, expression from all 8 ORFs in the terminal repeat was investigated in IgM+ WBC from koi with latent KHV infection. The spliced ORF6 was found to be abundantly expressed in IgM+ WBC from KHV latently infected koi. The spliced ORF6 transcript can also be detected in vitro during productive infection as early as 1 day post-infection. Mapping the ORF6 transcript from in vitro infection starts at -127 bp upstream of the ATG and ends 20 bp downstream of the polyadenylation signal. The hypothetical protein sequence of ORF6 contains a consensus sequence with homology to a conserved domain of EBNA-3B and ICP4 of other herpesviruses. This is the first report of identification of latent KHV in B cells and gene transcription during latency for herpesviruses outside of Herpesviridae in the Alloherpesviridae.

Poster Session

VIR-PT2034 - The importance of genetic variation of the human Coronavirus OC43 in neuroinvasion, neurotropism and neuropathology

alain Le Coupanec[1], Marc Desforges[1], Pierre Talbot[1]
[1]*INRS-Institut Armand-Frappier, Laval, Canada*

Human coronaviruses (HCoV) are respiratory pathogens that are neuroinvasive (ability to invade the central nervous system (CNS) from the periphery), neurotropic (ability to infect CNS cells), and potentially associated with development of neurological diseases (neurovirulent). The research presented herein aims to investigate the viral and cellular parameters of HCoV infection of the CNS, and possible neurological consequences. The S protein is one major factor of virulence in the CNS for several coronavirus species, including HCoV-OC43. Therefore, in an attempt to study the role of the protein in the spreading of virus within the CNS and to identify important amino acid residues related to this function, we compared the sequence of the gene encoding the viral S protein in the reference strain of HCoV-OC43 to sequences from viruses detected in clinical isolates from human brains and expectorations of the upper respiratory tract. In this context, we have identified a predominant mutation (G758R) in the S protein of clinical isolates of HCoV-OC43 from the upper respiratory tract. Using a molecular cDNA infectious clone system in which we have introduced this single mutation, we have generated the corresponding recombinant virus for further study. The neuroinvasive capacities of this mutant are modulated according to the age of mice at time of infection and that the virus is unable to spread efficiently from brain towards spinal cord, leading to modulation of the associated neurological symptoms. This study should allow us to further understand the interactions of HCoV-OC43 with the CNS and thus to better understand the possible consequences of CNS infection by HCoV in humans and the eventual development of potential neurological degenerative pathologies. (Supported by an operating grant from CIHR (III) and a Tier-1 Canada Research Chair to PJT)

Poster Session

VIR-PT2036 - Mouse coronavirus infections impair brain blood barrier innate immune responses and structural cerebrovascular endothelial cells functions

Christian Bleau[1], Mélanie Burnette[1], Lucie Lamontagne[1]
[1]Université du Québec à Montréal, Montréal, Canada

The blood-brain barrier (BBB) endothelial cells (ECs) is a primary innate immune antiviral barrier limiting brain invasion by hematogenous spread viruses. Some viruses have evolved strategies to bypass this vascular barrier and induce neuropathy. The evasion mechanisms from this innate barrier, however, are not well-known. Using different serotypes of the murine coronavirus mouse hepatitis viruses (MHV), we investigated, in vivo and in vitro, the viral-induced innate response and structural dysfunctions of ECs. C57BL/6 mice were infected i.p. with the hepatotropic-neurotropic MHV3, mildly-neurotropic MHV-A59 and the attenuated variant 51.6-MHV3 showing low tropism for ECs. Following i.p. infection, MHV3 viral nucleoprotein gene expression, but not MHV-A59 and 51.6-MHV3, was detected in brains of infected mice correlating with cerebral vascular lesions and marked decrease in ECs junction protein expression. Infections with the MHV-A59 and 51.6-MHV3, however, induced higher intracerebral expression of IFN-beta, IL-6, TNF-alpha and chemokines than in brain from MHV3-infected mice. Using EC line Bend.3 as an in vitro BBB model, MHV3 provoked higher cytopathic effects and decrease of transendothelial resistance, correlating with lower junction proteins mRNA expression, in contrast to that observed with lower virulent MHV-A59 or 51.6-MHV3. Moreover, MHV-A59 and 51.6-MHV3, but not MHV3, elicited an antiviral IFN-beta response as well as IL-6, TNF-alpha and chemokine production by ECs. Low antiviral and inflammatory responses observed in MHV3-infected EC correlated with viral receptor (CEACAM1a)- and TLR2-dependent tropism for ECs involving together, both caveolin and clathrin-dependent endocytic uptake, in contrast to that seen with MHV-A59 or 51.6-MHV3. Our findings highlight a new viral evasion mechanism from innate antiviral responses of EC from BBB in disturbing EC junction function and reducing antiviral IFN-beta and inflammatory responses, thus favouring viral spread to the brain.

Poster Session

VIR-PT2038 - Oncolytic viral sensitizer drug dependant on decrease of antiviral immune responses

Mohammed Selman[2], Ramya Krishnan[2], Rozanne Arulanandam[1], Jean-Simon Diallo[1]
[1]Ottawa Hospital Research Institute, Ottawa, Canada, [2]University of Ottawa, Ottawa, Canada

A high-throughput pharmacoviral screen identified a number of small molecules that modulate the cellular response to virus infection and oncolytic virotherapy. One of these molecules, termed virus-sensitizer 1 (VSe1), was found to target tumour innate immune responses and could enhance oncolytic virus (VSV) efficacy in animal tumour models and within primary human tumor explants, while remaining benign to normal tissues. In the present study, we examine the mechanism of action of VSe1 by demonstrating that it is able to increase the oncolytic potential of VSV by impacting both virus replication and spreading various cell lines. Microarray data analysis revealed that VSe1 can inhibit IRF7 regulated genes and interferon stimulated genes (ISGs)in the presence or absence of VSV infection. We show that in addition to inhibiting type I IFN production, VSe1 potentiates the antiviral state, through down regulation of the interferon-stimulated Jak-STAT pathway, by decreasing phosphorylation of STAT-1. Further understanding the mechanism of action of VSe1 will ultimately lead to improved, targeted combinatorial therapies.

Poster Session

VIR-PT2040 - Development of an anti-angiogenic gene therapy strategy for the treatment of ovarian cancer

Darrick Yu[1], James Petrik[2], Sarah Wootton[1]
[1]Pathobiology, Ontario Veterinary College, University of Guelph, Canada, [2]Biomedical Sciences, Ontario Veterinary College, University of Guelph, Canada

An integral step in the development of solid tumors is the recruitment of an additional blood supply. Therapies known to inhibit this process, known as anti-angiogenic cancer therapy, may therefore be highly effective in controlling tumor growth. Thrombospondin-1 (TSP-1) is an endogenous protein that possesses intrinsic anti-angiogenic activity and is highly involved in processes governing endogenous angiogenesis. TSP-1 is a modular protein with two major anti-angiogenic functional regions: one known as the three N-terminal Type I thrombospondin repeats domain (3TSR), and a second C-terminal domain that is known to bind to, and block, activation of CD47, a ubiquitous receptor known to function as a "don't eat me" signal. In this study, anti-angiogenic functional domains derived from TSP-1 were encoded within an adeno-associated virus (AAV) gene therapy vector in order to evaluate their ability to impact angiogenesis in a mouse ovarian tumor model. In particular, the following vectors were constructed and evaluated: AAV-TSP1 (full length thrombospondin-1), AAV-3TSR, AAV-CD47 binding domain, and AAV-3TSR-2A-CD47 binding domain, where 2A denotes placement of a self-cleaving peptide to promote separation of the two functional domains.

Poster Session

VIR-PT2042 - Bi-specific antibodies and anti-idiotypic antibodies: Two patents pending to improve virotherapy

Arno Thaller[1]
[1]*Praxisklinik, Markt Berolzheim, Germany*

The problems in virotherapy are shown in detail. Two of them are 1. the virus-transport to the tumour and 2. the production of anti-viral antibodies. Both problems can be solved by antibodies: 1. The adhesion site of most viruses is unspecific. NDV e.g. adheres to neuraminic acid, an epitope even on red blood cells! This means that hardly any virus is reaching its goal when given intravenously. A similar situation is given in the case of VSV. The adhesion site is LDL, present on a lot of cells! Intra-tumoural application or intra-arterial port-systems are necessary to achieve adequate titres in the tumour. Both ways are not without risks. The intratumoural application often needs radiologic assistance and bears the risk of infection and metastases. The port-systems bear the life-threatening risks of embolism and infection. Bi-specific antibodies can solve this problem by catching the unspecific adhesion site of the virus with one arm and with the other a tumour-specific epitope like PSMA - in the case of prostatic carcinoma - or Her-2-neu in the case of aggressive breast cancer. 2. The production of anti-viral antibodies is a problem that limits all virotherapy to virtually “one shot”. This problem can be met by the simultaneous application of anti-idiotypic antibodies. Until now the problem is addressed by suppression of all antibody production, e.g. by daily application of 250 mg cyclophosphamide. This dosage, however, suppresses the production of all antibodies, (the antiviral and the beneficial ones) and the CTLs. The prophylactic application of anti-idiotypic antibodies against anti-viral antibodies is a logic consequence. Both solutions are protected by patents pending. The ideas are based on fifteen years’ experience with oncolytic viruses. At present I have 14 different oncolytic viruses at my disposal.

Poster Session

VIR-PT2044 - HPV detection in anal intraepithelial lesions from HIV infected Cuban men

Celia María Limia-León[1], Yudira Soto-Brito[1], Vivian Kourí-Cardellá[1], Yanara García-Leyva[2], Orestes Andrés Blanco-González[1], María Victoria López-Soto[2], Consuelo Beatriz Correa-Sierra[1], Yoan Alemán-Campos[1], Lissette Pérez-Santos[1], Alina Álvarez-Valdés[1]

[1]Institute of Tropical Medicine "Pedro Kourı´", Havana, Cuba, [2]Hospital "Dr. Enrique Cabrera Cossío", Havana, Cuba

Background: An association between human Papillomavirus (HPV) infection and progression to anal intraepithelial neoplasia (AIN) and anal epidermoid cancer has been established, specifically in high-risk populations such as human immunodeficiency virus (HIV)-infected men. Objective: To know the prevalence of low and high-grade anal squamous intraepithelial lesions (ASIL), as well as HPV infection in a group of HIV infected Cuban men. Methods: Cross-section study to detect anal dysplasia and HPV infection was performed. Following to the inclusion criteria, patients who accepted to be tested for anal cytology were included and viral detection was done only in patients with satisfactory cytological diagnosis, according to Bethesda 2001 System. Finally, anal mucosa samples were collected from fifty six HIV-positive individuals. HPV DNA detection was determined by RT-PCR for 6 high-risk HPV types and end point PCR for low-risk HPV types (6 and 11). Results: The cytological diagnosis identified 5,4% negative for intraepithelial lesions or malignancy (NILM), 25% atypical squamous cells of undermined significance (ASC-US), 60,7% low-grade squamous intraepithelial lesions (LSIL), 5,4% high-grade squamous intraepithelial lesions (HSIL), and 3,5% atypical squamous cells can not exclude HSIL (ASC-H). The prevalence of HPV infection was 89% and the prevalence of any carcinogenic HPV genotype was 77%. HPV 16 was the most common genotype (52%), while HPV 18 infection was associated with the presence of HSIL (p=0.013). Notably, there were significant differences in the HPV viral loads with respect to the cytology results (p=0.000). Ano-receptive sexual relation was a risk factor for anal HPV infection (p=0.032). Conclusions: This is the first report of HPV detection in ASIL from HIV infected Cuban men. There is a high prevalence of ASIL and HPV infections in the study group. Most individuals were infected with high-risk HPV types. These findings support the inclusion of HPV vaccine in Cuba.

Poster Session

VIR-PT2046 - Association of age and gender with Torque teno virus detection in fecal material of patients with enteritis

Julie Brassard[1], Marie-Josée Gagné[1], Danielle Leblanc[1], Élyse Poitras[1], Alain Houde[1], Valerie F. Boras[2], G. Douglas Inglis[3]

[1]Agriculture and Agri-Food Canada, St-Hyacinthe, Canada, [2]Department of Laboratory Medicine, Chinook Regional Hospital, Lethbridge, Canada, [3]Agriculture and Agri-Food Canada, Lethbridge, Canada

Introduction: Torque teno virus (TTV) is a small virus belongs to Anelloviridea family. TTV is a disease orphan virus but it has often been associated with a variety of pathologies and co-infections, including hepatitis, asthma, idiopathic pulmonary fibrosis and autoimmune rheumatic disorders. TTV was recently identified as an infectious agent that could potentially be involved in cases of acute gastroenteritis. It is therefore important to evaluate the incidence of TTV in diarrheic human beings to elucidate the epidemiology of disease and to develop effective mitigation strategies. Objective: Contrast the prevalence of TTV in diarrheic and non-diarrheic human beings, and assess the prevalence and viral load of TTV in individuals with enteritis by age class and gender. Methods: Stool samples from human beings exhibiting signs of enteritis (954) and from non-diarrheic individuals (76) were collected in the Chinook Health Region, Alberta, Canada from May 2008 to April 2009. For each month, the distribution of diarrheic samples was: 20 samples from individuals between 0-16 years of age, 40 samples between 17-60 years of age, and 20 samples over 60 years of age. Viral genetic material was extracted, and detection and quantification of TTV were carried out by real-time PCR. Results: Of the diarrheic people, 39% tested positive for TTV, compared with 18% of non-diarrheic people ($p<0.001$). TTV was detected most often in diarrheic individuals that were 0-5 (58%) and 81+ (59%) years of age; TTV was detected less often (25%) and average viral loads were lower in individuals belonging to the 21-30 age class ($P<0.025$). Combined across age, the prevalence of TTV was significantly higher among men than women ($p<0.01$). Conclusion: This study revealed a significant association between TTV prevalence and enteritis. Also, TTV prevalence and viral load were higher in the very young and elderly suggesting that immunological status is important.

Poster Session

VIR-PT2048 - A population based study of Human Papilloma Virus (HPV) infection among women in a rural village in South India

Sabeena Sasidharan Pillai[1], Parvati Bhat[2], Veena Kamath[3], Aswathy Raj[1], Hindol Maithy[1], Giselle D"Souza[1], Kiran Chandra Bharani[1], Anjali Aithal[1], Govindakarnavar Arunkumar[1]

[1]Manipal Centre for Virus Research, Regional Reference Laboratory for Influenza, Manipal University, Manipal, India, [2]Dr. TMA Pai Hospital, Manipal University, Manipal, India, [3]Kasturba Medical College, Manipal University, Manipal, India

Background:Cervical cancer associated with Human Papilloma Virus (HPV) is the commonest cancer in rural India. We have planned a population based study to estimate the prevalence of HPV among women aged 18-65 years in rural India using urine as the sample. We also conducted knowledge, attitude and practice (KAP) survey on HPV and its health effects among the study participants. Materials and Methods: All women aged 18-65 years in the selected wards of the Perdoor village in Udupi district, Karnataka state, India was enrolled into the study after obtaining informed consent. While enrolling each participants was administered a KAP questionnaire and a nearly stream random voided urine sample was collected. The Urine sample was processed in the laboratory and was tested for HPV by PGMY09/11 and GP5+/6+ nested PCR and genotyped by sequencing. Results: A total of 247 women were recruited from August 2013 to January 2014. Urine sample for HPV screening was also collected and stored at -80C till tested. The urine HPV screening PCR was standardized and validated. The mean age of the participants were 36.3(SD=10) years.All the participants were from low (66%) and middle(33%) socioeconomic status.While 87.4% (215) participants were married, 8.5% (21) unmarried and 6.47% (16) women had initiated sexual activity prior to 18 years. Partners of 9.3% (23) participants had undergone circumcision but condom usage was reported by only 4.86%(12) women. None of the participants had heard of HPV infection and its health effects. Conclusions:This is the first major population based HPV study from rural India using urine and had an excellent acceptance. Despite the fact that cervical cancer is preventable, none of the participants had heard about HPV infection and its health effects. Outcome of this prospective study would greatly contribute evidence based public health action against cervical cancer prevention.

Poster Session

VIR-PT2050 - Sublingual immunization of trivalent human papillomavirus DNA encapsidated in nonreplicable baculoviral vaccine

Hee-Jung Lee[1], Hansam Cho[1], Yoonki Heo[1], Yeon Dong Cho[1], Jae-Sung Lee[2], Yu-Kyoung Oh[3], Young Bong Kim[1]
[1]Dept. of Bio-industrial technologies, Konkuk University, Seoul, South Korea, [2]Institute of Bioinnovation Reaserch, Kolon Life Science, Inc., South Korea, [3]Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, South Korea

We developed human endogenous retrovirus envelope protein-coated non-replicating recombinant baculovirus (AcHERV) for a multivalent human papillomavirus (HPV) DNA nanocarrier. Previously, we reported the immunogenicity of AcHERV-based multivalent human papillomavirus (HPV16, HPV 18, and HPV58) nanovaccine in which the L1 segments of HPV16, 18, and 58 were inserted into a single baculovirus genome of AcHERV. In this study, we generated an AcHERV-based trivalent DNA vaccine against HPV type16, 18, and 58 (AcHERV-Trivalent) and tested vaccine efficacy of HPV 16, 18, and 58 following intramuscular or sublingual immunization without adjuvant. The both Immunization of trivalent DNA vaccine induced IgG and IgA antibodies, neutralizing antibodies, IL-4, IFN-γ, and protection against challenge of HPV pseudoviruses. Serum IgG, IL-4, and neutralizing antibody responses of sublingual immunization were similar in intramuscular immunization. Vaginal IgA responses of sublingual immunization were superior to intramuscular immunization. Furthermore, all vaccinated groups by intramuscular or sublingual with trivalent DNA vaccine showed perfect protection against genital challenge with HPV16, HPV18, and HPV58 pseudoviruses. These results suggest that vaccine against HPV trivalent DNA vaccine and the potential of sublingual immunization as an efficient vaccination strategy for inducing mucosal immune responses.

Poster Session

VIR-PT2052 - ViralZone: recent updates to the virus knowledge resource.

Chantal Hulo[1], Patrick Masson[1], Edouard De Castro[1], Lydie Bougueleret[1], Ioannis Xenarios[1], Philippe Le Mercier[1]
[1]Swiss Institute of Bioinformatics, Geneva, Switzerland

ViralZone (viralzone.expasy.org) is a web resource that links sequence data with virus biology knowledge. The information is organized into viral fact sheets for each viral genus that describe virion shape, genome, molecular biology and epidemiology with links to the corresponding sequences in GenBank and SwissProt/UniProtKB. ViralZone provides detailed view of viral molecular biology through a 200 pages dictionary of controlled vocabulary that describe with figures the common steps of viral replication cycles and host-virus interactions. Users can navigate back and forth from genus fact sheet to relevant molecular vocabulary. Based on the controlled vocabulary assembled in ViralZone, we have created a ontology in Gene ontology (GO), and SwissProt/UniProtKB. Ontologies provide a formal representation of knowledge that is amenable to computational as well as human analysis. This viral molecular ontology will facilitate annotation of virus genes in sequence database and help large-scale analysis. More than 80,000 protein annotations have already been implemented in databases using this ontology. The recent update has added all molecular prokaryotic processes, with an update of phages protein annotation in SwissProt/UniProtKB.

Poster Session

VIR-PT2054 - Molecular characterization of attenuated Potato spindle tuber viroid strain from dahlia

Daiki Tsushima[1], Akito Taneda[2], Teruo Sano[1]
[1]Hirosaki University, Faculty of Agriculture and Life Science, Hirosaki, Japan, [2]Hirosaki University, Graduate School of Science and Technology, Hirosaki, Japan

The dahlia isolate of Potato spindle tuber viroid (PSTVd-D) showed mild disease symptoms on tomato plants (Solanum lycopersicom, cv. Rutgers) comparing to the Intermediate isolate (PSTVd-I). PSTVd-D shares 97% sequence identity to PSTVd-I and is different in nine nucleotides locating in the left- and the right-hand half of highly base-paired rod-like secondary structure; i.e., six nucleotides in the terminal left (TL) to the pathogenicity (P) domains and three in the variable (V) to the terminal right (TR) domains. Chimaeric PSTVd mutants were created in vitro by exchanging the left- and right-hand half of the molecule between PSTVd-I and -D, and assayed the pathogenicity to tomato plants. In the early stage of infection (i.e., 4 week post inoculation), a chimaeric mutant (PSTVd-I/D) consisting of the left-hand half of PSTVd-I and the right-hand half of PSTVd-D accumulated slowly and showed very mild symptoms similar to those incited by PSTVd-D. In contrast, a reversible chimaera (PSTVd-D/I) with the left-hand half of PSTVd-D and the right-hand half of PSTVd-I accumulated faster and exhibited severe symptoms similar to those by PSTVd-I. The result indicated that the virulence is regulated by an element in the right-hand half of the molecule which is responsible for replication/accumulation efficiency. Surprisingly, PSTVd-I/D which had shown very mild symptoms in the early infection started to show severe symptoms that is comparable to PSTVd-I in the latter stage of infection (5 – 8 weeks post inoculation), indicating that the virulence is also affected by an element in the right-hand half of the molecule, so called “pathogenicity region”, once the titer reached a certain level.

Poster Session

VIR-PT2058 - *Amalgaviridae*: a new family of dsRNA viruses

Sead Sabanadzovic[1], Nina Aboughanem-Sabanadzovic[2], Rodrigo A Valverde[3], Robert R Martin[4], Ioannis E Tzanetakis[5]

[1]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, USA, [2]Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, USA, [3]Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, USA, [4]Horticultural Crops Research Laboratory, USDA-ARS, Corvalis (OR), USA, [5]Department of Plant Pathology, Division of Agriculture, University of Arkansas, USA

A number of closely related viruses have been identified recently in several plant species from different parts of the world. These viruses are characterized by symptomless infections in the respective hosts and a genome made of a single, 3.5 kbp-long, bicistronic double stranded RNA (dsRNA) molecule. The presence of two partially overlapping open reading frames on the positive RNA strand is reminiscent of the genomic organization of viruses belonging to the family *Totiviridae*. Despite the similarities with totiviruses in genome organization, phylogenetic analyses of the viral RNA-dependent RNA polymerase indicate that these viruses belong to a new lineage of dsRNA viruses, evolutionarily closer to the extant partitiviruses (fam. *Partitiviridae*) than to the family *Totiviridae*. Taking into account that the properties of these viruses did not fit any of the extant taxa, a formal taxonomic proposal for the establishment of a new family (*Amalgaviridae*) has been recently submitted and approved by the Executive Committee of the International Committee on Taxonomy of Viruses. At the present time, family *Amalgaviridae* accommodates a single genus, gen. *Amalgavirus*, which is typified by *Southern tomato virus* and embraces several recognized species identified in blueberry, rhododendron and broad bean. Recent studies suggest common presence of amalgaviruses in cultivated and wild plants.

Poster Session

VIR-PT2060 - Does Cerumen have a risk for transmission of HIV?

Fatih Mehmet Hanege[1], M. Tayyar Kalcioglu[1], Fatma Sargin[2], Zafer Cetinkaya[3], Muhammet Tekin[1], Haluk Vahapoglu[2]

[1]Istanbul Medeniyet University, Medical Faculty, Goztepe Training and Research Hospital, Department of Otorhinolaryngology, Istanbul, Turkey, [2]Istanbul Medeniyet University, Medical Faculty, Goztepe Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey, [3]Istanbul Medeniyet University, Medical Faculty, Goztepe Training and Research Hospital, Department of Microbiology and Clinical Microbiology, Istanbul, Turkey

Objectives/Hypothesis: Human Immunodeficiency Virus (HIV) is a worldwide public health problem. HIV is mainly transmitted by sexual activity, parenteral exposures, and body secretions such as saliva and semen which have been studied before. Cerumen, however, has not been investigated for its capability to transmit HIV. The aim of this study is to evaluate the potential of cerumen in transmission of HIV infection. Study Design: This study was conducted with 42 newly diagnosed patients with confirmed positive blood HIV RNA as the study group, 27 treated patients with negative HIV RNA as the positive control, and 10 HIV non-infected patients as the negative control group. Methods: Seventy-nine cerumen specimens collected from all of the groups were prospectively analyzed for the presence of HIV RNA by polymerase chain reaction. Comparison was made against concordant blood HIV RNA results. Results: None of the 79 cerumen specimens were positive for HIV RNA. Conclusion: This study showed that cerumen has no significant risk for transmission of HIV infection, even in patients with high HIV RNA serum levels. However, standard infection control precautions should be applied carefully in all examinatory and surgical operations of the ears.

Workshop Sessions

BAM-WK106.01 - Of mice and mutants: unravelling leptospiral pathogenesis

Ben Adler[1]
[1]*Monash University, Clayton, Australia*

The availability of leptospiral genome sequences has allowed comparative genomics analyses of pathogenic, saprophytic and intermediate species, which identified a core set of leptospiral genes and suggested that intermediate species are more closely related to pathogenic species, but retain the enhanced metabolic capacity of saprophytic species. Genes of unknown function are over-represented in the pathogen-specific gene subsets, suggesting pathogenic mechanisms in leptospirosis are unique to Leptospira. Likewise, whole genome transcriptomics studies have found that genes upregulated under simulated in vivo conditions generally have no defined function. The recent advent of methods for constructing defined transposon mutants in pathogenic Leptospira has allowed for the first time an investigation of specific virulence factors invoilved in acute disease. These studies have now identified several essential virulence factors, including LPS, motility, catalase, heme oxygenase, the stress proteins ClpB and HtpG, the uveitis-associated protein LruA, and the OmpA-like protein Loa22. Also of interest has been the finding that many previously predicted virulence genes are not essential for the ability of Leptospira to cause disease, consistent with the notion of a degree of functional redundancy for virulence-associated genes. A high throughput screen of transposon mutants has allowed the identification of virulence genes of unknown function and has also identified for the first time genes required for renal colonization of the carrier host.

Workshop Sessions

BAM-WK106.02 - The study of vascular interaction and transmigration of the Lyme spirochete using intravital microscopy

George Chaconas[1]
[1]*University of Calgary, Calgary, Canada*

Lyme borreliosis is a common vector transmitted disease in the northern hemisphere. It is caused by the spirochete Borrelia burgdorferi and related species. Lyme spirochetes disseminate from the site of infection by invasion of the vasculature followed by transmigration into a variety of organ systems. This can result in a wide variety of disease manifestations in disseminated disease. We are studying vascular interactions and transmigration using genetically engineered, infectious, fluorescent spirochetes and high resolution intravital imaging in living mice. Studies on the spirochete proteins and on host proteins and macromolecules involved in adhesion and transmigration will be described.

Workshop Sessions

BAM-WK106.03 - Unmasking the stealth pathogen: characterization of *Treponema palladium* rare outer membrane proteins

Amit Luthra[1], Arvind Anand[1], Justin Radolf[1]
[1]Department of Medicine, University of Connecticut Health Center, Farmington, USA

In contrast to protein-rich outer membranes (OMs) of Gram-negative bacteria, the syphilis spirochete's OM contains a paucity of outer membrane proteins (OMPs). The dearth of OMPs is the ultrastructural basis for its reputation as the stealth pathogen. Several years ago, we devised a bioinformatics-based computational framework to identify T. pallidum (Tp) rare OMPs based on the assumption that they adopt the β-barrel configuration typical of OMPs in Gram-negative bacteria. Among the candidate OMPs identified were TP0326, a BamA ortholog, and members of the Tp repeat (Tpr) family. Using biophysical methodologies, along with surface localization, we have demonstrated that TP0326 meets all necessary criteria to establish it as a bona fide rare OMP and possesses the hallmark bipartite topology of a BamA. Using X-ray structures for other BamAs, we developed a topological model for the TP0326 β-barrel and identified an immunodominant, surface-exposed loop. SAXS analysis revealed that the periplasmic POTRA domain is much less flexible than its E. coli counterpart; we hypothesize that its limited flexibility serves to restrict incorporation of newly exported precursors into the OM. Using a similar combination of biophysical and surface localization methodologies, we have determined that TprC and TprI have a bipartite topology in which the C-terminal domain forms a β-barrel with porin-like properties, while the periplasmic N-terminal domain anchors the protein to the peptidoglycan. We hypothesize that the Tprs are functional orthologs of E. coli porins and that their substrate specificities collectively enable Tp to meet its nutritional requirements while presenting minimal surface antigenic targets during infection. Most recently, we have determined that TP0326, TprC, and TprI expressed in the E. coli OM retain their native bipartite topologies. We are now positioned for the next phase of our ongoing efforts to unmask the stealth pathogen—using genetic strategies to delineate the membrane topologies of rare OMPs.

Workshop Sessions

BAM-WK106.04 - Leptospira genome-wide bioinformatics for genes implicated in biofilm formation

Paula Ristow[1,2], Gregorio Iraola[3], Áurea Maria Almeida[1], Larissa Vasconcelos[1], Priscyla dos Santos Ribeiro[1], Bruno Lopes Bastos[1], Lucia Spangenberg[3], Hugo Naya[3]

[1]Universidade Federal da Bahia, Salvador, Brazil, [2]Fundação Oswaldo Cruz, Salvador, Brazil, [3]Institut Pasteur, Montevideo, Uruguai

Leptospirosis is endemic in Brazil and in other regions worldwide, with annual epidemic outbreaks. The etiological agent, pathogenic Leptospira, form biofilms in vitro and in vivo. Biofilms are the main lifestyle of prokaryotes. The molecular mechanisms of biofilm formation in Leptospira are still unknown. The objective of this work was to identify orthologs involved in biofilm formation in the genomes of saprophytic and pathogenic Leptospira spp.. We firstly performed a search for genes with proven biological role in biofilm formation in other bacteria using automated tools (search in the Gene database from the National Center for Biotechnology Information site, “R” and NCBI2R package) and a manual search in the literature. Gene sequences were compared using Basic Local Alignment Search Tool with the following complete genomes of Leptospira: L. interrogans (three genomes), L. borgpetersenii Hardjo (two), L. biflexa Patoc (two). From the 153 genes implicated in biofilm formation, Leptospira presented 19 orthologs. One of those orthologs (rhlC) coded for rhamnosyltransferase 2 and is only present in pathogenic Leptospira species. Rhamnolipids are biosurfactants that mediate the biofilm detachment. Four genes coded for heat shock proteins. One of those was clpD, a homolog of clpB, which codes a chaperon related to virulence attenuation in L. interrogans. Three genes were involved in cell signaling, two of them from two-component systems. We also found two global transcriptional regulators (etrA and csrA). Three genes were related to the biosynthesis of flagella (flgK, fliC and fliS). Six genes coded for enzymes, within them three for carbohydrate metabolism, which may be related to the synthesis of the matrix. Lastly, two genes coded for proteins of sulfur metabolism. Unveiling the molecular mechanisms of biofilm formation will contribute to understand the leptospiral biology. It also may be helpful to develop new biotechnologies for the control of leptospirosis.

Workshop Sessions

BAM-WK106.05 - Association of a MHC-DQa locus homolog with immune responses to Borrelia in Peromyscus leucopus, the reservoir host for Lyme disease

Vanessa Cook[1], Alan Barbour[1]
[1]*Department of Microbiology and Molecular Genetics University of California, Irvine, USA*

Association of MHC II locus polymorphisms with immune responses to infections has been studied in humans and laboratory mice, but little is known about these associations in natural reservoir hosts for zoonoses. *Peromyscus leucopus*, the white-footed mouse, is a major reservoir for *Borrelia burgdorferi*, the Lyme disease agent, and a target for wildlife vaccines. *P. leucopus* populations are genetically diverse and may differ in innate and adaptive responses to infections and immunizations. In this study, we aimed to assess diversity in *Peromyscus* on relevant quantitative traits, characterize host determinants of the outcome of infection and identify loci that contribute to susceptibility of infection and its outcome. Therefore, 30 outbred *P. leucopus* were immunized with keyhole limpet hemocyanin (KLH) and *B. burgdorferi* OspA on day 0. On day 14 animals were injected with *B. hermsii* and sacrificed on day 21. Infection and antibody responses were characterized by qPCR for bacteria in blood and spleen and ELISA for serum antibodies to borrelial FbpC protein as well as KLH and OspA. In addition, exon 2 of a MHC II DQa locus was sequenced and used for genotyping. The animals differed in bacterial burdens in blood and spleens. All produced antibody to KLH but varied markedly in antibodies to OspA and FbpC. Genotyping identified 8 MHC alleles among individual animals and distributed thusly: 9 homozygous for allele 1; 13 heterozygous for alleles 1+2; and 8 heterozygous with other combinations. The two groups with allele 1 had significantly lower antibody levels to OspA and FbpC than group 3. These findings indicate that polymorphisms of a MHC II locus are associated with differences in antibody responses to immunization and infection in a major reservoir for human infections.

Workshop Sessions

BAM-WK107.01 - Microbial ecosystem therapeutics as a new paradigm in medicine

Emma Allen-Vercoe[1], Elaine Petrof[2]
[1]University of Guelph, Guelph, Ontario, [2]Queen's University, Kingston, Ontario

It is increasingly realized that the gut microbiota plays an extremely important role in human health, and that damage to this delicate microbial ecosystem can result in disease. Yet, the microbial ecosystem in the gut is exceedingly complex and variable, so that understanding how ecosystem disturbance affects health is difficult. In this talk, I will describe the work we are doing to study human colonic ecosystems, and the reactions of these ecosystems to introduced stressors, in order to understand how ecosystem disturbance can lead to disease. Many diseases, including obesity, diabetes, regressive autism and inflammatory bowel disease are associated with a disturbed gut microbial ecosystem (sometimes called 'dysbiosis') which is in turn most often associated with reduced diversity in microbial composition. Therefore, one approach to treating disease could be through replenishment of the 'missing' microbiota. I will finish my presentation by describing our work to develop 'microbial ecosystem therapeutics' as a new approach to managing disease through re-balancing of the gut microbiota.

Workshop Sessions

BAM-WK107.02 - The bovine rumen microbiome

Itzhak Mizrahi[1]
[1]*Bet Dagan, Israel*

The mammalian gut microbiota is essential in shaping many of its host's functional attributes. Relationships between gut bacterial communities and their mammalian hosts have been shown in recent years to play an important role in the well-being and proper function of their hosts. A classic example of these relationships is found in the bovine digestive tract in a compartment termed the rumen. The rumen microbiota is necessary for the proper physiological development of the rumen and for the animal’s ability to digest and convert plant mass into basic food products, making it highly significant to humans and a perfect model system for the study of host - microbes interactions. In my lecture I will discuss some of our recent findings regarding this ecosystem, including gene mobility, the establishment of this microbial community and the changes occurring with the age and diet of the host.

Workshop Sessions

BAM-WK107.03 - The marine connection: occurrence of algal cell wall polysaccharide degrading systems in intestinal Bacteroidetes

Mirjam Czjzek[4,5], François Thomas[1], Etienne Rebuffet[2], Jan-Hendrik Hehemann[3], Murielle Jam[4,5], Gaëlle Correc[4,5], Tristan Barbeyron[4,5], Gurvan Michel[4,5]

[1]Biology Department, Mail Stop 52, Watson Laboratory, Woods Hole Oceanographic Institution Woods Hole, USA, [2]Department of Chemistry, Biochemistry and Biophysics, University of Gothenburg, Göteborg, Sweden, [3]Massachusetts Institute of Technology, Department of Civil and Environmental Engineering, Cambridge, USA, [4]UPMC University Paris 6, UMR 8227 Laboratory for Integrative Biology of Marine Models, Station Biologique de Roscoff, Bretagne, France, [5]CNRS , UMR 8227 Laboratory for Integrative Biology of Marine Models, Station Biologique de Roscoff, Bretagne, France

Marine heterotrophic bacteria play a crucial role in the carbon cycle, by recycling the organic matter produced by marine algae. This algal biomass is mainly composed of recalcitrant polysaccharides distinct from land plant polysaccharides. The catabolism of algal polysaccharides thus remains largely unknown, impairing the correct interpretation of marine (meta)genomic data. To answer this issue, our group is developing *Zobellia galactanivorans* as a model bacterium for the bioconversion of algal polysaccharides. This marine *Flavobacterium* was isolated in Roscoff (Barbeyron 2001); its genome confirmed the potential for polysaccharide bioconversion. Using phylogenetic and comparative genomic approaches, we have thus discovered and determined the crystal structure of the first porphyranases (Hehemann 2010) and 1,3-α-3,6-anhydro-L-galactosidases (Rebuffet 2011), which are involved in the degradation of agars and porphyrans, the main cell wall polysaccharides of red algae. Analysis of metagenomic data indicates that porphyranases and 1,3-α-3,6-anhydro-L-galactosidases are typical of coastal environment. These genes of marine origin have been also transferred to a gut bacterium isolated from Japanese individuals. Metagenomic analyses confirmed that porphyranases and 1,3-α-3,6- anhydro-L-galactosidases are absent in North American and Danish population but present in Spanish and Japanese populations with proportions of 10 and 38% respectively. The rationale of “marine” enzymes in gut microbes is linked to the high input of seafood in the diet of these two populations. In both populations, contacts between human-associated microbes and non-sterile seaweed- or animal-seafood have created a favorable condition for lateral gene transfers from marine bacteria to human gut Bacteroidetes. Horizontal gene transfers can thus provide gut bacteria with original sets of utensils to degrade otherwise refractory substrates found in the diet. A more complete understanding of the genetic gateways between food-associated environmental species and intestinal microbial communities sheds new light on the origin and evolution of Bacteroidetes as animals’ symbionts

Workshop Sessions

BAM-WK107.04 - The microbiome of breast tissue and the implications for health and disease

Camilla Urbaniak[1], Muriel Brackstone[2], Greg Gloor[3], Gregor Reid[4]
[1]Western University/Lawson Health Research Institute, London, Canada, [2]London Regional Cancer Program, London, Canada, [3]Western University, London, Canada, [4]Western University/Lawson Health Research Institute, London, Canada

The Human Microbiome project has allowed for a better understanding of the bacteria that colonize the human body. While the vast majority of such information relates to the gastrointestinal tract, there is also recent evidence of other 'tract-related' organs harbouring endogenous microbiota, such as the lungs and bladder, which were traditionally thought of as sterile. However, no dedicated study on the characterization of a potential microbiome within the breast, or any other "non tract-related" organ, has been reported. To investigate this, we used 16S rRNA sequencing, culture, denaturing gradient gel electrophoresis, qPCR and TOPO cloning to analyze the bacterial profiles in breast tissue from 81 women recruited from both Canada and Ireland. We discovered a diverse population of bacteria with profiles that differed between women with cancer and those free from disease ("healthy"). The main organisms associated with cancer were Escherichia/Shigella, Bacillus, Pseudomonas, Propionibacterium, Staphylococcus and Listeria. We are currently sequencing the genome of 3 different species of bacteria isolated from women with cancer to determine whether they have genetic properties that may promote breast cancer development or progression. We are also testing the ability of certain species to induce or prevent DNA damage as well as the ability of species isolated from "healthy" women to degrade environmental carcinogens commonly found in the mammary glands. These findings could lead to possible therapeutic strategies that modulate the breast microbiome in order to reduce the risk of breast cancer development/progression.

Workshop Sessions

BAM-WK107.05 - Antibiotics affect resistance genes found in the gut microbiome

Frédéric Raymond[1], Amin Amhed Ouameur[1], Maxime Déraspe[1], Naeem Iqbal[1], Maurice Boissinot[1], Ann Huletsky[1], Paul H. Roy[1], Marc Ouellette[1], Michel G. Bergeron[1], Jacques Corbeil[1]
[1]Centre de Recherche en Infectiologie de l'Université Laval, Québec, Canada

The human gut microbiome is affected by antibiotics and individuals respond differently to the same drug. To specifically address this question, second-generation cephalosporin Cefprozil was administered to 18 healthy volunteers for 7 days. We gathered stool samples before antibiotic exposure, immediately after exposure and three months later. Six untreated controls were also enrolled. An average of 15 billion nucleotides were sequenced on Illumina HiSeq for each sample. Metagenomes were assembled and profiled for taxonomy and resistance genes using the RAY Meta 2.0 assembler. Multivariate analysis showed consistent effects in antibiotic exposed participants compared to untreated controls, such as the increase of Clostridiaceae and the decrease of Veillonellaceae, Streptococcaceae, Lactobacillaceae, Coriobacteriaceae, Lachnospiraceae and Campylobacteraceae. While resistance gene content was much altered by the antibiotic, the impact of Cefprozil remained specific to individual participants. Using multiple factor analysis, increased abundance of taxa such as Escherichia coli, Enterobacter, Fusobacterium and Salmonella could be correlated with the presence of resistance genes. Other notable events include a radical shift from the Prevotella to the Bacteroides enterotype for one participant after antibiotic treatment, concomitant with an increased frequency of a blaTEM gene located within a mobile element. Point mutations in beta-lactamases such as blaCfxA were enriched after antibiotic treatment in several participants. Overall, these results suggest that although the microbiota is perturbed by antibiotics, the initial state of the microbiome plays a key role in its response to antimicrobial agents.

Workshop Sessions

BAM-WK108.01 - Wrapping divisome elements in artificial containers.

Manuel Pazos[1], Alicia Sánchez-Gorostiaga[1], Paolo Natale[1], Miguel Vicente[1]
[1]Centro Nacional de Biotecnología-CSIC,Madrid, Spain

Reconstruction of partial assemblies of the Escherichia coli divisome in the test tube, from simple nanodiscs to complex GUVs have been used. Nanodiscs allowed to measure the affinity between FtsZ polymers or oligomers and ZipA when tethered to a lipid bilayer (Hernández-Rocamora et al., 2012, J. Biol. Chem. 287: 30097-30104). In E. coli, FtsZ, ZipA and FtsA are the main proto-ring elements, they initiate assembly of the divisome. Permeable GUVs with attached ZipA shrink following FtsZ polymerization. They resemble the membrane invagination observed in cells producing high amounts of ZipA, in which the membrane permeability barrier is lost (Cabré et al., 2013, J. Biol. Chem. 288: 26625-26634). Maxicells are nucleoid-free cells in which protein synthesis is nevertheless possible (Sancar et al., 1979, J. Bacteriol. 137: 692–693) They retain some proteins and functions of the living cell. MinD and MinE, two components of one of the septum placement mechanisms, retain in maxicells their pole to pole oscillation. SlmA, one component of the alternative nucleoid occlusion septum placement mechanism, as well as the FtsZ protein itself, are degraded (Pazos, et. al., 2014, PLoS ONE 9(3): e91984. doi:10.1371/journal.pone.0091984). ZipA helps to stabilize FtsZ in maxicells. Stabilization requires the FZB domain of ZipA interaction with the C-terminal central hub FtsZ domain (Pazos et al., 2013, J. Biol. Chem. 288: 3219-3226). The non-essential ZapA and ZapB interact with each other and associate with FtsZ to stabilize the FtsZ-ring. In maxicells ZapB localizes directly to the division site independently from the nucleoid. A direct interaction of ZapB with FtsZ was found. Coinciding with FtsZ-ring disassembly, ZapA and ZapB migrate from midcell to the pole taking FtsZ with them (Pazos et al., 2013, Environmental Microbiol. 15: 3282–3291).

Workshop Sessions

BAM-WK108.02 - Non-canonical division in rod-shaped gammaproteobactertia

Nikolaus Leisch[2], Jolanda Verheul[1], Nika Pende[2], Tanneke den Blaauwen[1], Silvia Bulgheresi[2]
[1]University of Amsterdam, Bacterial Cell Biology, Amsterdam, The Netherlands, [2]University of Vienna, Department of Ecogenomics and System Biology, Vienna, Austria

The gammaproteobacterium .*Escherichia coli* has been investigated for decades as the standard model for bacterial growth and division. However more and more rod-shaped bacterial species (e.g. *Caulobacter crescents*, *Mycobacterium tuberculosis*, *Myxococcus xanthus*, *Bacillus subtitles*) are investigated and subtle differences in their division machinery and mode of division have been found (1). Recently, rod-shaped gammaproteobacteria have been described that divide longitudinally instead of transverse (2). These bacteria live in symbiosis with marine nematodes. A surprising variety in morphology of these bacteria can be found each specialized in the symbiosis with their own species of nematode. The symbionts can be attached to the surface of the symbiont with one pole (palisade-like) or with both poles (crescent-like). They can be short (2.5 μm) or very long (100 μm) and they can divide from one pole or both poles or symmetrically at mid cell despite their enormous length, depending on the species. Curiously, these variations are all achieved with more or less the same set of cell division proteins as present in *Escherichia coli*. 1. Blaauwen, den, T. (2013). Prokaryotic cell division: flexible and diverse. Current Opinion in Microbiology, 16(6), 738–744. 2. Leisch, N., Verheul, J., Heindl, N. R., Gruber-Vodicka, H. R., Pende, N., Blaauwen, den, T., & Bulgheresi, S. (2012). Growth in width and FtsZ ring longitudinal positioning in a gammaproteobacterial symbiont. Current Biology: CB, 22(19), R831–2.

Workshop Sessions

BAM-WK108.03 - Coordinating bacterial cell division with nutrient availability: a role for glycolysis

Leigh Monahan[1], Isabella V. Hajduk[1], Sinead P. Blaber[2], Ian G. Charles[1], Elizabeth Harry[1]
[1]The ithree Institute, University of Technology, Sydney, Ultimo, Australia, [2]Department of Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, Australia

Cell division in bacteria is driven by a cytoskeletal ring structure, the Z ring, composed of polymers of the tubulin-like protein FtsZ. Z ring formation must be tightly regulated to ensure faithful cell division, and several mechanisms have been described that influence the positioning and timing of Z ring assembly. Another important but as yet poorly understood aspect of cell division regulation is the need to coordinate division with cell growth and nutrient availability. In this study we demonstrate for the first time that cell division is intimately linked to central carbon metabolism in the model Gram-positive bacterium Bacillus subtilis. We show that a deletion of the gene encoding pyruvate kinase (pyk), which produces pyruvate in the final reaction of glycolysis, rescues the assembly defect of a temperature sensitive ftsZ mutant and has significant effects on Z ring formation in wild-type B. subtilis cells. Addition of exogenous pyruvate restores normal division in the absence of the pyruvate kinase enzyme, implicating pyruvate as a key metabolite in the coordination of bacterial growth and division. Our results support a model in which pyruvate levels are coupled to Z ring assembly via an enzyme that actually metabolizes pyruvate, the E1 alpha subunit of pyruvate dehydrogenase. We show that this protein localizes over the nucleoid in a pyruvate-dependent manner, and may stimulate more efficient Z ring formation at the cell centre under nutrient-rich conditions when cells must divide more frequently. Ultimately this helps to ensure the survival of newborn cells.

Workshop Sessions

BAM-WK108.04 - Structure of the AlfA filament: a novel bacterial actin architecture

Emeric Charles[1], Shunkai Yang[1], Jessica Polka[2], Jesse Hansen[1], R. Dyche Mullins[3], Justin Kollman[1]
[1]McGill University, Montreal, Canada, [2]Harvard University, Boston, USA, [3]UC San Francisco, San Francisco, USA

Many low copy number bacterial plasmids guard against stochastic loss at cell division by active partition into daughter cells. A common type of partition system makes use of actin homologs to drive plasmid movement. The bacterial actins themselves differ dramatically in both dynamic properties and structure. This variation suggests that different molecular mechanisms underlie plasmid partitioning by different bacterial actins, possibly representing unique constraints imposed by different host species. The bacterial actin AlfA drives plasmid partitioning in some Bacillus subtilus strains, and has been shown to have unique dynamic properties as well as a novel, highly twisted filament architecture. Here, we describe the structure of the AlfA filament generated by cryo-electron microscopy and homology modeling. AlfA's unusual filament architecture appears to be the result of a unique domain deletion within the monomer. AlfA filaments have a strong propensity to form well-ordered bundles, which is thought to be an important component of establishing a bi-oriented partitioning system. AlfA is linked to the plasmid by the DNA-binding adaptor protein AlfB. We have assembled the ternary complex of AlfA-AlfB-DNA, and present a preliminary model of interactions within the complex.

Workshop Sessions

BAM-WK108.05 - Bacterial cell wall assembly arrest in the ΔelyC mutant is caused by oxidative stress

Imène Kouidmi[1], Catherine Paradis-Bleau[1]
[1]*Université de Montréal, Montréal, Canada*

We recently performed a genome-wide screen in Escherichia coli to identify novel factors important for Gram-negative bacterial envelope assembly. We involved many factors of previously unknown function in this process. One factor renamed ElyC is extremely conserved among bacteria and was selected for further studies. An E. coli strain with a deletion of elyC is not viable at room temperature and mutant cells lyse at the beginning of stationary phase. Specific labeling of the cell wall with radioactive meso-diaminopimelic acid confirmed that the lysis is due to a cell wall assembly arrest. We observed that the defective phenotypes of the ΔelyC mutant worsen when increasing the oxygenation of the bacterial culture. We also showed that hydroxyl radicals accumulate in ΔelyC mutant cells at room temperature and that the level of oxidative stress is higher in the mutant than in the wild-type. We hypothesized that cell wall assembly arrest and cell lysis of the mutant cells are linked to oxidative stress. This was confirmed when we showed that inhibiting the production of hydroxyl radicals by the Fenton reaction with the iron chelator 2,2'-dipyridyl and growing the cells under anaerobic conditions suppress the defective phenotypes of the ΔelyC mutant. To understand how oxidative stress affects cell wall assembly, we took a candidate approach and identified dsbG as a multi-copy suppressor of ΔelyC mutant cell lysis. DsbG is a periplasmic reductase that protects proteins containing single cysteine residues against oxidation. The L,D-transpeptidases YbiS, ErfK and YnhG are known DsbG substrates involved in cell wall biogenesis. However, overexpression of dsbG in a triple mutant strain inactivated for ybiS, erfK and ynhG still suppresses the ΔelyC defective phenotypes. We are currently identifying the DsbG substrate(s) responsible for the suppression effect to clarify the link between ElyC, oxidative stress and bacterial cell wall assembly.

Workshop Sessions

BAM-WK108.06 - A novel essential protein links replication and chromosome partitioning in Caulobacter crescentus

James Taylor[1], Gregory Marczynski[1]
[1]McGill University, Montreal, Canada

We used a combined genetic and biochemical approach to identify a novel protein (ObpA) that binds to the chromosome replication origin and regulates replication in Caulobacter crescentus, a model for the bacterial cell cycle. ObpA is restricted to the alpha-proteobacteria and in this group it is a highly conserved protein, being encoded in almost all sequenced genomes. We show that the ObpA protein is essential for viability and is required for the initiation of chromosome replication. We have identified conserved amino-acid residues required for the ObpA-DNA interaction. ObpA represents a novel class of DNA-binding proteins and its high abundance in C. crescentus suggests additional roles elsewhere in the genome. We used ChIP-seq to identify the genomic binding targets for ObpA and found that it interacts preferentially with the chromosome partitioning locus (parS). Fluorescence microscopy shows that localization of a mCherry-ObpA fusion protein is dynamic over the cell cycle, moving ahead of the partitioning chromosome and finally forming a gradient from the poles to the mid-cell prior to division. ChIP-qPCR shows that ObpA binding to the replication origin occurs late in the cell cycle, prior to cell-division. We hypothesize that the partition-associated dynamics of this protein allow the origin to determine that partition has taken place and to prime the origin for the next round of chromosome replication.

Workshop Sessions

BAM-WK109.01 - Bacterial quorum sensing, social cheats and a solution to Darwin's dilemma – co-regulatory constraints on freeloaders in cooperating groups of bacteria

E. Peter Greenberg[1]
[1]*University of Washington, Seattle, USA*

In the bacterium Pseudomonas aeruginosa a gene regulator called LasR activates dozens of genes in response to the LasI-produced quorum-sensing signal 3OC12-HSL. The most overrepresented LasR-controlled functions code synthesis of exoproducts. Exoproducts are common goods that are made by individuals and shared amongst the group. Members of quorum-sensing groups are thus thought of as cooperators. Quorum-sensing cooperators are susceptible to invasion by social cheaters. LasR mutants emerge in and co-exist with LasR-wild-type cells. The LasR mutants benefit from cooperator-derived common goods without incurring a production cost. We wondered why a small percentage of LasR-controlled genes code for ability to grow on transported solutes where benefit is restricted to the cell in which the enzymes are produced (private goods). We hypothesized that one consequence of maintaining a few such activities under LasR control would be to restrain the emergence of social cheaters-to sanction cheaters in the group. We have shown that co-regulation of public and private goods by quorum sensing provides a strong constraint against social cheating. This might represent a form of social engineering in bacterial communities and it provides a plausible explanation for why a small percentage of functions controlled by quorum sensing are private goods. It is one solution to Darwin's dilemma. How can cooperation be stably maintained in groups where non-cooperators are predicted to have a fitness advantage? We have also begun to address the question, how can cooperation be broken in otherwise stable groups of cooperators and we have discovered ways in which a tragedy of the commons can reproducibly be induced in cooperatives of bacteria.

Workshop Sessions

BAM-WK109.02 - Regulation of biofilm, motility and virulence properties of EAL domain only proteins

Ute Römling[1]
[1]*Karolinska Institutet, Stockholm, Sweden*

Cyclic di-GMP is an almost ubiquitous second messenger involved in the regulation of many physiological processes. One of the most ancient roles of cyclic di-GMP signaling is probably the regulation of the transition between motility and sessility. In pathogenic bacteria c-di-GMP can regulate the transition between colonization and acute infection. Thereby, cyclic di-GMP is synthesized by GGDEF domain proteins and degraded by EAL domain proteins. In the gastrointestinal pathogen Salmonella typhimurium cyclic di-GMP regulates the transition from motility to sessility. In addition, cyclic di-GMP is involved in the regulation of first line virulence properties such as invasion of epithelial cells and induction of the proinflammatory cytokine IL-8. Screening mutants of S. typhimurium cyclic di-GMP metabolizing proteins for alterations of the invasion phenotype in epithelial cells recently discovered an unconventional phenotype for the EAL domain like protein STM1697 as STM1697 inhibits invasion, motility, and secretion of the type three secretion system 1 effector protein SipA, whereas it promotes rdar biofilm formation and CsgD expression. STM1697 can, however, functionally replace the EAL-like protein STM1344 and vice versa, whereby both proteins neither degrade nor bind c-di-GMP. With an interface with FlhD distinct from its paralogue STM1344, STM1697 binds to FlhD, a component of the master regulator of the flagella regulon FlhD4C2 and acts additively under numerous conditions. We predict that the stand alone EAL domain proteins STM1697 and STM1344 belong to a subclass of EAL domain proteins, which are involved in motility, biofilm and virulence regulation through interaction with proteins that regulate flagella function. Reference: Ahmad I, Wigren E, Le Guyon S, Vekkeli S, Blanka A, El Mouali Y, Anwar N, Chuah ML, Lünsdorf H, Frank R, Rhen M, Liang ZX, Lindqvist Y, Römling U. The EAL-like protein STM1697 regulates virulence phenotypes, motility and biofilm formation in Salmonella typhimurium. Mol Microbiol. 2013, 90:1216-32.

Workshop Sessions

BAM-WK109.03 - A bacterial thermosensor modulates biofilm formation in response to human body temperature

Joe Harrison[1], Trevor Randall[1], Laura Jennings[2], Weerayuth Kittichotirat[2], Roger Bumgarner[2], Geoffrey Winsor[3], Fiona Brinkman[3], Tara Winstone[1], Larissa Singletary[2], Matthew Parsek[2]
[1]University of Calgary, Calgary, Canada, [2]University of Washington, Seattle, USA, [3]Simon Fraser University, Vancouver, Canada

Enzymes that “make and break” the second messenger bis-(3’,5’)-cyclic-dimeric-guanosine monophosphate (c-di-GMP) have been identified in nearly every known bacterial phylum. Among many pathogens, low levels of c-di-GMP upregulate motility and acute virulence factors, whereas high levels promote extracellular polymer production and biofilm development. While much is known about the diguanylate cyclase (DGC) and phosphodiesterase domains that synthesize and degrade c-di-GMP, respectively, few signals to which these proteins respond have been identified. Here we sought these signals by searching for conditions that could trigger or suppress the Pseudomonas aeruginosa rugose small colony variant (RSCV) phenotype, which is expressed when c-di-GMP is elevated. This identified a temperature-dependent RSCV phenotype for P. aeruginosa CF39S, a sputum isolate recovered from a cystic fibrosis patient suffering from chronic respiratory infection. Using data from whole-genome optical mapping and single-molecule real-time sequencing, the 7.3 megabase CF39S genome, which harbors a 500 kilobase megaplasmid, was finished in silico. Using genetic, biochemical and bioinformatics-based comparisons of a closely related isolate that did not respond to temperatures, we linked this phenotype to a transposon encoded DGC, which we have named the thermosensing diguanylate cyclase A (TdcA). This enzyme possesses a hallmark GGDEF-domain that catalyzes the synthesis of c-di-GMP, and purified recombinant TdcA displays a 140-fold increase in activity when shifted from room temperature (25°C) to body temperature (37°C). Acquisition of TdcA confers thermal control of biofilm formation to laboratory P. aeruginosa strains. Moreover, RNA-seq of P. aeruginosa CF39S revealed that TdcA activity not only regulates genes for extracellular polymers and motility, but also genes of unknown function in the accessory genome. Altogether, this work identifies a signal that directly regulates a DGC, suggests that c-di-GMP may control genes outside the core genome, and provides insight into a mechanism for temperature-sensing that may modulate biofilm formation in response to a host.

Workshop Sessions

BAM-WK109.04 - Quorum Sensing Regulates Rhamnolipids Biosynthesis in a Nutritionally Dependent Manner in Burkholderia glumae

Arvin Nickzad[1], Eric Déziel[1]
[1]*INRS - Institut Armand-Frappier, Laval, Canada*

Burkholderia glumae is a plant pathogenic bacterium that produces a considerable amount of rhamnolipids, a surface-active glycolipid considered as an alternative to synthetic surfactant. Being non-pathogenic to humans, it has the potential to replace the best known rhamnolipid producer, Pseudomonas aeruginosa, which is an opportunistic human pathogen, and hence reduce the production costs attributed to biosafety conditions required by this level 2 pathogen. In B. glumae the expression of major virulence determinants, including toxoflavin, lipase and flagella are under control of a LuxR-LuxI-type Quorum Sensing (QS) system. TofI is the N-acyl homoserine lactone synthase directing the synthesis of the extracellular signal N-octanoyl homoserine lactone (C8-HSL) that is recognized and bound by its cognate transcriptional regulator TofR. The TofR-C8-HSL complex activates the expression of target genes. In the present study, we demonstrate that QS positively regulates the expression of rhamnolipids biosynthesis genes in B. glumae. A QS-defective (tofI::Ω) mutant that lacks the production of C8-HSL does not produce rhamnolipids in a minimal medium and rhamnolipid production is restored by adding C8-HSL. Unexpectedly, the tofI::Ω mutant regains the ability to produce rhamnolipids in a complex nutrient broth (NB) medium. These results indicate that, in contrast with the situation in P. aeruginosa, regulation of rhamnolipids by QS in B. glumae is not absolute but instead nutritionally conditional. This suggests the presence of previously unknown regulatory mechanisms for production of rhamnolipids that are independent of TofI/TofR QS system.

Workshop Sessions

BAM-WK109.05 - Rhodococcal quorum quenching versus pathogen quorum sensing

Corinne Barbey[1], Andrea chane[1], Nicole Orange[1], Marc Feuilloley[1], Jean-François Burini[1], Xavier Latour[1]
[1]*LMSM, Evreux, France*

Virulence functions in numerous human or plant-associated bacterial genera have been described to be regulated by autoinducer-1-based quorum sensing. This mechanism involves both the synthesis and perception of N-acyl-homoserine lactone signaling molecules leading coordinated gene expression in a bacterial population. The communication used by these pathogenic bacteria is a potentially useful target for the development of novel biocontrol methods. These therapies are not aimed at eradicating the pathogen, but rather at exploiting its mechanisms of cellular communication to reduce the expression of virulence systems while maintaining the microbial balance. In situ rhodococcal quenching was fully demonstrated only the last year (Barbey et al., 2013). Here, the environmental Gram-positive Rhodococcus erythropolis prevents plant disease by disrupting quorum sensing-based communication of soft-rot bacteria. Such biocontrol activity results from the action of a novel pathway, which we called the γ-lactone catabolic pathway. This pathway is responsible for cleaving the lactone bond of a wide range of compounds comprising a γ-butyrolactone ring coupled to an alkyl or acyl chain. The aliphatic products of this hydrolysis are then activated and enter fatty acid metabolism. Interestingly, new data reveal that the expression of the γ-lactone catabolic pathway is induced not by the invasion step or the presence of the pathogen, but by its communication. The presence of signaling molecules is sensed by a TetR-like transcriptional regulator and signals binding induces a changing of regulator conformation, which allows the expression of the γ-lactone operon. This regulation mechanism appears to be an economic system well suited to the roles played by the pathway in rhodococcal metabolism, fitness and detoxification. We present the γ-lactone catabolic pathway (proteomic approach), its regulation mechanism, and resulting biocontrol activity (transcriptomic and confocal laser scanning microscopy approaches). The current application of this method to control others pathogens will be also discussed.

Workshop Sessions

BAM-WK109.06 - Persistent and hyperinfectious subpopulations result from bistable expression of CsgD in Salmonella typhimurium

Keith MacKenzie[1,2], Yejun Wang[1], Dylan Shivak[1,2], Cynthia Wong[1], Leia Hoffman[1], Po-King Lam[1], Ebtihal Alshabib[3], Andrew Cameron[3], Wolfgang Köster[1,3], Aaron White[1,2]

[1]Vaccine and Infectious Disease Organization-International Vaccine Centre, Saskatoon, Canada, [2]Department of Microbiology and Immunology, University of Saskatchewan, Saskatoon, Canada, [3]Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada, [4]Department of Biology, University of Regina, Regina, Canada

Bacteria have evolved diverse strategies that allow them to live in nearly all niches on earth. For pathogens, survival is ultimately dependent upon transmission to new hosts. In the case of Salmonella Typhimurium, there are numerous examples of extreme persistence in food products and environmental reservoirs, but it is not well understood how this relates to virulence and their ability to cause infections. Here we show that the presence of specialized cell types may account for the ability of S. Typhimurium to survive and transmit in the face of unpredictable conditions. In our in vitro flask model of S. Typhimurium biofilm development, bistable expression of CsgD in culture results in unique subpopulations of multicellular aggregates and planktonic cells. We compared these two subpopulations using RNAseq and identified over 1500 genes – nearly 35% of the Salmonella genome - that were differentially expressed. Planktonic cells had higher expression of SPI-1 type three secretion system (T3SS) genes, whereas multicellular aggregates showed up-regulation of many genes associated with survival and persistence. Higher expression of T3SS proteins in planktonic cells was correlated with significantly increased virulence in competitive infections of C57Bl/6 mice and enhanced invasion of the Caco-2 human intestinal cell line. However, when cell populations were exposed to desiccation, multicellular aggregates survived significantly better and the virulence advantage of single cells was lost. Our results could explain some of the transmission properties of S. Typhimurium, with single cells adapted for direct person-to-person transfer, and multicellular aggregates adapted for environmental survival and indirect transmission. The coordination of cells to generate specialized subpopulations may represent a bet-hedging strategy in S. Typhimurium that would increase its chances of transmission each time it exits from an infected host.

Workshop Sessions

BAM-WK110.01 - Evolutionary engineering of yeast by genome shuffling

Vincent Martin[1]
[1]*Concordia University, Montreal, Canada*

Strain improvement based on random mutagenesis and selection has been used extensively to improve the phenotypic traits of industrial microbes. However, finding the productive mutations in these strains can be difficult because a large background of incidental mutations often accompanies a small number of potentially productive ones. Here, we demonstrate that strain evolution by meiotic recombination-based genome shuffling coupled with sequencing can be used to deconstruct complex phenotypes and provide concrete targets for strain development. We used genome sequence information from a strain of *Saccharomyces cerevisiae* evolved by genome shuffling, coupled to RNAseq gene expression analysis and sequencing of the mutation loci in parental populations to identify mutations that confer resistance to growth inhibitors found in lignocellulosic hydrolysate. Our findings provide new insights into the genes and biological determinants of lignocellulosic hydrolysate inhibitor-tolerance in yeast. These include: stress response transcriptional repressor, Nrg1p; NADPH-dependent glutamate dehydrogenase, Gdh1p; protein homeostasis constituents, including Ssa1p; and Ubp7p and Art5p, related to ubiquitin-mediated proteolysis. When cross-referencing our findings, compelling evidence supports a determinant role for a mutation in ubiquitin-specific protease gene UBP7, which, when reintroduced in a laboratory *S. cerevisiae* strain, effectively increased lignocellulosic hydrolysate inhibitor tolerance.

Workshop Sessions

BAM-WK110.02 - Yeast cell factories: matching biotech needs and microbial physiology

Danilo Porro[1], Paola Branduardi[1]
[1]University of Milano Bicocca, Department of Biotechnology and Bioscience. Milan, Italy

The major purpose of industrial microbial biotechnology is the design and the development of a cell factory for the environmental friendly production of fuels, chemicals, materials or pharmaceuticals. In this respect, the budding yeast Saccharomyces cerevisiae is a prominent platform for commercial production of biopharma proteins, industrial enzymes, primary and secondary metabolites. Further developments could be based either on (a) novel productions and/or (b) new processes. The main activities of our laboratory are dedicated to the production of fuels and organic acids from recombinant yeasts. It should be underlined that whatever is the final compound, an industrial bioprocess is often characterised by limiting or even extreme conditions and/or by a continuous change of the intra- and extra-cellular environments. All this is often causing a reduction of the production rate at the end of industrial fermentations. Using other words, the most suitable yeast cell factory is the host having the best fitness for that specific production/process. The researches related to the understanding of the positive effects related to the modulation of the antioxidants and energetic pools on the robustness of the yeast cell factories to different stress conditions will be presented.

Workshop Sessions

BAM-WK110.03 - Production of diamines and carotenoids from alternative carbon sources by microbial cell factories

Volker F. Wendisch[1]
[1]Genetics of Prokaryotes, Faculty of Biology & CeBiTec, Bielefeld University, Germany

Amino acid production amounts to about 1.95 million tons of L-lysine and 2.93 million tons of L-glutamate per year (1). Corynebacterium glutamicum is the work-horse in this large-scale biotechnological process. Traditionally, amino acids are produced from molasses or starch hydrolysates. Recently, pathways for access to alternative carbon sources such as glycerol, a by-product of the biodiesel process, starch, or pentoses present in lignocellulosics have been engineered by various laboratories and amino acid production e.g. from glycerol (2), and pentoses has been described. Moreover, feasibility of amino acid production from hexose/pentose mixtures present in hemicellulosic hydrolysates e.g. of rice straw has been demonstrated (3) as well as from the amino sugar glucosamine (4). Transcriptome analyses have guided metabolic engineering in several instances and led to the identification and subsequent verification of new targets to improve lysine production, to the identification of new pathways and uptake systems for dicarboxylates and PTS-independent glucose uptake (5). The characterization of genetic control mechanisms of carbon metabolism, which are distinct from those of the model bacteria E. coli and B. subtilis, enabled strain development for improved carbon substrate utilization. Recently, new products from C. glutamicum have been developed e.g. for the production of L-proline (6), isobutanol (7) or diamines (8) and carotenoids (9-10).
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Workshop Sessions

BAM-WK110.04 - Microbial fermentations: from gene to product. The glycopeptide antibiotic case.

Flavia Marinelli[1]
[1]*Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy*

Glycopeptides are currently considered drugs of last resort for life-threatening infections caused by multi-drug resistant Gram-positive pathogens such as *Staphylococcus aureus*, *Enterococci* spp. and *Clostridium difficile*. They arrest bacterial cell wall biosynthesis by binding to the acyl-D-alanyl-D-alanine terminus of the nascent peptidoglycan, blocking its extracellular polymerization, and subsequently inhibiting cell growth and division. Chemically, these agents are glycosylated, halogenated and in some cases lipidated non-ribosomal peptides produced by a diverse group of filamentous actinomycetes. First generation glycopeptides include vancomycin, made by *Amycolatopsis orientalis*, and teicoplanin, produced by *Actinoplanes teichomyceticus*. The spread of resistance to glycopeptides in enterococci since 1988 and the recent emergence of high level of glycopeptide resistance in clinical isolates of methicillin-resistant *Staphylococcus aureus* have prompted the search for second-generation glycopeptides (dalbavancin, oritavancin, telavancin) that are produced by chemical modification of natural products. Although glycopeptide scaffold has been completely synthesized, the complexity of these natural products renders fermentation the only viable route for producing them pharmaceutically. Low yield of fermentation is a serious limitation on the way to the development of these drugs and their novel derivatives. Several strategies have been applied in attempts to increase the production of glycopeptides including empirical mutagenesis of the producing organisms and selection of high-producing clones, optimization of fermentation media and conditions, and, more recently, rational engineering by DNA manipulation. In this presentation, classical and innovative approaches for the strain and fermentation improvement will be reviewed taking as models teicoplanin production in *Actinoplanes teichomyceticus* and the teicoplanin-like A40926 -produced by *Nonomuraea* sp. ATCC 39727- that is the precursor of the promising second-generation glycopeptide dalbavancin.

Workshop Sessions

BAM-WK111.01 - Uncoupling between core genome and virulome in extraintestinal pathogenic *Escherichia coli*

Jesús Mingorance[1], Natalia Fernández-Romero[1], Marta Mora[2], Jesús Rodríguez-Baño[3], Lorena López-Cerero[3], Alvaro Pascual[3]

[1]Servicio de Microbiología, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain, [2]Unidad de Microbiología Clínica y Enfermedades Infecciosas, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain, [3]Unidad Clínica de Enfermedades Infecciosas y Microbiología, Hospital Universitario Virgen Macarena, Sevilla, Spain

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are among the most frequently isolated bacterial pathogens in most hospitals. ExPECs are considered opportunistic pathogens that produce mostly urinary and bloodstream infections. We have studied the genetic diversity of more than six hundred clinical ExPEC isolates from five different collections using an MLST-based SNP pyrosequencing approach. The virulence factor gene content (the virulome) was studied by PCR detection of twenty five representative genes. SNP typing showed the same population structure in the different collections, typically half of the isolates belong to a few sequence types (5 to 8), while the other half is composed by a large diversity of sequence types that appear once or twice. Cumulative distributions of sequence types show saturation curves indicative of a limited diversity. Contrary to this, the virulome shows an extremely high diversity, with almost as many gene profiles as isolates, and linear, non-saturating, cumulative distributions, even within frequent sequence types, showing that genetic exchange is much higher in the virulome fraction of the genome than in the core genome.

Workshop Sessions

BAM-WK111.02 - Genomic diversity and microevolution of the opportunistic pathogen Pseudomonas aeruginosa

Nina Cramer[1], Sebastian Fischer[1], Philippe Chouvarine[1], Sarah Dethlefsen[1], Patricia Moran Losada[1], Antje Munder[1], Sebastian Suerbaum[1], Lutz Wiehlmann[1], Jens Klockgether[1], Burkhard Tümmler[1]
[1]*Medizinische Hochschule Hannover, Hannover, Germany*

Pseudomonas aeruginosa, the type species of pseudomonads, is an opportunistic pathogen that colonizes a wide range of niches. The P. aeruginosa population has an epidemic structure of thousands of clonal complexes which freely exchange genes by recombination or lateral transfer. Twenty clones make up about 40% of the contemporary population. We have sequenced the genomes of representative strains of the 15 most frequent clonal complexes in the P. aeruginosa population and of the five most common clones from the environment of which so far no isolate from a human infection has been detected. These 6.4 – 7.4 Mbp large genomes encode about six to seven thousand ORFs and at least close to a thousand ncRNAs. The P. aeruginosa pangenome consists of a conserved core of at least 4,000 genes, a combinatorial accessory genome of a further 10,000 genes and 30,000 or more rare genes that are present in only a few strains or clonal complexes. This differential genetic repertoire of clones maintains a habitat-independent gradient of virulence in the P. aeruginosa population. Intraclonal genome diversity is mainly caused by the variable composition of the accessory genome. Individual clones are endowed with distinct repertoires of functional protein variants. For example, the most common clone C is more versatile than the second most frequent clone PA14 in genetic elements that confer niche adaptation. Loss-of-function mutations typically accumulate in the P. aeruginosa genome during chronic infections, but unexpectedly these strains were not outcompeted by clonal variants from other habitats in competitive fitness experiments.

Workshop Sessions

BAM-WK111.03 - Microbial life-style and disease

Søren Molin[1]
[1]*Department of Systems Biology Technical University of Denmark, Kongens Lyngby, Denmark*

When bacteria migrate from one environmental setting to another they initially adapt by regulating gene expression to accommodate the new challenges and opportunities. If the bacteria persist in the new environment adaptive evolutionary processes may further increase the fitness of the population. We are studying the interplay between gene regulatory adaptive mechanisms and adaptive evolution of bacteria infecting human airways based on a unique collection of longitudinal bacterial isolates covering more than 200,000 generations of growth in the airways of patients suffering from cystic fibrosis (CF). Investigations of 600 complete genomes representing both the very early stages of colonization as well as the late stages until the patients die have among other things shown the following: 1) Genetic drift dominates the evolutionary processes 2) Patho-adaptive mutations are frequently identified in regulatory genes 3) Early mutations in global regulators change the life-style of the bacteria 4) The life-style changes impact on biofilm development Based on our findings we suggest that several global regulatory factors at the same time ensure immediate epigenetic adaptation after an environmental shift, and long-term optimized genetic adaptation by genetic changes in the corresponding genes.

Workshop Sessions

BAM-WK111.04 - The Pseudomonas aeruginosa international consortium: the 1000 genomes project

Julie Jeukens[1], Brian Boyle[1], Irena Kukavica-Ibrulj[1], Jo Fothergill[2], Matthew Moore[2], Geoff Windsor[3], Jane Turton[4], Stephan Heeb[5], Hardeep Naghra[5], Roger Levesque[1], Josie McKeown[5], Miguel Camara[5], Paul Williams[5], Craig Winstanley[2], Nicholas Tucker[4], Fiona Brinkman[3]

[1]Institut de biologie intégrative et des systèmes, Université Laval, Québec, Canada, [2]University of Liverpool, Liverpool, UK, [3]Simon Fraser University, Vancouver, Canada, [4]University of Strathclyde, Glasgow, Scotland, [5]University of Nottingham, Nottingham, UK

The *P. aeruginosa* international consortium 1000 genomes project is producing an extensive collection of genomes from a single species for studying genome evolution, comparative genomics of antibiotic resistance and virulence genes by cataloguing SNPs, indels and genome rearrangements. Given the potential link between environmental strains and evolution towards infection by *P. aeruginosa* in animals and in humans, we need to better understand the role of environmental strains in genome evolution, how patients become infected and identify prognostic markers for better evidence-based decisions on patient care. We are sequencing the genomes of more than 500 *P. aeruginosa* isolates from Cystic Fibrosis (CF) patients and 500 strains from other human and animal infections and environmental strains. We created a strain database combining basic information including, date of isolation, geographical origin, clinical details, phenotypic data and sequencing information. The Pseudo database was constructed using Opal, the data repository component of OBiBa (obiba.org). We have selected 1000 *P. aeruginosa* isolates in order to represent maximum genomic diversity by comprehensively sampling geographic origin, VNTR/AT typing, host, *in vitro* phenotype and *in vivo* behaviours. High quality draft genome sequencing is performed using Illumina MiSeq systems and combining 40-48 genomes per run with 300 bp paired-end libraries for coverage of 35-40 X, followed by genome assembly using an integrated pipeline. Priority genomes will be selected and sequenced using PACBIO RS technology to generate complete genome assemblies for annotation. A pipeline being implemented at Pseudomonas.com will be used for a standardized high throughput comparative genomics annotation and easy access to data for clinical applications. Clinical exploitation of genomics data will be supportive to molecular epidemiology performed for surveillance and outbreak investigation in CF and has the potential for future genotypic antimicrobial susceptibility testing for *P. aeruginosa*, as well as the identification of novel therapeutic targets and prognostic markers.

Workshop Sessions

BAM-WK111.05 - Population diversification, adaptation and transmission of a Pseudomonas aeruginosa epidemic strain studied using patient samples and model systems

Craig Winstanley[1], Emily Jones[1], Chloe James[2], Joanne Fothergill[1], David Williams[1], Sam Haldenby[1], Ben Evans[1], Martin Walshaw[3], Aras Kadioglu[1], Steve Paterson[1], Michael Brockhurst[4]
[1]University of Liverpool, Liverpool, UK, [2]University of Salford, Salford, UK, [3]Liverpool Heart & Chest Hospital, Liverpool, UK, [4]University of York, York, UK

Since its emergence in the mid 1990s, we have studied the transmissible Liverpool Epidemic Strain (LES) of Pseudomonas aeruginosa, a Cystic Fibrosis (CF)-adapted clone that is widespread amongst CF patients in the UK and has been reported in North America. We have analysed populations of the LES from the sputa of multiple chronically infected adult CF patients. Following the establishment of a chronic infection in the lungs of CF patients, populations of P. aeruginosa LES adapt and diversify due to mutation. Hence, multiple single strain isolates from the same patient sample can exhibit diversity in phenotypes such as quorum sensing, mucoidy and antibiotic resistance. Using phenotypic analysis and whole genome sequencing we have compared LES populations between patients and followed population changes over time, including during periods of exacerbation. Our data suggest that populations are highly variable between patients and dynamic within patients over time periods of several months. In addition, we have analysed archived, historical isolates and isolates from multiple geographical locations to determine the distribution of specific mutations identified using genome sequencing. These studies give us insights into transmission pathways within and between CF units. In parallel, we have also studied adaptation of P. aeruginosa LES populations using in vitro (artificial sputum medium biofilm) and in vivo (mouse inhalation) model systems. In both patient and model systems, there is selection for mutations in key regulators, including quorum sensing system regulatory proteins and 2-compenent regulatory systems. By studying the behaviour of P. aeruginosa populations both real patient samples and model systems we can gain insights into the factors that drive adaptation. This study was supported by the Wellcome Trust.

Workshop Sessions

BAM-WK112.01 - Ecotoxicological impact of pesticides on natural microbial communities responsible for pesticide biodegradation in river sediments

Fabrice Martin-Laurent[1], Marion Devers[1], Nadine Rouard[1], Olivier Crouzet[2], Nathalie Cheviron[2], Stephane Pesce[3]
[1]INRA, UMR Agroécologie, Dijon, France, [2]INRA, UR Pessac, Versailles, France, [3]Irstea, UR Maly, Lyon, France

The general use of pesticides in various crops allows ensuring the quality of the crop production but has environmental impact, pesticide residues being often found in water resources, notably river in the agrosystems. As a result of repeated exposure to pesticides microorganisms can adapt to their degradation being responsible for enhanced degradation, an environmental friendly process diminishing the persistence of pesticide residues in the environment. This process can occur in the soil but also in the sediment of the river regularly exposed to pesticide contamination. To our best knowledge the ecotoxicological impact of multiple pesticides contamination on the degrading activity of microbial community of river sediment adapted to enhance biodegradation is not documented. In order to explore this question, we established an experiment using a sediment collected in the Morcille river (Beaujolais, France) known to harbor a microbial community adapted to the enhanced biodegradation of atrazine, 2,4-D and diuron. This sediment was either treated with glyphosate, or tebuconazole, or dimetomorphe or a mixture of these three pesticides or not treated (control) (four replicates per treatment). Samples were regularly collected to estimate the impact of pesticide contamination on (i) the ability of the microbial activity of the sediment to mineralize atrazine, 2,4-D and diuron by radiorespirometry and (ii) the abundance of atrazine, 2,4-D and diuron pesticide-degrading community was monitored by quantitative PCR targeting atzA/trzN, tfdA, and puhA/puhB genes using as template DNA extracted from the sediments. The results of this study will be presented and a first conclusion is that pesticide biodegradation potential of river sediment could constitute an interesting microbial functional bioindicator of pesticide effects. Acknowledgment: This work was funded by the APR Pesticide 2011 of the French Ministry of Environment within the framework of the Ecophyto program.

Workshop Sessions

BAM-WK112.02 - Mechanism of algal blooms in the Three Gorges Reservoir, China based on water quality and phytoplanctonic community

Lei Zhang[1,2], Zhiqiang Xia[1], Li Fu[1], Jianjun Yu[1], Douglas Haffner[2], Paul Hamilton[3]
[1]Southwest University, Chongqing, China, [2]Greak Lakes Institute for Environmental Research, University of Windsor, Windsor, Canada, [3]Canadian Museum of Nature, Ottawa, Canada

Since its impoundment of the Three Gorges Reservoir (TGR), Yangtze River, China in 2003, the water quality in the reservoir has been deteriorating given with algal blooms occurring in tributaries averaging once year in 2003 to several times a year. A survey, including 22 tributaries and 6 sections of the mainstream of the TGR was implemented in March of 2013. Combined with a long term, fixed site and high frequency (every 10-15days) field investigation in the eutrophic Pengxi River, the largest tributary on north shore of TGR, from April to Dec, 2013 an investigation of the mechanism of algal bloom in the TGR was implemented. During this study, 16 out of 22 tributaries had harmful algal blooms, none were observed in the mainstream. 7 Seven downstream tributaries approaching the Three Gorges Dam had algal blooms dominated all by Peridiniopsis sp., while in contrast the blooming algae in tributaries located in middle and upstream of TGR were more diverse dominated by Cryptophyta, Chlorophyta and Bacillariophyta, respectively. Neither total nitrogen (ranging from 0.30-5.40 mg/L, with an average of 1.51 mg/L in the main stream, and 1.86 mg/L in tributaries), nor total phosphorus (from 0.074-0.572 mg/L, 0.181 mg/L main stream, and 0.167 mg/L tributaries) or the ratio of (TN/TP) were significantly related to algal bloom outbreaks. On May 22, 2013, a major algal bloom outbreak was observed in the Pengxi River, and was associated with the onset of stratification with the initial development of the bloom confined to the metalimnion. It was concluded that a stable stratified water column is critical for algal blooms to occur in the TGR where physical processes regulate the timing of algal bloom and nutrient levels determine the size of the blooms.

Workshop Sessions

BAM-WK112.03 - Caracterization of the denitrification genes in Methylophaga nitratireducenticrescens sp. JAM1

Florian Mauffrey[1], Christine Martinau[1], Céline Villeneuve[1], Richard Villemur[1]
[1]*INRS Institut Armand-Frappier, Laval, Canada*

Methylophaga nitratireducticrescens JAM1 is a methylotrophic bacterium that has been isolated from a methanol-fed denitrification system treating seawater at the Montreal Biodome. It composed more than 50% of the denitrifyng biofilm. Strain JAM1 has the particularity among Methylophaga species to grow under denitrifying conditions in the presence of nitrate and methanol. Growth of strain JAM1 under denitrifying conditions by reducing nitrate into nitrite was shown to be correlated with the presence of two nitrate reductase narG genes. The genome of strain JAM1 was sequenced and consists of one chromosome (3,137,192 bp). The strain JAM1 genome sequence confirmed the presence of two nar operons but, interestingly, also two nor operons (nitric oxide reductase) and one nos operon (nitrous oxide reductase). In addition, a nirK sequence encoding an 82-amino-acid truncated nitrite reductase was found, which could explain that strain JAM1 cannot achieve complete denitrification. Our work aims to study the denitrification genes in strain JAM1. RT-PCR assays showed that all the denitrification genes were expressed, including the truncated nirK. To assess the impact of the two narG on strain JAM1, gene knockout strategy was used to delete each narG. narG1 and narG2 knockout mutants showed significant differences in their growth and nitrate reduction rates compared to the wild type strain. The levels of narG1 and narG in respective mutants and the wild type strain cultivated under different growth conditions were measured by RT-qPCR. Our results showed that the expression of the narG1 or narG2 in the respective mutants differs from wild type suggesting a particular regulation system in strain JAM1.

Workshop Sessions

BAM-WK112.04 - Aquatic metagenomes implicate Thaumarchaeota in global vitamin B12 production

Andrew Doxey[1], Daniel Kurtz[2], Michael Lynch[3], Laura Sauder[4], Josh Neufeld[5]
[1]University of Waterloo, Waterloo, Canada

Vitamin B12 (cobalamin; cbl) is a complex metabolite and essential cofactor across many branches of life, including most aquatic algae. Eukaryotic algae and other cbl auxotrophs rely on environmental cbl supplied from a relatively small set of cobalamin-producing prokaryotic taxa. Although several bacteria have been implicated in cobalamin biosynthesis and associated with algal symbiosis, the involvement of Archaea in cobalamin production is less well understood, especially with respect to the Thaumarchaeota. Based on the discovery of a complete thaumarchaeal cbl pathway in the metagenome of an aquarium biofilter, we hypothesized that Thaumarchaeota, which are ubiquitous and abundant in aquatic environments, play an important role in cbl biosynthesis within aquatic ecosystems. To test this hypothesis, we examined cbl synthesis genes across sequenced thaumarchaeal genomes and over 50 metagenomes from a diverse range of marine, freshwater, and hypersaline environments. Our analysis demonstrates that all available thaumarchaeal genomes possess cbl synthesis genes, predominantly from the aerobic pathway, suggesting a widespread genetic capacity for cbl synthesis. Furthermore, although bacterial cbl genes dominated most surface marine metagenomes, thaumarchaeal cbl genes dominated metagenomes from polar marine environments, increased with depth in the marine water column, and displayed a potential seasonality with increased winter abundance observed in time-series datasets (e.g., L4 surface water in the English Channel). Our results also suggest niche partitioning between thaumarchaeal, euryarchaeal, proteobacterial, and cyanobacterial cbl genes across all metagenome datasets analyzed. These results provide strong first evidence for specific biogeographical distributions of thaumarchaeal cbl genes, expanding our understanding of the global biogeochemical roles played by Thaumarchaeota in aquatic environments.

Workshop Sessions

BAM-WK112.05 - The non-phosphorylated IIANtr protein represses the alkylresorcinol synthesis in Azotobacter vinelandii

Luis Muriel-Millán[1], Soledad Moreno[1], Guadalupe Espín[1]
[1]*Instituto de Biotecnología-UNAM, Cuernavaca, México*

Azotobacter vinelandii is a gammaproteobacterium that undergoes a differentiation process to form desiccation-resistant cysts, which consist of a central body surrounded by two layers (intine and exine). During the encystment, the bacterium synthesizes the phenolic lipids alkylresorcinols (ARs) which replace the membrane phospholipids and are components of the exine layer. The arsABCD operon encodes the enzymes necessary for ARs synthesis and its transcription is activated by ArpR. The nitrogen-related phosphotransferase system (PTSNtr) is a global regulatory system in bacteria. It is comprised by EINtr (ptsP), NPr (ptsO) and IIANtr (ptsN) proteins that participate in a phosphoryl transfer chain from phosphoenolpyruvate, where IIANtr appears to be the terminal phosphoryl acceptor. Here, we characterized the role of PTSNtr on AR synthesis in A. vinelandii. The mutations on ptsP and ptsO genes (that are expected to impair the phosphorylation of IIANtr) abolished the AR synthesis, while in the ptsN mutant the ARs levels increased as compared with that of the wild type strain. Inactivation of ptsN restored AR levels in the ptsP- mutant. By using qRT-PCR we found that mutations on ptsP and ptsO decreased the mRNA levels of arsA and arpR as compared with showed by the wild type strain. The mutation on ptsN increased the mRNA levels of arsA and arpR in the wild type and ptsP- mutant strains. These data were confirmed by using transcriptional fusions of arsA and arpR with gusA reporter gene; mutations on ptsP and ptsO diminished the transcription of arsA and arpR, and a ptsN mutation increased them. A strain that harbors a ptsN H68A, that produce a non-phosphorylatable IIANtr, presented a phenotype of non-production of ARs and decreased transcription of arsA and arpR. We conclude that non-phosphorylated IIANtr negatively regulates the AR synthesis by repression of arpR expression, the transcriptional activator of arsABCD biosynthetic operon.

Workshop Sessions

BAM-WK112.06 - Biology and genome of vB_RleM_PPF1, a temperate rhizobiophage infecting and lysogenizing Rhizobium leguminosarum

Anupama Halmillawewa[1], Marcela Restrepo[1], Ben Perry[2], Christopher Yost[2], Michael Hynes[1]
[1]Department of Biological Sciences, University of Calgary, Calgary, Canada, [2]Department of Biology, University of Regina, Regina, Canada

In the course of experiments to isolate new bacteriophages that infect Rhizobium leguminosarum, several strains were used as trapping hosts to enrich phage from soil samples from the Canadian prairies. One strain, F1, which was isolated in Saskatchewan, was susceptible to infection by most of the phages isolated using other strains, but was infected only by a group of highly related phages that had a narrow host range when used as a trapping host. One of these F1 isolated phages was chosen for further examination. This phage, vB_RleM_PPF1 (lab name PPF1) was able to lysogenize strain F1 at a high frequency. Electron microscopy revealed it to belong to the Myoviridae, and to have a head diameter of 83±5 nm and a tail length of 130±5 nm. Although it did not form plaques on the majority of our R. leguminosarum strains, it was able to lysogenize strain VF39. The genome of PPF1 was sequenced using 454 technology, and assembled into one contig of 54.5 kb, with a GC content of 61.9%, and putatively encoding 94 open reading frames. The site of integration of PPF1 was identified to be in a tRNApro gene by genomic sequencing of a lysogenic F1 strain to produce a draft sequence. The attP and attB regions share an identical sequence of 50 bp, which overlaps with the 3' end of the tRNApro gene. Conserved integrase, terminase, and phage structural genes were identified in the annotation of the genome, but the majority of predicted genes encoded proteins of unkown function or had no orthologs in databases. Integration of the phage in multiple independent lyosgens of strain F1 was revealed by PCR to take place at the same site in all cases. Induction of prophage was detectable after Ultraviolet light treatment.

Workshop Sessions

MEM-WK304.01 - Synthesis and role of galactosaminogalactan in biofilm formation of Aspergillus fumigatus

Don Sheppard[1]
[1]*McGill University, Montreal, Canada*

Aspergillus forms adherent biofilms on pulmonary epithelial cells and a variety of other substrates. Comparative analysis of biofilm deficient mutants,revealed a correlation between adherent biofilm formation and the production of the exopolysaccharide galactosaminogalactan. Galactosaminogalactan is a secreted and cell wall bound polysaccharide composed of variable combinations of galactose and N-acetyl galactosamine. Comparative transcriptome analysis of these biofilm deficient strains identified a cluster of co-regulated genes with predicted functions in carbohydrate synthesis and modification. Deletion of uge3, a gene within this cluster predicted to encode a glucose epimerase, abrogated galactosaminogalactan production. Uge3 deletion resulted in altered cell wall morphology with a loss of capsular exopolysaccharide and increased exposure of B-glucans. Loss of galactosaminogalactan resulted in a marked reduction in adherence to pulmonary epithelial cells and other substrates, and attenuated virulence in a neutropenic mouse model of invasive aspergillosis. Examination of the other elements of the GAG biosynthetic cluster identified a gene encoding a putative carbohydrate deacetylase, agd3. Deletion of agd3 resulted in a strain that produced of non-deacetylated galactosaminogalactan and was attenuated in virulence. Non-deacetylated galactosaminogalactan was unable to adhere to the surface of hyphae, and could not support biofilm formation. In silico analysis of Agd3 predicts a signal peptide suggestive of secretion of this enzyme. Consistent with this observation, co-culture of the Δagd3 mutant with culture supernatants from the galactosaminogalactan deficient Δuge3 mutant restored biofilm production. Structural modelling revealed that the polysaccharide deacetylase domain of Agd3 is structurally similar to bacterial deacetylases involved in the production of bacterial exopolysaccharide. These data suggest that deacetylation is required for galactosaminogalactan function by generating a cationic polysaccharide which adheres to negatively charged surfaces such as membranes and plastics. These studies suggest that the production of deacetylated exopolysaccharide is a convergent evolutionary strategy developed by both bacteria and fungi for adherence and biofilm formation.

Workshop Sessions

MEM-WK304.02 - Regulatory, biosynthetic and functional crosstalk between biofilm exopolysaccharides of Aspergillus fumigatus and Pseudomonas aeruginosa

Brendan Snarr[1], Natalie Bamford[2], Fabrice Gravelat[1], Mark Lee[1], Shantelle LaFayette[1], Dao Nguyen[1], Matthew Parsek[3], Lynne Howell[2], Donald Sheppard[1]
[1]McGill University, Montréal, Canada, [2]The Hospital for Sick Children, Toronto, Canada, [3]University of Washington, Seattle, USA

Background: Mixed-species biofilms are now recognized as important elements of chronic infections. In patients with chronic lung disease, the mold Aspergillus fumigatus and the Gram-negative bacterium Pseudomonas aeruginosa are two biofilm-producing organisms commonly co-cultured from sputum samples. Acqusition of these organisms is associated with poor clinical outcomes. We hypothesize that A. fumigatus and P. aeruginosa interact via the hexosamine-rich exopolysaccharide components of their biofilms, specifically A. fumigatus galactosaminogalactan (GAG) and P. aeruginosa pellicle (Pel). Methods: Mutant strains of both organisms deficient in key enzymes required for the synthesis and activation of their respective exopolysaccharides were used. Interactions between these organisms were observed using confocal microscopy. The abilities of purified proteins and sterile culture supernatants to rescue, inhibit or disrupt biofilm formation of these organisms was investigated. Results: P. aeruginosa was found to bind to the surface of A. fumigatus in a GAG-dependent manner. Further, A. fumigatus GAG was able to rescue the adherence of biofilm-deficient mutants of P. aeruginosa. In addition, GAG biosynthetic enzymes directly influenced biofilm production by P. aeruginosa. The A. fumigatus secreted hexosamine deacetylase Agd3 enhanced biofilm formation of a hexosamine deacetylase-deficient P. aeruginosa mutant, while the secreted A. fumigatus regulatory protein Sph3, prevented the formation of P. aeruginosa biofilms in a dose dependent fashion. Conclusions: These studies suggest the exciting hypothesis that cooperative biofilm formation between A. fumigatus and P. aeruginosa may involve interactions at the regulatory, biosynthetic and functional level. Understanding these interactions may improve our understanding of the pathogenesis of chronic pulmonary infections with these organisms.

Workshop Sessions

MEM-WK304.03 - Leishmania promastigotes induce cytokine secretion in macrophages through the degradation of Synaptotagmin XI

Guillermo Arango Duque[1], Mitsunori Fukuda[2], Salvatore J. Turco[3], Albert Descoteaux[1]
[1]INRS-Institut Armand-Frappier and Centre for Host-Parasite Interactions, Laval, Canada, [2]Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, Japan, [3]Department of Biochemistry, University of Kentucky, Lexington, USA

Leishmania parasites cause a spectrum of debilitating diseases found worldwide. The GPI-anchored zinc metalloprotease GP63 is a pathogenicity factor that cleaves various host cell molecules, enabling Leishmania to subvert macrophage functions such as antigen cross-presentation. Moreover, lipophosphoglycan is a glycophospholipid that promotes parasite survival in the phagosome. In macrophages, Synaptotagmins (Syts) are type-I membrane proteins that regulate vesicle docking and fusion in processes such as exocytosis and phagocytosis. We recently discovered that Syt XI is a recycling endosome- and lysosome-associated protein that negatively regulates the secretion of TNF and IL-6. Here, we show that Syt XI is directly degraded by GP63 and excluded from Leishmania parasitophorous vacuoles by lipophosphoglycan. Infected macrophages were found to release TNF and IL-6 in a GP63-dependent manner. To demonstrate that cytokine release is dependent on GP63-mediated degradation of Syt XI, siRNA-mediated knockdown of Syt XI before infection revealed that the effects of siRNA knockdown and GP63 degradation were not cumulative. Furthermore, in mice, intraperitoneal injection of GP63-expressing parasites led to an increase in TNF and IL-6 secretion and to an augmented influx of neutrophils and inflammatory monocytes to the inoculation site. Both of these cell types have been shown to be infection targets and aid in the establishment of infection. In sum, our data reveal that GP63 induces proinflammatory cytokine release and increased infiltration of inflammatory phagocytes. This study provides new insight on how Leishmania exploits the immune response to establish infection.

Workshop Sessions

MEM-WK304.04 - Gluey, glutinous, sweet, oily and colorful, This Is what makes the mycelium of Aspergillus fumigatus sticky

Jean-Paul Latgé[1]
[1]*Institut Pasteur, Paris, France*

The polysaccharide cell wall is the extracellular armour of the fungal cell. It is essential in the protection of the fungal cell against aggressive external stresses and plays also a key role during infection. In A.fumigatus, the cell wall is almost exclusively composed of polysaccharides. The fibrillar core is composed of a branched β-(1,3)-glucan to which chitin, β-(1,3)-/β-(1,4)-glucan and galactomannan are covalently bound. The alkali-soluble amorphous fraction is mainly composed of α-(1,3)-glucans which cover the mycelial surface in association with galactosaminogalactan and galactomannan. For a long time, it was considered that the cell wall was a fixed structure which would trigger the defense response when it was sensed as non-self by the host. It is now known that the cell wall polysaccharide composition and localization continuously change to adapt to their environment. Changes in the polysaccharide composition and especially their localization are ways for the fungus to avoid immune aggressions in a way similar to the antigenic variation phenomenon seen in parasites which allows them to escape the host immune response by continuously altering the composition of their surface antigens. The continuous reshuffling of the cell wall composition during environmental changes or in cell wall mutants suggest also that many conclusions based on the following dogma:” we use a mutant deleted in the cell wall gene X and the phenotype seen is due to the lack of the product of the encoding gene X “should be considered with extreme caution. In aerial conditions, the mycelium grows as a network of agglutinated and hydrophobic hyphae embedded in an extracellular matrix rich in polysaccharides, hydrophobins and melanin. In my talk, I will the role of the different components of the ECM in the adherence of hyphae between themselves to form a “biofilm” which could be simply called colony in filamentous fungi!

Workshop Sessions

MEM-WK305.01 - From bad to good fungus: new insights into genetic factors leading to biocontrol lifestyle in fungi

Richard Bélanger[1], François Lefebvre[1], David Joly[2], Guus Bakkeren[2]
[1]Universite Laval, Quebec, Canada, [2]Université de Monction, Moncton, Canada, [3]Agriculture and Agri-Fod Canada, Summerland, Canada

In agriculture, fungal pathogens are responsible for billions of dollars in crop losses annually. Conversely, the same fungal pathogens can be parasitized by other fungi, the latter being considered beneficial because of their biocontrol properties. In some instances, pathogenic and biocontrol fungi can be closely related phylogenetically, a situation that offers a unique window into the genetic determinants that dictate specific lifestyles in fungi. For example, the anamorph species Pseudozyma flocculosa is described as a non-pathogenic Ustilaginale with the rare property of being an effective biocontrol agent of powdery mildew fungi. At the same time, it is also closely related to Ustilago maydis, a maize-infecting model organism in fungal genetics. However, in spite of this genetic relatedness, P. flocculosa and U. maydis do not appear to share much in terms of development and lifestyle. For this reason, we exploited the opportunity to sequence and annotate the genome of P. flocculosa and compare it to that of U. maydis and other Ustilaginales, in order to unravel the features that could account for such distinct lifestyles among related organisms. Through comparative analyses, genomic features of P. flocculosa were found to be very similar to those reported for U. maydis, S. reilianum and U. hordei. In spite of being a non plant-pathogen, P. flocculosa has conserved many genetic traits associated to mating and pathogenicity. On the other hand, minor yet intriguing differences between P. flocculosa and phytopathogenic species included the absence of homologs to secreted effector proteins proven to influence virulence in U. maydis. In addition, genetic elements unique to P. flocculosa suggest the presence of specific factors that could explain how the fungus has evolved biocontrol properties.

Workshop Sessions

MEM-WK305.02 - Bioactivity, biochemical and ultrastructural characteristics of Trichoderma atroviride conidia influenced by different culturing conditions: factors affecting biocontrol potential

Amir Daryaei[1], Richard E Falloon[1, 2], Travis R Glare[1], Eirian E Jones[1], Hossein Alizadeh[1], Ian C Hallett[2]
[1]Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand, [2]New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand

Identification of production and storage factors that affect conidial germination and bioactivity (fitness) will assist the success of biological control agents. Effects of abiotic factors (temperature, nutrients, water activity, pH) on conidial fitness of Trichoderma atroviride LU132 during production were studied. Conidia from the culturing regimes which resulted in greatest and least bioactivity against Rhizoctonia solani in dual culture were selected to assess effects of storage conditions on conidial fitness over time. Fitness of the test conidia was examined after storage at 30˚C and at 0 or 50% relative humidity (RH) over 6 months. Fitness declined over time, and the decline was greater for 50% RH than 0% RH. The greatest number of conidia and greatest germination resulted from growth at 25°C, but greatest bioactivity resulted from conidia produced at 30˚C. Different C to N ratios (5:1 or 160:1) did not affect these parameters. However, fewer conidia were produced at 30˚C, and the least germination and bioactivity resulted from conidia produced at 20°C. The different environmental factors were not independent. For example, conidial production at 30°C is probably accompanied by water stress, oxidation, and rapid pH change which may also affected fitness. Further study was performed based on temperature and hydrocarbon type. Biochemical and ultra-structural studies were carried out to determine relationships between quality variations and cellular characteristics for conidia produced in different culturing conditions. Results of fatty acid, and sugar (trehalose and polyols) analyses, and ultrastructural observations are presented in this paper.

Workshop Sessions

MEM-WK305.03 - Bioherbicide Phoma macrostoma for field crops

Russell Hynes[1], Michelle Hubbard[1], Karen Bailey[1]
[1]*Agriculture & Agri-Food Canada, Saskatoon, Canada*

Field trials were conducted to determine the crop tolerance of alfalfa to the bioherbicide Phoma macrostoma, the efficacy of the bioherbicide on Canada thistle, Cirsium arvense, and wild mustard, Sinapsis arvensis, and the effect of bioherbicide application timing on weed control. Phoma macrostoma was harmful to alfalfa (cv. Longview) in the year of establishment significantly reducing the number of plants. The plant reductions were dependent on the bioherbicide rate applied. Two year-old alfalfa was unaffected by the application of P. macrostoma. The effect of bioherbicide application time on weed efficacy was tested in a no-crop situation using tillage just prior to each application time thus creating a situation whereby the bioherbicide was applied prior to weed emergence. Phoma macrostoma effectively reduced Canada thistle, wild mustard, and other broadleaved weed seedlings under field conditions when applied prior to weed emergence. The bioherbicide was most effective on reducing plant number and biomass of wild mustard by 75-100%. The biomass of other broadleaved weed seedlings was reduced by 75%. The control of Canada thistle was more variable with reductions in weed cover ranging from 52-80% and the reductions in biomass from 35-69%. Early to mid-season applications of the bioherbicide reduced the number of plants of Canada thistle (48-78% reduction) and dandelion, Taraxacum officinale, (81-99% reduction). Other broadleaved weeds were reduced by 96% but only with the early application. Wild mustard was reduced by 96-100% at all application times. The bioherbicide was also able to reduce biomass of all weeds with the reductions exceeding 80% at the early and mid-season applications. Late applications were less effective in reducing weed numbers and biomass.

Workshop Sessions

MEM-WK305.04 - Mechanisms of biological suppression of damping-off and root rot diseases of cucumber and radish with soil bacteria

Salah Eddin Khabbaz[1,2], Liang Zhang[1], Aiming Wang[1], Pervaiz A. Abbasi[1]
[1]1 Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Canada, [2]General Commission for Scientific Agriculture Research, Hama, Syria

Indigenous soil bacteria have the potential to be developed into biofungicides for the management of soilborne diseases of various crops. More than 200 antagonistic bacteria were isolated from a commercial potato field and screened for their growth inhibition of plant pathogens under laboratory conditions. Among them, Pseudomonas fluorescens (Pf 9A-14), Pseudomonas sp. (Psp. 8D-45), and Bacillus subtilis (Bs 8B-1) were the most effective in promoting plant growth and suppressing Phytophthora root rot of cucumber and Rhizoctonia damping-off of radish under growth room conditions. All three isolates showed maximum inhibition of mycelia growth for both pathogens by the production of volatile compounds. GC-MS analysis of three bacterial isolates revealed over 13 volatile compounds. PCR analysis confirmed the presence of antibiotic biosynthetic genes such as phenazine carboxylic acid (PCA), 2, 4 diacetylpholoroglucinol (2,4 DAPG), pyrrolnitrin, and pyoluteorin in three bacteria. The peat and talc formulations of these antagonistic bacteria applied as seed or amendment treatments to the infested peat-based mix suppressed Phytophthora root rot of cucumber and Rhizoctonia damping-off of radish and enhanced plant growth. Seed treatment of mixture peat formulation of all three bacteria significantly increased the percentage of healthy seedlings by 285.4% and 862.3%, and plant fresh weights by 158.3% and 126.5% respectively, as compared to the infested controls of Phytophthora and Rhizoctonia.

Workshop Sessions

MEM-WK305.05 - Growth of Cladosporium species on indoor surfaces is dominated by more xerotolerant species belonging to the Cladosporium sphaerospermum complex

F.J.J. Segers[1], M Meijer[1], J Houbraken[1], K.A. Laarhoven van[2], H Huinink[2], O.C.G. Adan[2], R.A. Samson[1], H.A.B. Wösten[3], J Dijksterhuis[1]

[1]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, [2]Eindhoven University of technology, Eindhoven, The Netherlands, [3]Molecular Microbiology and Kluyver Centre for Genomics of Industrial Fermentation, Utrecht university, Utrecht, The Netherlands

Indoor fungi cause cosmetic and structural damage in a considerable part of the European dwellings. Prolonged exposure of inhabitants to allergens and mycotoxins produced by such fungi is a potential threat to human health. Cladosporium species are among the dominant indoor fungi and thrive on various types of surfaces including glass, gypsum, wall paper, paint and wood. Cladosporium species can mostly be divided into three different species complexes, namely the Cladosporium cladosporioides complex, the Cladosporium herbarum complex and the Cladosporium sphaerospermum complex. A number of 145 isolates from different indoor environments were identified and a comparison was made between air samples and swab samples. Identification was done by the sequencing of 3 loci namely, internal transcribed spacer (ITS), and a partial sequence elongation factor 1α (TEF) and actin (ACT). Here Cladosporium halotolerans and Cladosporium sphaerospermum were most dominant in swab samples, while no isolates were found from the suggested indoor and allergenic species Cladosporium herbarum. A selection of indoor Cladosporium species was grown on malt extract agar medium complemented with glycerol to lower the water activity (aw). By studying the growth at different aw we can determine what the optimal aw is and what the lowest possible aw for growth is. Species from the Cladosporium sphaerospermum complex show growth at lower aw, compared to species from the other two complexes. This suggests a relation between the ability of these fungi to grow on indoor surfaces and the habit to grow better at lower water activity. Keywords: Cladosporium, Indoor fungi, Water activity and Phylogeny

Workshop Sessions

MEM-WK306.01 - Fungal melanin as an energy transducer and radioprotector

Ekaterina Dadachova[1], Ruth Bryan[1], Arturo Casadevall[1], Ekaterina Revskaya[1], Igor Shuryak[2]
[1]Albert Einstein College of Medicine, New York, USA, [2]Columbia University, New York, USA

Ionizing radiation is known for its cytotoxic and mutagenic properties. However, our group and others have demonstrated that prolonged sub-lethal irradiation stimulates the growth of melanin-pigmented (melanized) fungi, supporting the hypothesis that interactions between melanin and ionizing photons generate energy useful for fungal growth, and/or regulate growth-promoting genes. We are investigating the energy transduction properties of melanin and creating melanin-based radioprotectors for cancer patients undergoing radiation therapy. There are no quantitative models of how fungal proliferation is affected by ionizing photon energy, dose rate, and presence/absence of melanin on the same genetic background. We developed such a model, and tested it using experimental data on melanin-modulated radiation-induced proliferation enhancement in the fungus Cryptococcus neoformans, exposed to a wide range of X-ray dose rates and peak energies. Our analysis demonstrates that radiation-induced proliferation enhancement in C. neoformans behaves as a binary “on/off” phenomenon, which can be triggered by dose rates <0.002 mGy/h, and stays in the “on” position until growth inhibition occurs >5000 mGy/h. We also investigated the possibility of creating an oral radioprotector based on the fungal melanin that would act as an internal shield and protect the tissues via Compton scattering followed by free radical scavenging. CD-1 mice were fed melanin-containing black edible mushrooms Auricularia auricila-judae before 9Gy total body irradiation. The location of the mushrooms in the body before irradiation was determined by in vivo fluorescent imaging. Black mushrooms protected 80% of mice from the lethal dose, while control mice or those given melanin-devoid mushrooms died from gastrointestinal syndrome. The role of melanin in radioprotection was proven by the fact that mice given white mushrooms supplemented with melanin survived at the same rate as mice given black mushrooms. The ability of melanin-containing mushrooms to provide remarkable protection against radiation suggests that they could be developed into oral radioprotectors.

Workshop Sessions

MEM-WK306.02 - Application of metabolomics to fungal solid state fermentation of winery derived waste

Avinash Karpe[1, 2], David Beale[2], Ian Harding[1], Enzo Palombo[1]
[1]Commonwealth Scientific and Industrial Research Organization (CSIRO), Highett, Australia, [2]Faculty of Life and Social Sciences, Hawthorn, Australia

During fermentation, Saccharomyces cerevisiae generates about 12-15% ethanol from raw material (grapes or other feedstock), leaving the major part (approx. 85%) consisting of spent wash, seeds, marcs and pomace as landfill waste. This predominantly lignocellulose waste can be degraded by lignocellulolytic organisms to produce commercial biofuels and useful bioactive compounds. A Solid State Fermentation (SSF) was performed to degrade the winery biomass waste derived from grape varieties of Vitis vinifera vars. Shiraz, Grenache and Cabernet by fungal cultures of Trichoderma herzianum, Aspergillus niger, Penicillium chrysogenum and P. citrinum. Quantitative chemical analysis was performed for lignins, reducing sugars and quantitative enzyme assays were performed for cellulose, β-glucosidase and xylanase. The degradation ability of all these fungi on the grape varieties was monitored using metabolic profiling. It was observed that the SSF process resulted in considerable degradation of all grape substrates. Reducing sugars increased by 35.9 and 15.6 Kg/m3 in P. chrysogenum and A. niger cultures, respectively, resulting in high cellulase activities of 97.6 U and 45.1 U, respectively. During the same degradation period, the highest β-glucosidase activity (211.5 U) was demonstrated by P. citrinum. Xylanase, a member of β-glucosidase subfamily, also displayed proportionally high activity (822.4 U) in the P. citrinum culture. Although, none of the aforementioned fungi have been reported for ligninase activities, P. chrysogenum displayed minor lignin mineralization, confirmed by the metabolite profiling. The metabolite profiles and chemometric analyses of fungal degradation obtained by gas chromatography-mass spectrometry indicated differential patterns for different grape varieties. The experiment showed the potential of metabolomics for investigating the activities of different fungi over diverse substrates. This could help in developing a consortial microbial pattern of biomass conversion to commercially important molecules, such as ethanol and hydrogen, which are considered as important biofuel molecules.

Workshop Sessions

MEM-WK306.03 - Cellobiose sensing and cellulase regulation in Neurospora crassa

Sam Coradetti[1], Yi Xiong[1], James Craig[1], N Louise Glass[1]
[1]UC Berkeley, Berkeley, USA

More efficient industrial production of fungal cellulases and associated hydrolytic enzymes is a prerequisite for large scale production of second-generation cellulosic biofuels. We study molecular mechanisms of plant cell wall sensing and deconstruction in the model fungus Neurospora crassa to facilitate coordinated expression of complex enzyme cocktails in engineered fungal strains. We have identified two transcription factors that are critical for cellulose sensing in N crassa, CLR-1 and CLR-2. CLR-2 is a direct regulator of cellulases. Expression of CLR-2 is sufficient for cellulase secretion in N crassa without the presence of cellulose or other inducers. CLR-2 is highly conserved among ascomycete fungi and was essential for cellulase expression in Aspergillus nidulans. CLR-1 plays an essential role in sensing extracellular cellulose via its hydrolyzed product cellobiose and regulates cellulase expression via CLR-2. While conserved in sequence across most ascomycete fungi, CLR-1 function was not conserved in A nidulans. Proteomic and genetic strategies to elucidate the molecular mechanisms of CLR-1 function and cellobiose sensing in N crassa will be discussed.

Workshop Sessions

MEM-WK306.04 - Recent Developments in Biofuels and Bio-based Chemicals Production by Yeasts

Charles Abbas[1]
[1]*Archer Daniels Midland Research Decatur, USA*

In the past decade, the demand for energy has fueled a worldwide resurgence in interest in the production of biofuels and bio-based chemicals from renewable feedstocks. The increased focus on the use of renewable feedstocks, such as lignocellulosics, is due in part to recent advances in pretreatment and hydrolysis technology. With the increased search for alternatives to fossil fuels, renewable feedstocks have also received governmental mandates and subsidies that aim to create incentives for their commercial production. Second generation biofuels include, in addition to cellulosic ethanol, long-chain alcohols, terpenoid hydrocarbons, and diesel-length alkanes. Advances in technology have also led the chemicals industry to leverage new feedstocks that provide a cost advantage while delivering a sustainable chemical product. Today, the vast majority of industrial chemicals are produced with fossil fuel-based feedstocks; but with the volatility of petroleum-based feedstocks, there is increased demand for more sustainable products. The development of new yeast strains of *Saccharomyces cerevisiae* by genetic engineering and the use of other genera of yeasts that can utilize a broader range of feedstocks have been the focus of research efforts. The chemicals of primary interest are the C3 and C4 chemicals that represent the fundamental building blocks of key segments of the industrial chemical industry, which today have annual sales valued at more than $10 billion. Used for a broad range of industrial and consumer products, these chemicals are on the list of top ten chemicals developed by the U.S. Department of Energy (DOE). The production of these chemicals has been already demonstrated at bench- and pilot-scale and at demonstration-scale facilities.

Workshop Sessions

MEM-WK306.05 - Co-cultivations of fungi: microscopic analysis and influence on protein production

I. Benoit[1,2], T. Gruntjes[1,2], A. Vinck[1], J. van Veluw[1], H.A.B. Wösten[1], A. Tsang[3], R.P. de Vries[1,2]
[1]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, [2]Microbiology & Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht, The Netherlands, [3]CSFG, Concordia University, Montreal, Canada

During their natural life cycle most fungi encounter other microorganisms and live in mixed communities with complex interactions, such as symbiosis or competition. Industrial fermentations, on purpose or by accident, can also result in mixed cultures. Fungal co-cultivations have been previously described for the production of specific enzymes or metabolites [1, 2], however, little is known about the interactions between two species that are grown together. The ascomycetes *Aspergillus niger* and *Aspergillus oryzae* are two of the most important industrial fungi worldwide and both have a long history of strain improvement to optimize enzyme and metabolite production. The basidiomycetes *Gloeophyllum trabeum* and *Phanerochaete chrysosporium* are respectively a brown and a white rot, harboring different enzymatic tools to degrade the plant biomass. We have co-cultivated the two Aspergilli and *A. niger* with *G. trabeum* and *P. chrysosporium*. The morphology and mechanism of the interaction of these mixed cultures in agricultural by-product is addressed using flow cytometry, microscopy and proteomics. References (1) Ge et al., 2009, Bioresource Technology 100(5): 1872-1874 (2) Hu et al., 2011, International Biodeterioration & Biodegradation 65(1): 248-252

Workshop Sessions

MEM-WK307.01 - The impact of DNA sequencing on yeast species descriptions

Marc-André Lachance[1]
[1]*Department of Biology, University of Western Ontario, London, Canada*

DNA sequencing now makes it possible to classify yeasts phylogenetically and to identify yeasts rapidly and accurately. Already, the availability of sequence information has provided the impetus to do away with the dual nomenclature traditionally applied to yeasts and other fungi. The approach also has had a formidable impact on the number of new species descriptions. Here, I shall address how sequence analysis has affected the quality of descriptions, with special emphasis on species delineation. To this effect, I have reviewed yeast species descriptions published in selected journals since the beginning of the broad implementation of DNA sequencing in yeast systematics in the late 90s. The following generalizations can be made. Although a clear species concept is rarely stated explicitly, the observation by Kurtzman and Robnett (1998) that “good” yeast species seldom exhibit polymorphisms of more than three substitutions has served as the pivotal argument in describing most new species. Some authors have also invoked the criterion of reciprocal monophyly (phylogenetic species concept), although such attempts are sometimes made from datasets with poor taxon sampling. The genealogical concordance criterion, although commendable, does not seem to have been used, whereas the ability or mating pairs to generate fertile offspring has occasionally been applied to heterothallic, haplontic species. In most cases, sequence-based species delineation will have improved nomenclatural stability, at least at the species level. On the negative side, the ease with which DNA sequences can be obtained occasionally leads to neglect of other features that make species descriptions useful. Future improvement in the quality of species descriptions should come from increased diligence in characterizing life cycles and growth characteristics of new species, assessing within-species variation, and exploring geographic and ecological distributions.

Workshop Sessions

MEM-WK307.02 - Biodiversity of common food and airborne Fungi

Robert A Samson[1], Cobus Visagie[1], Keith A Seifert[2]
[1]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, [2]Biodiversity, Agriculture and Agri-Food Canada Ottawa, Canada

The fungal genera and species, which produce mycotoxins or cause spoilage of food, have been well studied. This is also true for the fungi occurring in indoor environments causing biodeterioration of buildings and has been regarded as causal agents of various health hazards. Identification of these fungi up to species level is essential because it can help to explain at what ecological and physiological conditions these fungi can grow in. Our current knowledge of the total mycobiota of food and indoor environments shows that it consists of a total of 100-150 taxa. However, recent polyphasic taxonomic studies have shown that the diversity of taxa is much greater than was previously thought. Particularly in the well-known genera of the Trichocomaceae applying a polyphasic approach, which combines phenotypic and molecular analyses, discovered a much greater biodiversity. Examples are presented of some common taxa, which proved to be cryptic, such as the Aspergillus candidus complex and A. niger complex. In addition, data are presented from an extensive study of the mycobiota of house dust which revealed 49 species of Penicillium, 59 species of Aspergillus and 17 species of Talaromyces. Many of these taxa are new to science.

Workshop Sessions

MEM-WK307.03 - The hybrid nature of the Eukarya and a consilient view of life on Earth

James McInerney[1]
[1]National University of Ireland, Maynooth, Ireland

The origin of the eukaryotic cell, which is known as eukaryogenesis, has puzzled scientists for more than 100 years, and many hypotheses have been proposed. Recent analyses of genomic, transcriptomic, proteomic and evolutionary data enable the safe elimination of some of these hypotheses, whereas support for other hypotheses has increased. Using new methods and data, I evaluate the available theories for their compatibility with empirical observations and conclude that cellular life consists of two primary, paraphyletic prokaryotic groups and one secondary, monophyletic group that has symbiogenic origins — the eukaryotes.

Workshop Sessions

MEM-WK307.04 - Diversity and dispersal of the Ophiostomatoid Fungus, Knoxdaviesia proteae, within the fruiting structures of Protea repens

Janneke Aylward[1,3], Leanne L Dreyer[1,3], Emma T Steenkampe[2,3], Michael J Wingfield[3], Francois Roets[3,4]
[1]Dept of Botany and Zoology, Stellenbosch University, RSA, [2]Dept of Microbiology & Plant Pathology, University of Pretoria, RSA, [3]DST/NRF CTHB, University of Pretoria, RSA, [4]Dept of Conservation Ecology & Entomology, Stellenbosch University, RSA

Knoxdaviesia proteae is a member of a polyphyletic assemblage of Protea-associated ophiostomatoid fungi that occur in the Southern Hemisphere. This fungus appears to occur exclusively within the fruiting structures of the indigenous South African plant, Protea repens. It primarily employs mycophagous mites for dispersal, but long-distance dispersal may be facilitated by exploiting beetles as vehicles for mite movement. The intricate associations between K. proteae and its arthropod vectors have recently been elucidated, but the ecology, extent of dispersal and reproductive strategy of K. proteae and other Protea-associated ophiostomatoids remain largely unknown. In order to investigate this system at a molecular level, we developed 12 polymorphic microsatellite markers for K. proteae using an ISSR-PCR enrichment strategy coupled with 454 pyrosequencing. These molecular markers were subsequently used to explore the population structure in 198 K. proteae isolates sampled from two P. repens populations in the Cape Floristic Region (CFR), South Africa. The aims were to 1) determine whether the dispersal strategy employed by the fungus may cause individual P. repens trees to harbour genetically discrete groups of K. proteae and 2) establish the origin of the initial fungal colonizers of new P. repens plants. The results showed that massive genetic and genotypic diversity exists within this species. Further, the high level of gene flow and recombination observed within K. proteae leads to weak population structure, even between geographically distant populations. The results of the dispersal and recolonization studies showed that K. proteae dispersal is random and widespread, emphasising the role of beetles in its movement. Biodiversity in the CFR is thus not restricted to the fauna and flora, but also extends to the microscopic elements of this floristic region. The results from this study may serve as a model for other Protea-associated fungi and promote further fungal studies in this biodiversity hotspot.

Workshop Sessions

MEM-WK307.05 - Pyrosequencing revealed geographical distribution and ecological diversification of fungal communities on barley and malt from western Canada

Wen Chen[1], Christopher T. Lewis[1], André Lévesque[1], Nan Zhang[1], Kelly Turkington[2], Janice Bamforth[3], Don Gaba[3], Sheryl A. Tittlemier[3], Aaron MacLeod[3], Tom Gräfenhan[3]

[1]Agricutlure and Agri-Food Canada, Ottawa, Canada, [2]Agricutlure and Agri-Food Canada, Lacombe, Canada, [3]Grain Reserach Laboratory, Canadian Grain Commission, Winnipeg, Canada

The composition of microbial communities on cereal grains are expected to reflect the environment in which they are grown and processed, and to include members that contribute to the safety and quality of end products. We applied 454 pyrosequencing technology to the internal transcribed spacer (ITS) region of ribosomal DNA to characterize epiphytic fungal communities naturally associated with barley grain. We profiled true fungal communities of 64 barley samples grown in six different locations across the Canadian Prairies over two growing seasons (2011-12). Of these, eight samples harvested in 2011 have undergone the malting process. We extracted over 1.3 million quality trimmed ITS1 and ITS2 sequences, which were then clustered, based on a 97% similarity cutoff, into approximately 7,000 ITS1/ITS2 operational taxonomic units (OTUs) after exclusion of OTUs representing a single sequence. Representative sequences of each OTU were processed using the QIIME pipeline and a curated UNITE ITS reference database in order to create a taxonomic profile of the true fungi. The barley grain profiles included a core fungal community that consisted mainly of wide spread yeast or yeast-like fungi (e.g. Aureobasidium and Cryptococcus) and genera containing species that are known to be associated with plants (e.g. Epicoccum and Alternaria) including important pathogens such as Fusarium and Pyrenophora. Ordination analyses (e.g. Canonical Correlation Analysis, CCA) showed that the mycoflora of raw barley grain varied greatly between geographic locations, with the abundance of certain genera (e.g. Fusarium) significantly correlated to seasonal precipitation and the total fungal biomass. The abundance of other genera, such as Puccinia and Epicoccum, increased significantly when barley was harvested using straight cut method at a later date. The observed increase of fungal load, especially by species of Fusarium and Alternaria, on late harvested barley (due to weathering) and in malt can negatively affect malting quality.

Workshop Sessions

VIR-WK211.01 - Structure-function analysis of human JC polyomavirus attachment to sialic-acid containing receptors

Melissa Maginnis[1], Luisa Stroh[2], Barbel Blaum[2], Christian Nelson[3], Ursula Neu[2], Gretchen Gee[3], Thilo Stehle[2], Walter Atwood[3]

[1]Department of Molecular and Biomedical Sciences, University of Maine, Orono, USA, [2]Interfaculty Institute for Biochemistry, University of Tubingen, Tubingen, Germany, [3]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, USA

JC polyomavirus (JCPyV) is a ubiquitous human pathogen and the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML). JCPyV infection is dependent on engagement of cell-surface sialic acids, yet the specificity of this interaction was unclear. Utilizing a glycan array screen, we determined that the major viral capsid protein (VP1) of the JCPyV prototype strain (Mad-1) binds specifically to α2,6-linked lactoseries tetrasaccharide c (LSTc). We defined the specific residues in VP1 that are responsible for mediating direct interactions with the α2,6-linked sialic acid of LSTc. Interestingly, viral isolates from the cerebral spinal fluid of individuals with PML often contain mutations in the sialic acid-binding pocket of VP1 that are thought to arise from positive selection. However, PML-associated mutations block VP1 binding to LSTc and reduce JCPyV infectivity. The JCPyV WT3 strain was previously reported to interact with α2,3- or α2,8-linked sialic acids expressed on gangliosides, which serve as receptors for the majority of polyomaviruses discovered to date. We utilized a structure-function approach to determine whether JCPyV can interact with additional sialic acid receptors or whether there are strain-specific differences in oligosaccharide receptor usage. Structure-function analysis and affinity saturation using VP1 pentamers and viruses with a Mad-1 or WT3 capsid revealed that both strains exhibit low affinity binding to carbohydrate structures on gangliosides, but have the highest affinity for LSTc. Exogenous ganglioside expression does not enhance JCPyV infection, and reduction of ganglioside expression by siRNA treatment does not reduce infection. These data lend further support for the importance of α2,6-linked LSTc in JCPyV infection. Additionally, these findings demonstrate that while the human polyomaviruses share structural similarities and engage their oligosaccharide receptors in a similar manner, the specificity and affinity of virus-carbohydrate interactions play an important regulatory role in viral tropism and pathogenesis.

Workshop Sessions

VIR-WK211.02 - JC polyomavirus infection of human renal proximal tubular epithelial cells: an in vitro model of persistence

Benedetta Assetta[1], Walter J. Atwood[1]
[1]*Brown University, Providence, USA*

The human JC polyomavirus (JCPyV) is the etiological agent of progressive multifocal leukoencephalopathy (PML), a fatal neurodegenerative disease. JCPyV establishes an asymptomatic persistent infection in the kidneys of immunocompetent individuals. It is unclear whether JCPyV becomes latent in the kidney with cycles of reactivation, or whether it maintains a low level of replication with detectable bursts of infection. In the present study, we sought to establish an in vitro model of JCPyV persistence using a primary cell line of human renal proximal tubular epithelial cells (HRPTEC). HRPTEC express the receptor motif LSTc and the serotonin receptors 5-HT2AR, 5-HT2BR, and 5-HT2CR required to start an infectious cycle. We infected HRPTEC with lab-adapted brain and kidney strains of JCPyV and found that all strains were able to replicate. To test whether JCPyV could spread in HRPTEC a growth assay was performed. Infection of human BK polyomavirus (BKPyV), which causes polyomavirus-associated nephropathy (PVN) in immunocompromised hosts, is well characterized in HPRTEC and was used as positive control. Cells were infected with JCPyV or BKPyV and expression of late protein VP1 was assessed every 3 days for 15 days. At day 9, there was a significant burst in BKPyV infection and most of the cells lysed as a result. On the other hand, JCPyV spread more slowly through the HRPTEC than BKPyV, and viral growth reached a plateau at day 15. No significant cytolysis was visible at any time point tested for JCPyV. These preliminary findings represent a promising first step towards the establishment of a model of JCPyV persistence in the kidney that will be used to characterize viral and cellular factors involved in this process. JCPyV is an important human pathogen and it is therefore crucial to gain new insights into the mechanism of JCPyV persistence and reactivation in immunocompetent individuals.

Workshop Sessions

VIR-WK211.03 - Human defensins and initial innate recognition of JC polyomavirus infection

Stephen R Zins[1], Walter J Atwood[1]
[1]*Brown University, Providence, USA*

JC polyomavirus (JCPyV) is a double-stranded DNA virus that establishes a persistent infection in immunocompetent individuals. Immunocompromised patients are at risk for reactivation of JCPyV and subsequent development of the fatal disease Progressive Multifocal Leukoencephalopathy (PML). The initial innate immune response to JCPyV infection has not been studied, and little is known about the early host recognition of the virus. Defensins are naturally occurring small molecules that show an incredible ability to neutralize a wide variety of pathogens, including several classes of viruses. We have shown that the human alpha defensin HD5 neutralizes JCPyV infection in human fetal glial cells (site of lytic infection) by disrupting viral trafficking and stabilizing the viral capsid. It has also recently been shown that kidney epithelial cells (a site of proposed persistent JCPyV infection) naturally express HD5 and that expression increases upon infection with bacteria. It was originally thought that epithelial cells primarily expressed only beta defensins. This work builds upon preliminary data that long-term infection of human renal proximal tubular epithelial cells (HRPTEC) and human renal cortical cells (HRCC) results in a significant down-regulation of human beta defensin 3 (HBD3). HBD3 is known to be immunomodulatory and it is thought that JCPyV establishes a persistent infection in part due to dampening the host immune system. This work elucidates the role of defensins upon initial JCPyV infection in the kidney, and explores the initial host-pathogen response of human polyomaviruses by investigating the initial innate response to the virus and subsequent immune modulation.

Workshop Sessions

VIR-WK211.04 - Taxonomy of Circoviridae: restructuring and expansion

Philippe Biagini[1]

[1] *UMR 7268, Viral Emergence and Co-Evolution Unit, Aix-Marseille University / French Blood Agency / CNRS, Marseille, France*

Viruses with circular single-stranded DNA genomes infect human and non-human hosts (animals, insects, bacteria, plants). On the basis of molecular and structural considerations, they are grouped into distinct taxonomic families. The family Circoviridae was named after porcine circovirus which was biochemically characterized in 1982. Official description of the family was published in 1995, with several viral species grouped into a single genus, Circovirus. The progressive characterization of new viruses identified in birds and pigs led to the subsequent creation of two genera, Circovirus and Gyrovirus, incorporating distinct species. According to latest results obtained by metagenomics and next generation sequencing, it is however evident that the genetic diversity characterizing viruses with circular single-stranded DNA genomes is extremely wide. This is exemplified by the recent discovery of cycloviruses, sharing significant similarities to circovirus isolates (genome organization, presence of Rep and Cap genes...) despite a high genetic diversity. Such findings are in favor with a restructuring of the family Circoviridae. Taxonomic aspects, updates, and future directions are exposed.

Workshop Sessions

VIR-WK211.05 - Prospective, comprehensive and effective viral monitoring in Cuban children undergoing solid organ transplantation

Consuelo Correa Sierra[1], Vivian Kourí Cardellá[1], Lizet Sánchez Valdés[1], Pedro A Martínez Rodríguez[1], Gretel González Muñoz[1], César E Silverio[2], Norma Hondal[2], José Florín[3], Lissette Pérez Santos[1], Mabel González Alemán[2]
[1]Tropical Medicine Institute "Pedro Kourí", Havana City, Cuba., [2]University Pediatric Hospital "William Soler", Havana City, Cuba., [3]University Pediatric Hospital of "Centro Habana", Havana City, Cuba.

In Cuba, viral monitoring in the post-transplant period was not routinely performed. The aim of this research is to identify the most frequent viruses that affect transplanted children, by implementing a viral follow-up during the post-transplant period. The study population included all Cuban pediatric patients who underwent solid organ transplantation between November 2009 and December 2012. A total of 34 transplanted pediatric patients, kidney (n=11) and liver (n=23), were prospectively monitored during a 34-week period for viral DNAemia and DNAuria by simultaneous detection of cytomegalovirus (CMV), Epstein-Barr virus, herpes simplex virus, varicella-zoster virus, human herpesvirus 6, human adenovirus and polyomaviruses (BKV and JCV) using quantitative real-time polymerase chain reaction (qRT-PCR). Viral genome of at least one virus was detected in 21 of 34 recipients, 18 patients excreted virus in urine while 12 presented DNAemia. Liver recipients had detectable viral DNA in 65.2% and kidney recipients in 54.5%. CMV and BKV were the most frequent viruses detected in 41.2% and 35.3% of patients, respectively; in addition, CMV viruria was more frequently detected among kidney recipients and BKV viruria in liver recipients, although this difference was not statistically significant. CMV was the virus mainly associated with DNAemia and its excretion in urine (with a cut off value of 219 copies/mL) was associated with detection in plasma ($p<0.001$); furthermore, CMV viruria was predictive of CMV viremia (OR:8.4, CI:2.4-29.1, $p=0.001$). CMV was found causing direct complications in 3 patients (CMV-syndrome). All treated patients showed clinical and/or virological evidence of response to the antiviral therapy. There was no association between high viral load and clinical complications, due to the prompt initiation of preemptive ganciclovir. This comprehensive viral monitoring program effectively prevents the development of critical viral disease, and thus, urges the implementation of qRT-PCR as routine for viral monitoring of pediatric transplanted Cuban organ recipients.

Workshop Sessions

VIR-WK211.06 - The prevalence of Human Papilloma Virus among women attending cervical screening (Pap Smear) service in Abakaliki Southeastern Nigeria

B.A.F. Ngwu[1], G. O. Ezeifeka[2]

[1]Department of Medical Microbiology, Faculty of Clinical Medicine, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria, [2]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka Anambra State, Nigeria

The prevention and control of cervical cancer depends on the prevention and control of its causative agent Human Papilloma Virus (HPV) especially the high risk types 16, 18. In this study the prevalence of the HPV especially high risk types were studied among women attending cervical screening (Pap smear) centre in a Federal Teaching Hospital Abakaliki, Ebonyi State Nigeria. Consents were obtained and blood samples were aseptically collected for HPV assay using HPV IgG ELISA kits from Cusabio Co. Ltd Germany. The results showed that out of the 360 women aged between 20 years and 63 years with a mean age of 35 years who came for Pap smear screening, only 25% (90) participated in the study. The overall result showed that 70.8% (64) were positive and 29.2% (26) were negative. The high risk HPV 16 and HPV 18 have the prevalence of 22.2% and 21.2% respectively. The result is significantly positive for all the HPV types 6, 11, 16 and 18, but not significally positive for the high risk HPV types 16, and 18. We conclude that there is poor uptake of cervical screening services in southeastern Nigeria (25%) and there is an urgent need for medical education and sensitization of women in this region to know the risk factors for HPV transmission and the need to take up pap smear screening services provided by the teaching hospital and other health providers to prevent the transmission of HPV and thereby control cervical cancer.

Workshop Sessions

VIR-WK212.01 - Molecular mechanisms of a novel viral enterotoxin

Stacey Schultz-Cherry[1], Victoria Meliopoulos[1], Shauna Marvin[1], Pamela Freiden[1], Rebecca DuBois[1], Stephen W. White[1]

[1]St Jude Children's Research Hospital, Memphis, USA

According to the WHO and UNICEF, 1.5 million children under the age of 5 die from diarrhea annually. Astroviruses are associated with at least 10% of all sporadic cases and >25% of all hospitalized cases. Astroviruses are rapidly evolving RNA viruses linked to outbreaks of diarrhea, which can be obtained nosocomially, directly from infected individuals, and through contaminated food and water. Although typically an acute disease associated with diarrhea, astrovirus infections can also be associated with nephritis, hepatitis, and fatal encephalitis. Immunocompromised children are even more susceptible often developing persistent infections that lead to wasting or even systemic infections. There are currently no "cures" for astrovirus. My laboratory was the first to demonstrate that astroviruses induce diarrhea by a novel mechanism; they possess an enterotoxin that disrupts intestinal epithelial barrier function independent of cellular damage or an inflammatory response. This occurs within 24 hr post-infection due to reorganization of the tight junction protein occludin and the actin cytoskeleton. Intriguingly, certain clinical isolates disrupt barrier function faster than the lab-adapted viruses. This is the first evidence showing that a viral coat protein is an enterotoxin. Work to identify the "toxigenic" domain of the coat protein suggest that spike region, which is structurally distinct amongst the astrovirus species but shares structurally similarity with the hepatitis E capsid, may also act as a toxin. Mechanistically, binding of the capsid protein to epithelial cells leads to increased phosphorylation of focal adhesion kinase and activation of myosin light chain kinase within 6 hr post-infection. Surprisingly, there is also an increased level of vascular endothelial growth factor (VEGF) within this time frame. Studies are underway to determine the role for these signaling pathways in astrovirus pathogenesis. Finally, we demonstrate that astroviruses and the toxin may act independent of species barriers.

Workshop Sessions

VIR-WK212.02 - Development of a screening assay of norovirus-binding enteric bacteria bearing histo-blood group antigen-like substances

Daisuke Sano[1], Mohan Amarasiri[1], Takeshi Yoshimura[1], Toyoko Nakagomi[2], Osamu Nakagomi[2], Satoshi Okabe[1]
[1]Hokkaido University, Sapporo, Japan, [2]Nagasaki University, Nagasaki, Japan

Human norovirus (HuNoV) occurs in multiple genotypes, and an initial step of replication, the binding to histo-blood group antigens (HBGAs) as a probable receptor, seems genotype dependent. Since some enteric bacteria capture HuNoV particles through their HBGA-like substances, they may modulate the life cycle of HuNoVs, from the attachment to the intestinal epithelium to egressing into the natural environment to reach a new host. It is critical to clarify how diverse human enteric bacteria can capture HuNoVs through bacterial HBGA-like substances for understanding to what extent HBGA-positive bacteria impact on the life cycle of HuNoVs. Thus, we aimed at developing an assay in which whole bacterial cells were examined for their binding capacity to HuNoVs. In this assay, the mixture of bacterial cells with NoV VLP was filtered through a NANOSEP MF GHP 0.45 μm filter, and the amount of VLPs in the filtrate was measured by a norovirus-antigen ELISA. With this filtration, we examined the interaction between three strains of bacteria (Enterobacter sp. SENG-6, E. coli O86 and an E. coli MG1655 mutant AKN123) and GII.6 VLP. SENG-6 has an A-type antigen-dominant HBGA, O86 a B-type antigen-dominant HBGA, and AKN123 HBGA-negative, while GII.6 VLP recognizes A, B, and H(O) antigens. GII.6 VLP was mixed with each bacterial suspension, and shaken for 15 min at 4˚C prior to the filtration. As a result, the amount of GII.6 VLP was significantly lower in the filtrate of the mixtures with Enterobacter sp. SENG-6 and E. coli O86 than in the filtrate of the mixtures with E. coli AKN123, an HBGA-negative strain ($p<0.01$). These results indicate that the proposed assay using GII.6 VLP can easily discriminate bacterial strains that can bind to NoV in the HBGA-binding group. This assay will help evince the distribution of norovirus-binding bacteria in intestinal microbiota and natural environments.

Workshop Sessions

VIR-WK212.03 - The genetic variability of noroviruses in a patient with underlying chronic norovirus infection

Darja Duh[1], Mojca Cimerman[1], Andrej Golle[1], Nina Nečimer[2], Božena Kotnik Kevorkijan[3]
[1]Institute of Public Health Maribor, Maribor, Slovenia, [2]Roche farmacevtska družba d.o.o., Ljubljana, Slovenia, [3]University Medical Centre Maribor, Maribor, Slovenia

Noroviruses, causing acute gastroenteritis in children and adult worldwide, are RNA viruses with 7.5 kb genome organized into 3 ORFs. Least researched but most important for medicine, pharmacy and science is chronic norovirus infection with prolonged virus shedding. Occurring in immunocompromised patients, such infection is characterized by high viral sequence diversity in ORF2/3 and a mixture of related genetic variants referred to as quasispecies. Whether viral diversity is driven by immune selection or other mechanisms and whether transmission of genetic variants is bottleneck event or co-infection of quasispecies remains to be elucidated. Noroviruses cannot be cured. Since hospitalization is necessary in severe chronic cases, nosocomial potential is implicated for chronic infection. Better understanding of genetic diversity and transmission of noroviruses will be beneficial to development of drugs and possible norovirus vaccine. Therefore high resolution and precision techniques like next generation sequencing are needed for chronic norovirus infection studies. One study described the use of NGS in the case of chronic norovirus infection; however, stool was sampled within one year of initial infection and only partial sequences were analyzed. It was previously determined that the break point in viral evolution most likely occurred in the second year of chronic norovirus infection. We are presenting the case of immunocompromised patient with a 3 year history of norovirus infection detected by real time RT-PCR. NGS was successfully performed on 3 RNA samples extracted from stool taken in the beginning of each year. More than 200.000 HQ reads per each sample were obtained by GS FLX+ System with average read length of 400 bp. Detailed genome analysis is in process. We are anticipating that the analysis will reveal specific variable parts which will then be further sequenced from a series of 6 RNA samples taken within 1 month in the second year of infection.

Workshop Sessions

VIR-WK212.03 - WITHDRAWN - DNA vaccination against human norovirus elicits strong humoral responses in experimental animals

Gabriel Parra[1], Joseph Kendra[1], Karin Bok[1], Stanislav Sosnovtsev[1], John Ballantyne[2], Michael Chambers[2], Leonard Ruiz[3], Kim Green[1]

[1]Caliciviruses Section, NIAID, NIH, Bethesda, USA, [2]Aldevron, Fargo, USA, [3]International Medica Foundation, Shoreview, USA

Noroviruses are the most important cause of acute gastroenteritis in all age groups worldwide. Strains from genogroups GI and GII have been shown to infect humans, with the GII.4 genotype as the most prevalent. The persistence of GII.4 in the human population has been attributed to the continuous emergence of new variants that replace old variants every 3-5 years. The expression of the major capsid protein (VP1) results in self-assembly of virus-like particles (VLPs), which are under investigation as vaccine candidates. To bypass the need for VLP production, we tested DNA vaccination as an alternative approach for norovirus immunization. We showed that DNA vaccinated animals presented titers of up to $2x10^5$ against strains from the same genogroup, which was comparable to that of animals immunized with the corresponding VLPs. To further characterize the humoral response after DNA immunization, we measured the antibody responses against a panel of time-ordered GII.4 VLPs and S/P chimeric VLPs. Animals that were immunized with VLPs or DNA corresponding to strain Hu/NoV/GII.4/MD145-12/1987/US presented similar titers (range $2.5x10^4$–$1.3x10^5$) against GII.4 strains that circulated prior to 1996; however, VLP immunization performed better eliciting antibodies against newer strains (i.e. FarmingtonHills_2002 and Sydney_2012). Reactivity profiles of sera from immunized animals against chimeric VLPs suggested that both domains of the VP1, Shell and Protruding, elicit humoral responses after DNA vaccination. To determine the potential of DNA vaccines to elicit protective responses, we tested the sera in hemagglutination inhibition (HAI) assays (a surrogate of norovirus neutralization). Slightly lower HAI titers were detected in DNA-vaccinated animals when compared to those receiving VLPs, but differences were not statistically significant. In conclusion, DNA vaccines provide another strategy for norovirus immunization that could be used alone or in combination with VLP-based vaccines to stimulate broadly reactive immunity against these diverse and rapidly evolving viruses.

Workshop Sessions

VIR-WK212.04 - Specific conserved surface residues of the feline calicivirus (FCV) capsid are required for receptor binding and/or infection.

Zhengchun Lu[1], Emily Desmet[1], Meleana Hinchman[1], John Parker[1]
[1]*Cornell University, Ithaca, USA*

Feline calicivirus (FCV) is a common pathogen of cats that causes mild to severe disease. Previously we showed that virulent FCV isolates appear to have faster kinetics of growth in tissue culture than non-virulent isolates. We hypothesized that these differences in growth were conferred by variations in the virus capsid proteins. To test this hypothesis we used a full-length infectious cDNA clone derived from the low-virulence FCV-Urbana strain, to prepare a chimeric virus that contained the FCV-Urbana backbone and the genes encoding the VP1 and VP2 of the high-virulence FCV-5 strain. Under both single and multiple cycle growth conditions, we found that the recombinant FCV-5-Urbana chimeric virus infected cells more efficiently than the parental virus (average 10 fold higher yield, $p < 0.001$). Virus-receptor interactions are major determinants of tropism and in vivo virus spread. FCV uses fJAM-A as its functional receptor. To identify conserved capsid surface residues that are essential for interaction with fJAM-A, we used the chimeric virus to generate a panel of 14 recombinant viral mutants with alterations in conserved surface residues along the dimer interface of VP1. Two virus mutants were recovered and grew with similar kinetics to the wild type chimera. However, twelve mutants were non-viable. For the non-viable mutants, we prepared recombinant capsid virus-like particles (VLPs). We then analyzed the capacity of these mutant VLPs to bind soluble fJAM-A. Three mutants did not bind fJAM-A. Four mutants had an 80% reduction in binding relative to the wild-type VLP. Intriguingly, five mutants bound at levels similar to wild type. These findings suggest that highly conserved capsid surface residues not required for fJAM-A binding are important for infection.

Workshop Sessions

VIR-WK212.05 - Strong constraints on changes in capsid protein of norovirus pandemic lineage GII.4_2006b after the onset of outbreaks

Hironori Sato[1], Masaru Yokoyama[1], Hiromi Nakamura[1], Kazushi Motomura[1,2,3]
[1]*National Institute of Infectious Diseases, Tokyo, Japan,* [2]*Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections, Nonthaburi, Thailand,* [3]*Osaka University, Osaka, Japan*

Little is known about how a new epidemic variant of norovirus (NoV) evolves after the onset of the outbreaks. To address this issue, we examined here diversification of 8 viral proteins using 250 near-full length genomic sequences of the NoV pandemic variant GII.4_2006b in the stool specimens those were collected in Japan between 2006 and 2011. We found that viral capsid protein, which is generally considered to be highly variable, remained as homogeneous as the three viral enzymes during repeated epidemics. Accumulations of amino acid substitutions were strongly restricted, leading to frequent emergence of distinct 2006b variants carrying the identical capsid protein throughout the study period. The results suggest that relative fitness of the NoV GII.4_2006b capsid proteome reached a near peak level at the onset of epidemics and inevitably resisted changes to maintain the high fitness. The findings provide a molecular basis for understanding and controlling NoV pandemic variant.

Workshop Sessions

VIR-WK212.06 - Assessment of gastroenteric viruses from wastewater directly discharged into Uruguay River, Uruguay

Rodney Colina[1], Matías Victoria[1], Fernando López Tort[1], Andrés Lizasoain[1], Marize Miagostovich[3], Jose Leite[3], Juan Cristina[2]

[1]University of Republic, North Regional, Salto, Uruguay, [2]University of Republic, Faculty of Sciences, Montevideo, Uruguay, [3]Oswaldo Cruz Institute, Rio de Janeiro, Brasil

The aim of this study was to assess the viral contamination of group A rotavirus (RVA), norovirus (NoV) and human astrovirus (HAstV) in sewage directly discharged into Uruguay River, and to characterize RVA genotypes circulating in Uruguay. For this purpose, sewage samples (n=96) were collected biweekly from March 2011 to February 2012 in four Uruguayan cities: Bella Unión, Salto, Paysandú and Fray Bentos. Each sample was concentrated by ultracentrifugation method. Qualitative and quantitative RT-PCR for RVA, NoV and HAstV were performed. A wide dissemination of gastroenteric viruses was observed in the sewage samples analyzed with 80% of positivity, being NoV (51%) the most frequently detected followed by RVA with a frequency of 49% and HAstV with 45%. Genotypes of RVA were typed using multiplex semi-nested RT-PCR as follows: P[8] (n=15), P[4] (n=8), P[10] (n=1), P[11] (n=1), G2 (n=29) and G3 (n=2). The viral load ranged from 10(3) to 10(7) genomic copies/liter and they were detected roughly with the same frequency in all participant cities. A peak of RVA and HAstV detection was observed in colder months (June to September), whereas no seasonality was observed for NoV. This study demonstrates for the first time, the high degree of gastroenteric viral contamination in the country; highlighting the importance of developing these analyses as a tool to determine the viral contamination in this hydrographic boundary region used by the local populations for recreation and consumption, establishing an elevated risk of gastroenteric diseases for human health.

Workshop Sessions

VIR-WK212.07 - A unique mechanism of norovirus escape from antibody neutralization

Abimbola Kolawole[1], Ming Li[2], Chunsheng Xia[2], Audrey Fischer[3], Nicholas Giacobbi[4], Chenchen Yu[1], Joshua Wolfe[3], Andrew Feldman[3], Thomas Smith[2], Christiane Wobus[1]

[1]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, USA, [2]Donald Danforth Plant Science Center, St. Louis, USA, [3]Applied Physics Laboratory, Johns Hopkins University, Baltimore, USA, [4]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, USA

Human noroviruses cause pandemics every 2-3 years. Emergence of new epidemic strains is in part mediated by mutations in the viral capsid that allow escape from antibody neutralization and herd immunity. To better understand how noroviruses evolve resistance to antibody neutralization, we investigated the structural basis for the escape of murine norovirus (MNV) from antibody neutralization. Previous results indicate that the neutralizing monoclonal antibody (mAb) A6.2 binds to the A'B' and E'F' loops of the MNV-1 protruding (P) domain of the capsid. To identify specific residues in the P domain that play a role in neutralization escape, 22 recombinant MNVs were generated with amino acid substitutions in the A'B' or E'F' loops. Viruses were analyzed for their ability to escape mAb A6.2 neutralization. Six mutations in the E'F' loop, V378F, A382K, A382P, A382R, D385G, and L386F, mediated escape from mAb A6.2 neutralization. To elucidate underlying structural mechanisms for these results, the atomic structure of the A6.2 Fab was determined and fitted into the previously generated pseudo-atomic model of the A6.2 Fab / MNV-1 virion complex. Previously, two distinct conformations, 'A', and 'B', of the atomic structures of the MNV-1 P domain were identified due to flexibility in the two P domain loops. A superior stereochemical fit of the A6.2 Fab to the 'A' conformation of the MNV P domain was observed. Structural analysis of our observed escape mutants indicates changes towards the less preferred 'B' conformation of the P domain. The shift in the structural equilibrium of the P domain towards the conformation with poor structural complementarity to the antibody strongly supports a unique mechanism for antibody escape. This occurs via antigen flexibility instead of direct antibody-antigen binding.

Workshop Sessions

VIR-WK213.01 - Infection by chlorovirus PBCV-1

James Van Etten[1]
[1]*University of Nebraska, Lincoln, USA*

Chloroviruses, family *Phycodnaviridae* are large (290-370 kb) dsDNA-containing, plaque-forming viruses that infect certain isolates of unicellular, eukaryotic, chlorella-like green algae. The viruses are ubiquitous in freshwater environments from around the world and can reach titers as high as 10^5 infectious particles per ml of water. The prototype chlorovirus PBCV-1 encodes ~400 proteins and 11 tRNAs. Many chlorovirus-encoded proteins are either the smallest or among the smallest proteins of their class. Their small sizes and the finding that many virus-encoded recombinant proteins are "user friendly" in the laboratory have resulted in intense biochemical and structural characterization of several chlorovirus-encoded proteins including a K^+ channel protein. This talk will focus on some of the very early events associated with PBCV-1 infection.

Workshop Sessions

VIR-WK213.02 - A deeply conserved transcription factor regulates the replication of Potato spindle tuber viroid RNA genome

Ying Wang[1], Jian Wu[1], Biao Ding[1]
[1]*The Ohio State University, Columbus, USA*

As single-stranded, circular, non-coding RNAs, viroids can invade host plants and co-opt cellular machineries to achieve replication and systemic spreading. Thus, viroids can serve as a unique model to study the interactions between infectious RNAs and hosts. Potato spindle tuber viroid (PSTVd), a type species of the family Pospiviroidae, encodes no proteins and presumably relies solely on host factors for infection, the mechanisms of which remains mostly unknown. Here, we identified a conserved transcription factor, Transcription Factor IIIA (TFIIIA), that is pivotal for PSTVd replication. Our analyses showed that TFIIIA from Nicotiana benthamiana (NbTFIIIA) interacted with PSTVd in vitro and in vivo. Down-regulated expression of NbTFIIIA led to reduced accumulation of PSTVd and over-expression of NbTFIIIA led to increased PSTVd accumulation in N. benthamiana. Together, our study uncovered the first host transcription factor that is essential for the replication of a viroid in Pospiviroidae.

Workshop Sessions

VIR-WK213.03 - New possible *Potyvirus* species infecting wild and cultivated plants in Iran

Alireza Golnaraghi[1], Taban S. Khosroshahi[1], Sara Yazdani-Khameneh[1], Anahita Hamedi[1], Marjan Diyanat[2], Kazusato Ohshima[3]

[1]Department of Plant Protection, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University (IAU), P.O. Box 14515-775, Tehran, Iran, [2]Department of Weed Science, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University (IAU), Tehran, Iran, [3]Laboratory of Plant Virology, Faculty of Agriculture, Saga University, Saga, Japan

The genus *potyvirus* is one of the largest virus genera, including about two hundred confirmed species and tentative species, and several new possible potyvirus species which are discovered annually. In the present work, surveys were conducted to determine potyviruses associated with mosaic, mottling and yellowing symptoms of cultivated and wild plants grown in different geographical regions in Iran. The potyvirus infection of the symptomatic plants was initially tested by the broad-spectrum reacting potyvirus antibodies (Bioreba, Switzerland). Potyvirus infections were confirmed by reverse-transcription polymerase chain reaction (RT-PCR) using previously described universal primers NIb2F (5'-GTITGYGTIGAYGAYTTYAAYAA-3') and NIb3R (5'-TCIACIACIGTIGAIGGYTGNCC-3'). The nucleotide sequences of 350-bp DNA amplicons derived from 24 samples were determined and compared with other sequences available in the GenBank using BLASTN analysis. Phylogenetic analysis, performed by the maximum-likelihood algorithm, was used to confirm the results of database searches. Potyviruses were unequivocally identified to the species level in 21 samples (identity scores of more than 95%). These viruses were *Bean common mosaic virus*, *Bean yellow mosaic virus*, *Lettuce mosaic virus*, *Potato virus Y*, *Turnip mosaic virus* and *Watermelon mosaic virus*. Potyvirus infections were also detected in samples Fa-Nar25, Lo-W19 and Th-W508 (obtained from *Narcissus tazetta*, *Tragopogon sp*. and *Brassica nigra*, respectively), but these samples could not be clearly assigned to species. The sequences of the three samples matched that of *Narcissus yellow stripe virus* (JQ395042), *Plum pox virus* (AJ243957) and *Turnip mosaic virus* (AB440239), but only with identity scores of 78%, 76% and 83%, respectively. The results seem to show the possible occurrence of new virus species or strains in Iran, and indicate that the universal primers are able to amplify a part of potyvirus genome from some recognized and unrecognized species of the *potyvirus* genus.

Workshop Sessions

VIR-WK213.04 - Investigation of the role of Argonaute proteins in Virus-induced gene silencing and recovery in Arabidopsis

Xiaofang Ma[1,2,3], Marie-Claude Nicole[1], Guoping Wang[2,3], Ni Hong[2,3], Peter Moffett[1]
[1]Département de biologie, Université de Sherbrooke, Sherbrooke, Canada, [2]National Key Laboratory of Agromicrobiology, Huazhong Agricultural University, Wuhan, China, [3]College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China

A major mechanism of antiviral immunity in plants is effected by RNA silencing which relies on the recognition and degradation of viral double-stranded RNA into virus-derived small RNA (vsRNA) by DICER-like (DCL) enzymes. Once incorporated into complexes containing members of the Argonaute (AGO) family of endonucleases, these vsRNA act as g uides to target viral RNA for degradation or inhibition of translation. Endogenous mRNAs can also be targeted by vsRNAs in a phenomenon known as virus-induced gene silencing (VIGS). The Arabidopsis genome encodes for ten AGO and four DCL protein, which play roles in multiple RNA silencing-related phenomena. We have infected single, double and triple ago and dcl mutants with Tobacco rattle virus carrying a fragment of the PDS gene (TRV-PDS), which induces a visible VIGS phenotype. We also infected the same mutants with TRV-GFP, which acts as a readout for the phenomenon known as “recovery”, wherein the plant is able to silence the expression of the viral genetic material. Our results suggest that RNA silencing may not be the only mechanism responsible for virus recovery as all Arabidopsis mutants tested in this study, including the triple dicer mutant dcl2/dcl3/dcl4, in which RNA silencing defense against viruses was purported to be abolished, underwent recovery from TRV-GFP. Polysome analysis suggests that TRV-GFP expression is at least partially repressed at the translational level in recovered plants. Genetic analysis of VIGS indicated that VIGS is compromised in the ago1 and dcl2/dcl3/dcl4 mutants, indicating important roles for these genes in VIGS of endogenous genes. Our results suggest that the regulation of viral RNA translation and accumulation by RNA silencing is controlled by different components than those which target endogenous genes mRNAs. Dissecting these different pathways will be the subject of future studies.

Workshop Sessions

VIR-WK213.05 - Cauliflower mosaic pararetrovirus infects symbiotic as well as free-living microalgae in nature

Karel Petrzik[1], Jan Vondrak[2], Jana Kviderova[3,4], Jaromir Lukavsky[3]
[1]Biology Centre AS CR, Ceske Budejovice, Czech Republic, [2]Institute of Botany AS CR, Pruhonice, Czech Republic, [3]Institute of Botany AS CR, Trebon, Czech Republic, [4]Centre for Polar Ecology, University of South Bohemia, Czech Republic

The lichen symbiotic alga Trebouxia aggregata (Archibald) Gärtner (phylum Chlorophyta, family Trebouxiaceae) has been identified as host of the well-known herbaceous plant virus Cauliflower mosaic virus (CaMV, family Caulimoviridae). The alga had been isolated from Xanthoria parietina lichen and maintained in a culture collection for more than 70 years. CaMV was detected in this collection entry and is now completely sequenced. Its genome is 8020 nt long and differs by 173 nt from the closest European CaMV-D/H isolate from cauliflower. No site under positive selection was found on the CaMV genome from T. aggregata. We therefore assume that the virus's presence in this alga did not last enough for fixation of specific changes in its genome. The virus from T. aggregata is mechanically transmissible to herbaceous host and induces disease symptoms there. Immunogold labeling analysis of ultrathin sections of T. aggregata detected CaMV only outside the central crenulate chloroplast. Apart from this symbiotic alga, CaMV capsid protein sequences were amplified from many other non symbiotic algae species maintained in a collection of autotrophic organisms, including Oonephris obesa, Elliptochloris sp., Microthamnion kuetzingianum, Chlorella vulgaris, and Pseudococcomyxa sp. CaMV-free Chlorella vulgaris, Pseudococcomyxa simplex and Micractinium pusillum strains were treated with purified CaMV to establish virus infection. We found the virus to multiply there and remain detectable even after five passages. The virus infection is morphologically symptomless on many algae and the photosynthesis activity is slightly decreased in comparison to CaMV-free alga culture. This is the first proof as to the natural presence of CaMV in algae and the first demonstration of artificial infection of algae with this virus. This work was supported with P501/12/1747 project of the Czech Science Foundation.

Workshop Sessions

VIR-WK213.06 - Potyvirus move systemically through xylem vessel as membrane associated structures

Juan Wan[1], Daniel Garcia[1], Romain Grangeon[1], Hojatollah Vali[2], Jean-Francois Laliberte[1]
[1]INRS-Institut Armand Frappier, Laval, Canada, [2]Facility for Electron Microscopy Reserch, McGill University, Montreal, Canada

RNA viruses remodel cellular membranes for their replication. Turnip mosaic virus (TuMV) is a positive-strand RNA virus belonging to the family potyviridae. TuMV infection induces the formation of at least two types of structures: a large perinuclear globular structure and small motile vesicles that are associated with the cortical endoplasmic reticulum (ER). These structures contain viral replication complexes and have been designated as viral factories. However, little is known about the finer details of these factories. In this study, Nicotiana benthamiana plants were infected by TuMV and systemically infected. Stem and leaf tissues were processed for confocal and electron microscopy, to investigate TuMV factories formation in different types of tissues. The leaf cross-sections showed that TuMV factories are found in all of the different leaf tissues. Transmission electron microscopy coupled with electron tomography showed that TuMV virions accumulate in the vacuole and acquire an envelope by hijacking the tonoplast. Moreover, longitudinal sections of stem showed that TuMV-induced structures were also present in xylem vessels, which contained viral RNA and lipids. Transmission electron microscopy showed the accumulation of membranous structures and virions in the xylem vessels. Sap collected from infected plants contained viral proteins and was infectious. To test whether xylem vessels, which are dead tissues, could be involved in TuMV systemic infection, we steam-treated a small section of the stem to induce cell death of phloem tissues. We observed systemic infection above the steam-treated stem section after 10 days. These data indicates that TuMV moves systemically in the plant through xylem vessels as a membrane-associated complex.

Workshop Sessions

VIR-WK213.07 - Host responses to infection by South African cassava mosaic virus: a transcriptomic and small RNA study

Chrissie Rey[1], Farhahna Allie[1], Erica Pierce[1], Sarah Rogans[1]
[1]*School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa*

Cassava mosaic disease is caused by several geminiviruses and is the major constraint on cassava production in sub-Saharan Africa and Asia. Plant host responses to viral pathogens, using genomic approaches, provides useful information that may be applied to control plant diseases. In this study, we exploited next generation sequencing (SOLiD or Illumina platforms) to explore unique and conserved transcript and miRNA population responses to the geminivirus, South African cassava mosaic virus (SACMV) in its natural perennial host, cassava (Manihot esculenta Crantz). Infection was monitored in T200, a susceptible landrace, at three time points post infection (12, 32 and 67 dpi). The multiplexed sequencing run produced a total of 523MB of paired-end reads for the SACMV infected T200 cDNA library. Of these, approximately 50.7 % of the reads mapped to the cassava reference genome available at phytozome. GOslim functional groups illustrated that differentially expressed genes in T200 were overrepresented in the cellular component category for defence, plasma membrane and nucleus. Analysis revealed that 4181 transcripts were differentially expressed (>2-fold changes at $p<0.05$) across all three time points. There was a positive correlation between the number of regulated genes at each time point during infection, the increase in viral titre and the severity of symptoms. Alterations were observed, amongst others, in the expression of defence-related genes, transcription factors, R genes, and metabolic pathways. Analysis of small RNA libraries identified alterations in cassava miRNAs, which included conserved and novel cassava-specific families, following infection. Differences were noted in miRNA levels and patterns between different time points during the infection period, and endogenous targets were predicted in the cassava genome for many miRNA families. Collectively, transcriptome and miRNA data demonstrates a network of altered pathways which can be used to construct an integrated multi-component model for host factors involved in host susceptibility to SACMV.

Workshop Sessions

VIR-WK213.08 - Studying the biogenesis and architecture of viral replication organelles with new probes for electron microscopy

Isabel Fernandez de Castro[1], Daniel Barajas[2], Peter D. Nagy[2], Cristina Risco[1]
[1]National Center of Biotechnology, Madrid, Spain, [2]University of Kentucky, Lexington, United States

Early in infection, viral polymerases and cofactors interact with cell membranes, where they build functional replication complexes (RC) in replication organelles. In our lab we are studying the biogenesis and structure of the replication organelles built by several RNA viruses such as Bunyaviruses, Rubella virus and the Tombusvirus Tomato Bushy Stunt Virus (TBSV). Previous works have demonstrated that the TBSV replication complex consist of the two viral replication proteins (p33 and p92) and 6–10 host proteins. p33 replication protein recruits the TBSV (+) RNA to the site of replication, which is the cytosolic surface of peroxisomal membranes. In this work we have used a clonable tag for electron microscopy based on the small metal-binding protein metallothionein (MT) and the method METTEM (Metal Tagging Transmission Electron Microscopy) to study the assembly of the TBSV replication organelle in the cell. Yeast strains expressing p33-His-MT tags were studied by METTEM. We detected p33 molecules in a complex system of endomembranes with spherules that connected with the endoplasmic reticulum and the plasma membrane. Thanks to the high sensitivity and resolution of protein localization with the MT clonable tag we confirmed that p33 molecules are associated with planar membranes where they do not seem to be active in replication; p33 also concentrated in characteristic spherules where they actively replicated the viral genome. In addition, we have obtained morphological evidences for the participation of the Endosomal Sorting Complex Required for Transport (ESCRT) proteins in the construction and maintenance of functional TBSV spherules and RC. Powerful imaging technologies such as correlative light and electron microscopy (CLEM), electron tomography and new probes for in situ molecular mapping without antibodies, are giving us a new way to visualize how individual macromolecules come together to build the viral RC.

Workshop Sessions

VIR-WK214.01 - Identification of the DDX21 Helicase as a novel host restriction factor against Influenza A viruses that is countered by the viral NS1 protein

Guifang Chen[1], Chien-Hung Liu[1], Ligang Zhou[1], Robert Krug[1]
[1]*Molecular Biosciences, Austin, USA*

A major goal of influenza A virus research is to identify specific host proteins that play critical roles in virus replication. Replication of influenza viral RNA is catalyzed by the three polymerase proteins (PA, PB1 and PB2) in association with the nucleoprotein (NP). Some viral polymerases are found in macromolecular complexes that also contain the viral NS1 protein bound to the 30kDa subunit of the cleavage and polyadenylation specificity factor (CPSF30). We purified these infected cell macromolecular complexes to identify host cell proteins that interact with viral protein(s) in these complexes, with the goal of determining the roles of these host-viral interactions. We show that the DDX21 RNA helicase is associated with these purified infected cell macromolecular complexes, and demonstrate that this constitutively expressed protein is a novel host restriction factor that inhibits influenza A virus replication by suppressing viral RNA synthesis and hence viral protein synthesis at early times after infection. DDX21 binds the PB1 protein and as a result inhibits assembly of the tripartite viral RNA polymerase. Further, we show that this antiviral activity is countered by the multifunctional viral NS1 protein, which binds DDX21 and displaces PB1 from DDX21. We identify the binding site for DDX21 on the NS1 protein, which is in the N-terminal RNA-binding domain, and demonstrate that a recombinant virus encoding a NS1 protein with a mutated DDX21 binding site does not counteract endogenous DDX21-induced inhibition of viral protein synthesis at later times of infection. Our results strongly support a novel mechanism by which the sequential interaction of PB1 and NS1 with endogenous DDX21 regulates viral gene expression in infected cells. In this mechanism influenza A virus, via the binding of DDX21 by the NS1 protein, transforms the DDX21 host restriction factor into a host regulator of viral gene expression.

Workshop Sessions

VIR-WK214.02 - Isolation and characterisation of the positive-sense replicative intermediate of a negative-strand RNA virus

Ashley York[1], Narin Hengrung[1,2], Frank Vreede[1], Juha Huiskonen[2], Ervin Fodor[1]
[1]Sir William Dunn School of Pathology, University of Oxford, Oxford, UK, [2]Division of Structural Biology, University of Oxford, Oxford, UK

Negative-strand RNA viruses include several major human and animal pathogens that cause substantial morbidity and deaths worldwide, as well as several important plant pathogens that are responsible for plant diseases with considerable economic impact. A defining feature of these viruses is that their single-stranded RNA genomes are of opposite polarity to messenger RNA and are replicated through a positive-sense intermediate. The replicative intermediate is thought to exist as a complementary ribonucleoprotein (cRNP) complex, however, the isolation of such complexes from infected cells has never been accomplished. To understand the overall molecular architecture of the influenza virus ribonucleoprotein complexes and the basic molecular requirements of influenza virus genome replication, we set out to isolate the replicative cRNP intermediate of a negative-strand RNA virus from infected cells for the first time. Recombinant influenza A viruses were generated by reverse genetics to contain an RNA tag which binds the Pseudomonas aeruginosa phage 7 (PP7) coat protein with nanomolar affinity, the interaction of which is exploited for the RNA-based affinity purification of the influenza A cRNP from infected cells with high specificity. This technological advance enabled the structural and functional characterisation of this elusive but essential component of the viral RNA replication machine. The cRNP exhibits a filamentous double-helical organisation with defined termini, containing the viral RNA-dependent RNA polymerase (RdRp) at one end and a loop structure at the other end. In vitro characterisation of cRNP activity yielded mechanistic insights into the workings of this RNA synthesis machine. In particular, we found that cRNPs show activity in vitro only in the presence of added RdRp. Intriguingly, a replication-inactive RdRp mutant was also able to activate cRNP-templated viral RNA synthesis. A model of influenza virus genome replication that relies on the trans-activation of the cRNP-associated RdRp is proposed.

Workshop Sessions

VIR-WK214.03 - Identification of adaptive mutations in the influenza A virus NS1 gene that increase cytoplasmic localization and differentially regulate host gene expression

Nicole Forbes[1,2], Mohammed Selman[1,2], Martin Pelchat[1], Jian Jun Jia[1], Alain Stintzi[1], Earl Brown[1]
[1]University of Ottawa, Ottawa, Canada, [2]Ottawa Hospital Research Institute, Ottawa, Canada

The NS1 protein of influenza A virus (IAV) is a multifunctional virulence factor. We have previously characterized gain-of- function mutations in the NS1 protein arising from the experimental adaptation of the human isolate A/Hong Kong/1/ 1968(H3N2) (HK) to the mouse. The majority of these mouse adapted NS1 mutations were demonstrated to increase virulence, viral fitness, and interferon antagonism, but differ in binding to the post-transcriptional processing factor cleavage and polyadenylation specificity factor 30 (CPSF30). Because nuclear trafficking is a major genetic determinant of influenza virus host adaptation, we assessed subcellular localization and host gene expression of NS1 adaptive mutations. Recombinant HK viruses with adaptive mutations in the NS1 gene were assessed for NS1 protein subcellular localization in mouse and human cells using confocal microscopy and cellular fractionation. In human cells the HK wild-type (HK-wt) virus NS1 protein partitioned equivalently between the cytoplasm and nucleus but was defective in cytoplasmic localization in mouse cells. Several adaptive mutations increased the proportion of NS1 in the cytoplasm of mouse cells with the greatest effects for mutations M106I and D125G. The host gene expression profile of the adaptive mutants was determined by microarray analysis of infected mouse cells to show either high or low extents of host-gene regulation (HGR or LGR) phenotypes. While host genes were predominantly down regulated for the HGR group of mutants (D2N, V23A, F103L, M106I+L98S, L98S, M106V, and M106V+M124I), the LGR phenotype mutants (D125G, M106I, V180A, V226I, and R227K) were characterized by a predominant up regulation of host genes. CPSF30 binding affinity of NS1 mutants did not predict effects on host gene expression. To our knowledge this is the first report of roles of adaptive NS1 mutations that impact intracellular localization and regulation of host gene expression.

Workshop Sessions

VIR-WK214.04 - The genome packaging mechanism of influenza B virus

Sumiho Nakatsu[1], Hiroshi Sagara[2], Takeshi Noda[1,3], Yoshihiro Kawaoka[1,4,5,6]
[1]Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Medical Proteomics Laboratory, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo, Japan, [3]PRESTO, Japan Science and Technology Agency, Saitama, Japan, [4]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, USA., [5]International Research Center for Infectious Diseases, Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [6]ERATO Infection-Induced Host Responses Project, Saitama, Japan

Like influenza A virus, influenza B virus primarily infects humans, causing annual epidemics and harming our health. The influenza B virus genome possesses eight segmented, single-stranded, negative-sense RNAs. Each viral RNA segment associates with viral nucleoproteins and polymerases, forming a ribonucloprotein (RNP) complex. When progeny virions bud from infected cells in the late stage of infection, they need to package eight kinds of RNPs to be infectious. Recently, we and others showed that influenza A virus selectively packages a complete set of eight RNPs. However, the genome packaging mechanism of influenza B virus remains unknown. Here, to elucidate the genome packaging mechanism of influenza B virus, we used electron microscopy to compare the RNPs in the virions of influenza A and B viruses in terms of (1) the lengths and diameters of the purified RNPs, (2) the number of the RNPs within the virions and (3) the spatial configuration of the RNPs within the virions. No significant difference was observed between the lengths and diameters of the two types of virus. In contrast, ultrathin sections of progeny virions revealed differences between the viruses with respect to the number of packaged RNPs within their virions. Specifically, electron tomography of progeny virions revealed that some influenza B virions packaged less than eight RNPs, whereas almost all influenza A virions packaged eight RNPs arranged in a specific pattern. These result suggest that, like influenza A virus, influenza B virus generally packages eight RNPs within its virions, but that some influenza B virions fail to package eight different RNPs, implying that the genome packaging mechanisms of these two types of influenza virus may differ slightly.

Workshop Sessions

VIR-WK214.05 - The eight facets of the influenza A virus cap-snatching process

Dorota Sikora[1], Lynda Rocheleau[1], Earl G. Brown[1], Martin Pelchat[1]
[1]University of Ottawa, Faculty of Medicine, Ottawa, Canada

Influenza A Virus (IAV) causes yearly epidemics and significant worldwide mortality. IAV has a segmented genome composed of eight single-stranded negative sense viral RNAs (vRNAs). At the earliest step of IAV replication, a complex formed by the viral RNA-dependent RNA polymerase (RdRp) and any of the vRNAs interacts with host RNAP II, cleaves the 5' end of host pre-mRNAs, and uses these capped RNA fragments as primers for viral mRNA synthesis, using a process called cap-snatching. Because the capped RNA fragments also contain 10-15 nucleotides downstream of the cap, sequence heterogeneity is found at the host-derived 5' ends of viral mRNAs. To investigate whether RNA selection occurs during cap-snatching, we performed high-throughput sequencing of the host primers found at the 5' ends of the eight viral mRNAs following 5'-RACE on IAV transcripts from human (A549) or mouse (M-1) cells infected with A/Hong Kong/1/1968 (H3N2). While the virus-encoded sequence is conserved, our results indicate that the host primers are divergent between the eight viral transcripts, and this in both human and mouse -derived samples. We observed noticeable differences in the length distributions, the nucleotides motifs and the identity of the host primers between the eight viral mRNAs. Interestingly, mapping the reads to known transcription start sites indicates that most of the host primers originate from genes associated with cell regulation processes. Because RdRp complexes containing any of eight vRNAs do not target the same host mRNAs, our results suggest negligible competition amongst vRNA templates during cap-snatching. Noteworthy, despite this, cap-snatching from all IAV transcripts target gene encoding proteins involved in common biological processes. By targeting genes that play key roles in cell cycle regulation, IAV could inhibit the first lines of host defense early after infection, which could lead to enhanced replication and pathogenesis.

Workshop Sessions

VIR-WK214.06 - Identification of host cellular proteases involved in Influenza A virus replication by RNAi screen

Mable WS Hagan[1,2], Charlene Ranadheera[1,2], Anders Leung[2], Michael Carpenter[1,3], Darwyn Kobasa[1,2]
[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada, [2]Special Pathogens Program, Public Health Agency of Canada, Winnipeg, Canada, [3]Section of Viral Diseases, Public Health Agency of Canada, Winnipeg, Canada

Activation of influenza virus hemagglutinin (HA) by host cellular proteases is essential for influenza infection. Recently, members of the type II transmembrane serine proteases (TTSPs) have been shown to cleave influenza HA in vitro. Furthermore, HA cleavage has been described to occur in different cellular compartments and at various points during the influenza replication cycle, suggesting the involvement of multiple proteases during influenza infection. The goal of this study is to identify other cellular proteases involved in the crucial HA activation step for seasonal and pandemic influenza viruses. Most cell lines used to culture influenza viruses do not express proteases that can proteolytically activate the viral HA and the in vitro culture of most influenza viruses is dependent on the addition of exogenous trypsin for HA activation. To facilitate the detection of proteases that are important in influenza virus growth, we utilized Caco-2 cells that promote trypsin-independent growth of seasonal (H1N1, H3N2) and pandemic (2009 H1N1, 1918 H1N1) influenza viruses. Furthermore, a seasonal H1N1 GFP-expressing influenza virus was generated and used as a tool to screen a human siRNA protease library. Initial screens identified several proteases that play a role in influenza biology, including TMPRSS2 as previously reported by others. Interestingly, validation of potential proteases by reduction in infectious viral titers, analysis of HA cleavage by Western blot, and the generation of stably knocked down cell lines revealed novel proteases needed for efficient influenza replication in processes other than HA activation. Therefore, cellular proteases may play an important role in the influenza life cycle in addition to HA activation. By identifying host proteases that are required for influenza replication, we hope to expand our knowledge of essential processes in influenza biology, and ultimately provide new targets for therapeutic intervention.

Workshop Sessions

VIR-WK214.07 - Identification of PB2 mutations responsible for the efficient replication of H5N1 influenza viruses in human lung epithelial cells

Reina Yamaji[1], Shinya Yamada[1], Mai Q Le[2], Mutsumi Ito[1], Yuko Sakai-Tagawa[1], Yoshihiro Kawaoka[1, 3, 4, 5]
[1]Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, [3]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, USA, [4]ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, [5]Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Highly pathogenic H5N1 avian influenza viruses have caused outbreaks among poultry worldwide, resulting in sporadic infection of humans with a mortality rate of about 60%. However, the efficient transmission of H5N1 viruses among humans has yet to occur, suggesting that further adaptation is required for H5N1 viruses to transmit efficiently among humans. The viral determinants for efficient replication in humans remain poorly understood. Here, we report that introduction of PB2 from an H5N1 influenza virus isolated from humans in Vietnam (A/Vietnam/36285/2010) into an avian H5N1 virus (A/wild bird/Anhui/82/2005) increased the ability of that virus to grow in human lung epithelial cells. We identified amino acid residues at positions 249, 309, and 339 of the PB2 from A/Vietnam/36285/2010 as responsible for the efficient replication. Our finding indicates that H5N1 viruses that acquire these mutations may adapt well in human cells. Moreover, these amino acids changes in PB2 could serve as important molecular markers for assessing the pandemic potential of H5N1 field isolates.

Workshop Sessions

VIR-WK215.01 - Premature triggering of an enveloped virus fusion protein as a new mechanism of antibody neutralization

Paul Young[1], Jie Li[2], Daniel Watterson[1], Daniel Ericsson[1], Matthew Cooper[1], Xiao-Yan Che[2], Bostjan Kobe[1]
[1]University of Queensland, Brisbane, Australia, [2]Southern Medical University, Guangzhou, China

Antibody-mediated neutralization of virus infectivity plays a key role in host defence and protection against re-infection, and is an essential component of effective vaccine strategies. However, the structural basis of the different mechanisms by which neutralization occurs is poorly understood. We have generated a panel of mouse monoclonal antibodies (mAbs) to the dengue virus (DENV) envelope (E) protein domain III (EDIII), and have performed structural and functional characterization of two that neutralize all four DENV serotypes (3E31 and 2D73). The target of these antibodies, the dengue virus E protein, is responsible for host-cell receptor binding, as well as cellular entry following low pH-triggered conformational changes that drive membrane fusion. We have experimentally demonstrated that one of these cross-reactive neutralizing antibodies, 2D73, can trigger premature fusion in a pH-independent manner, while the other, 3E31, inhibits fusion. The two mAbs recognize overlapping but distinct epitopes and their structural characterization provides insight into a new mechanism of enveloped virus neutralization via premature triggering of the fusion protein. These findings lay the foundation for new strategies for vaccine and therapeutics development

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Tuesday, 29 July 2014 **14:50 - 15:03 Room 511 D**

Workshop Sessions

VIR-WK215.02 - The variable region of the 3' untranslated region is a critical virulence factor in the Far-Eastern subtype of tick-borne encephalitis virus in mouse model

Mizuki Sakai[1], Kentaro Yoshii[1], Yuji Sunden[1], Kana Yokozawa[1], Minato Hirano[1], Hiroaki Kariwa[1]
[1]Graduate School of Veterinary Medicine, Hokkaido University, Sapparo, Japan

Tick-borne encephalitis virus (TBEV) is a major arbovirus that causes thousands of cases of severe neurological illness in humans annually. However, virulence factors and pathological mechanisms of TBEV remain largely unknown. To identify the virulence factors, we constructed chimeric viruses between two TBEV strains of the Far-Eastern subtype, Sofjin-HO (highly pathogenic) and Oshima 5-10 (low pathogenic).The replacement of the coding region for the structural and non-structural proteins from Sofjin into Oshima showed a partial increase of the viral pathogenicity in a mouse model. Oshima-based chimeric viruses with the variable region of the 3' untranslated region (3'-UTR) of Sofjin, which had a deletion of 207 nucleotides, killed 100% of mice and showed almost same virulence with Sofjin. Replacement of the variable region of 3'-UTR from Sofjin into Oshima did not increase viral multiplication in cultured cell and a mouse model at the early phase of viral entry into the brain. At the terminal phase of viral infection in mice, the virus titer of the Oshima-based chimeric virus with the variable region of the 3'-UTR of Sofjin reached a level identical to that of Sofjin, and showed a similar histopathological change in the brain tissue. This is the first report to show that the variable region of the 3'-UTR is a critical virulence factor in mice. These findings encourage further study to understand the mechanisms of the pathogenicity of TBEV and develop preventative and therapeutic strategies for TBE.

Workshop Sessions

VIR-WK215.03 - Nutlin-3 (a MDM2 antagonist) could inhibit reproduction of classical swine fever virus in vitro

Jieyuan Jiang[1], Leilei Yang[1], Wenliao Li[1], Li Mao[1]
[1]IInstitute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Nanjing, China

MDM2 binds the p53 tumor suppressor protein with high affinity and negatively modulates its transcriptional activity and stability. Nutlin-3 is a MDM2 antagonist, a p53 activator and an apoptosis inducer. Inhibition of the MDM-p53 interaction results in the stabilization of p53, cell cycle arrest and apoptosis. Classical swine fever virus is one number of pestivirus that is single sense RNA virus and cause serious death infections of swine. Here, nultin-3 was added to the ST cell monolayers for 2 hours before CSFVs infected the cells. The total RNA was extracted from all of the experiments that includes: Group A was normal control (no nultin-3, and no infection); Group B was virus infection, without nultin-3 addition; C added with nultin-3, without virus infection; and D with nultin-3 added and CSFV infection. The Real-time RT-PCR exanimation showed that the viral RNA in Group D was significantly reduced compared to the group C in the experiments and as following: -△△Ct was -0.235+/10.049, -2.4775+/-0.484, -6.0175+/-0.929, -8.3425+/-0.732 at 12hr, 24hr, 48hr and 72hr, respectively). Determined with Western blotting, In the Nultin-3 treated groups, p53 proteins were increased significantly compared to the groups without nultin-3 addition. It showed that nultin-3 indeed positively inhibited MDM2 functions and increased p53 protein levels in the cells, and the increased p53 protein levels could obviously inhibit the CSFV replication and decreased CSFV titers in the cell culture.

Workshop Sessions

VIR-WK215.04 - Infection enhancement mediated by homologous antibodies targeting the receptor-binding domain of the dengue virus envelope glycoprotein

Jaime Henrique Amorim[1], Denicar Fabris[1], Rúbens Alves[1], André Silva[1], Margareth Capurro[1], Luís Carlos S. Ferreira[1]

[1]*University of São Paulo, São Paulo, Brazil*

So far, neutralizing antibodies targeting the envelope glycoprotein of dengue virus (DENV) have represented the major goal of most vaccine formulations. In particular, antibodies capable of binding the receptor-binding domain III of the envelope protein (EDIII) have been considered a premier target for anti-DENV vaccines. In the present report, we show that EDIII-specific antibodies capable of neutralizing DENV2 in vitro do not confer protective immunity. Otherwise, EDIII-specific antibodies promoted homologous antibody-dependent enhancement by vectorizing virus particles to Fc-γ- receptor bearing cells, as demonstrated with animals immunized with purified recombinant EDIII, inactivated virus particles, and with human convalescent serum samples. In this presentation we will give insights into the mechanisms of antibody-mediated infectivity enhancement as well as protective immunity and offer a basis for more rational approaches aiming the development of effective dengue vaccines.

Workshop Sessions

VIR-WK215.05 - Silencing of neurotropic flavivirus replication in the central nervous system by microRNA-targeting

Alexander Pletnev[1], Natalya Teterina[1], Olga Maximova[1]
[1]*Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, USA*

In recent years, microRNA-targeting has become an effective strategy for selective control of tissue-tropism and pathogenesis of both DNA and RNA viruses. The success in silencing of viral replication in specific tissue or cells depends on the ability of the virus to escape the miRNA-mediated suppression by generating deletions or mutations within the miRNA-targets. Here, using neurotropic mosquito- or tick-borne flavivirus as a model, we demonstrate that simultaneous miRNA targeting of the viral genome in the open reading frame and 3'-noncoding regions for brain-expressed miRNAs had an additive effect and produced a more potent neurovirulence attenuation of the virus compared to separate targeting of those regions. Multiple miRNA co-targeting of the viral genome within these two distantly located regions completely abolished the neurotropism as no viral replication was detected in the developing central nervous system of neonatal mice infected directly into the brain. Furthermore, no viral antigens were detected in neurons, and neuronal integrity in the brain of mice was well preserved. These findings offer a new general strategy for successful attenuation of neurotropic viruses. This miRNA co-targeting approach can be adapted for other viruses in order to minimize their replication in a cell- or tissue-type specific manner, but most importantly, to prevent virus escape from miRNA-mediated silencing.

Workshop Sessions

VIR-WK215.06 - Japanese encephalitis virus NS1' protein increases virus production and viral fitness in avian cells

Yuki Takamatsu[1], Kenta Okamoto[1], Dhin Tuan Duc[1], Fuxun Yu[1], Daisuke Hayasaka[1], Leo Uchida[1], Takeshi Nabeshima[1], Corazon C. Buerano[1], Kouichi Morita[1]

[1]*Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan*

Japanese encephalitis virus (JEV), which belongs to the genus Flavivirus of the family Flaviviridae, is a leading cause of meningo-encephalitis in Asian countries. The flavivirus non-structural protein 1 (NS1) plays a role in virus replication and in the elicitation of immune response. The NS1' protein found among the members of the JEV subgroup is an extended form of NS1 and is generated by a -1 ribosomal frameshift. This protein is known to be involved in viral pathogenicity, however, its specific function is still unknown. Here, we describe an investigation on the molecular function of NS1' protein through the production of JEV NS1' expressing and non-expressing clones and their infection in avian and mammalian cells. Efficient NS1' protein expression was observed in avian cells and was found to increase JEV production in both avian cultured cells and embryonated chicken eggs. NS1' protein also increased viral fitness in avian cells. NS1' protein was observed to co-localize with NS5 protein and resulted in enhanced JEV RNA replication in avian cells. These findings clearly indicate that NS1' enhances the production of JEV in avian cells and may facilitate the amplifying/maintenance role of birds in the virus transmission cycle in nature.

Workshop Sessions

VIR-WK215.07 - Upregulation of BiP/GRP78 that enhances XBP1 mRNA splicing during the unfolded protein responses in C6/36 cells with dengue 2 virus infection

I-Hsuan Chiang[1], Tien-Huang Chen[2], Wei-June Chen[1]
[1]Chang Gung University, Kwei-San, Taiwan, [2]Centers for Disease Control, Taipei, Taiwan

The dengue virus (DENV) is one of some 70 members in the family Flaviviridae; which causes dengue fever and occasionally dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). In turn, DENV essentially propagates alternating in mosquito and mammalian cells during the replication cycle in nature. Nevertheless, the fate in association with DENV infection apparently differs between the cell types. DENV-infected mammalian cells mostly end up with apoptosis mediated by the ER stress while deleterious effects are usually trivial in mosquito cells. In spite, DENV- infected C6/36 cells appear the ER-stress that induces the unfolded protein responses (UPR); moreover, chaperones such as BiP/GRP78 are upregulated. Very importantly, the present presented that upregulated BiP/GRP78 is involved in assistance of XBP1 splicing in mosquito cells under the the ER stress induced by DENV infection. The spliced form of XBP1 (sXBP1) normally functions to activate transcriptions to express downstream genes. Protein disulfide isomerase (PDI) is supposed to be the one that is involved in the reduction of disulfides during the disposal of misfolded proteins by ER-associated degradation (ERAD). As presented in this study, viral protein E, but not protein C, was clearly in association with the amount of BiP/GRP78 in C6/36 cells. Presumably, misfolded viral protein E that contains 6 disulfide bonds was processed and transported to the cytosol for degradation hypothetically via PDI-mediated retro-translocation pathway. Taken together, BiP/Grp78 is uregulated in DENV-infected C6/36 cells, playing as a regulator of XBP1 mRNA splicing and its target gene PDI. This event contributes to quality control of proteins, particularly viral protein E, leading to appropriate load of viral proteins and assembled virions in mosquito cells in which the induced ER stress is usually lower.

Workshop Sessions

VIR-WK215.08 - Investigating the origin of an emerging virulent strain of West Nile virus in Australia

Natalie Prow[1], Judith Edmonds[1], Yin Setoh[1], Helle Bielefeldt-Ohmann[2], Willy Suen[2], Andrew van den Hurk[3], Cheryl Johansen[4], Peter Kirkland[5], Alexander Khromykh[1], Roy Hall[1]

[1]School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Australia, [2]School of Veterinary Science, The University of Queensland, Gatton, Australia, [3]Public Health Virology, Queensland Health and Forensic and Scientific Services, Coopers Plains, Australia, [4]School of Pathology and Laboratory Medicine, The University of Western Australia, Perth, Australia, [5]Elizabeth Macarthur Agriculture Institute, Menangle, Australia

The mosquito-borne West Nile virus (WNV) is responsible for outbreaks of viral encephalitis in humans and horses with particularly virulent strains causing recent outbreaks in Eastern Europe, the Middle East and North America. In Australia, a strain of WNV, Kunjin (WNVKUN), is endemic in Northern Australia and infection with this virus is generally asymptomatic. However in early 2011, following extensive flooding, an unprecedented outbreak of encephalitis in horses occurred in south-eastern Australia, resulting in more than 1,000 cases and a mortality rate of 10-15%. A WNV-like virus (WNVNSW2011) was isolated and is most closely related to the indigenous WNVKUN. However, at least two amino acid changes associated with increased virulence were present in the WNVNSW2011 sequence. To investigate the origins of WNVNSW2011, we analysed a panel of WNVKUN strains isolated from different regions of Australia between 1960 and 2012, comparing their neuroinvasiveness in established mouse models and their genetic sequence for the presence of known virulence markers for WNV. Some strains of WNVKUN from eastern Australia exhibited similar levels of virulence to WNVNSW2011, including a strain of WNVKUN isolated in 2012. In contrast, recent isolates of WNVKUN from WA were relatively attenuated. These data suggest that virulent strains of WNVKUN are circulating in Eastern Australia and therefore the risk of future outbreaks due to WNVKUN still remain. Genetic analysis identified two possible virulence and/or evolutionary markers in the 3'UTR and NS5 gene. Putative virulent motifs will be incorporated into existing infectious clones to understand mechanisms of disease development. These data will provide valuable information about viral factors leading to the emergence of virulent strains of WNV.

Workshop Sessions

VIR-WK216.01 - A Unique SUMO-2-interacting Motif within LANA is essential for KSHV Latency

QIliang Cai[1], Erle Robertson[2]
[1]Fudan University, Shanghai, China, [2]University of Pennsylvania, Philadelphia, USA

Kaposi's sarcoma-associated herpesvirus (KSHV) stabilizes hypoxia-inducible factor alpha (HIF-1alpha) during latent infection, and HIF-1alpha reactivates lytic replication under hypoxic stress. However, the mechanism utilized by KSHV to block lytic reactivation with the accumulation of HIF-1alpha in latency remains unclear. Here, we report that LANA encoded by KSHV contains a unique SUMO-interacting motif (LANASIM) which is specific for interaction with poly-SUMO-2 and facilitates LANA UMOylation at lysine 1140. Proteomic and co-immunoprecipitation analysis further reveal that the poly-SUMO2 modified transcription repressor KAP1 is a critical factor recruited by LANA-SIM. Deletion of LANA-SIM led to functional loss of both LANA-mediated viral episome maintenance and lytic gene silencing. Moreover, hypoxia reduced KAP1 SUMOylation and resulted in Sin3A dissociation from LANA-SIM-associated complex. RNA interference-based knockdown of KAP1 or Sin3A in KSHV-infected PEL cells disrupts viral episome stability and increases the efficiency of hypoxia-induced lytic reactivation. Additionally, RBP-Jkappa enhances LANA-SIM-mediated inhibitory function on the HIF-1alpha-responsive element within RTA promoter. Therefore, the LANA-SIM motif plays an essential role in KSHV latency and is a potential drug target against KSHV-associated cancers.

Workshop Sessions

VIR-WK216.02 - Evidence for Immuomodulatory function in the first full genome assembly of Equine herpesvirus 5

Zelalem H Mekuria[1], Paola K. Vaz[1], James R Gilkerson[1], Phillip Markham[1], John Markham[1], Sangwon Lee[1], Carol Hartley[1]
[1]*University of Melbourne, Faculty of Veterinary Science, Melbourne, Australia*

Infection of the horse by equine gammaherpesviruses -2 and- 5 (EHV-2 and EHV-5) is common throughout the world, EHV-5 is commonly found in respiratory samples from clinically normal as well as sick horses, however recently it has been associated with equine multinodular pulmonary fibrosis (EMPF). Partial sequence information for EHV-5 DNA polymerase gene and full gene sequence of gB, gH and DNA terminase genes is available in public data base. This study describes the first full genome sequence of EHV-5 assembled using illumina platform. EHV-5 isolate 2-141 was propagated on primary equine fetal kidney cells, purified before the genomic DNA was extracted. Reads assembled Denovo to give EHV-5 consensus (Geneious 6.1.2 Java version 1.6.0_ 35-b10 (64 bit)). Ab initio gene prediction and annotation performed by Geneious, BLASTn, BLASTx and tBLASTx algorithm. Final assembly yielded a 170 kbp sequences with an average G+C content of 50% throughout with some fluctuation in short repeat regions. Eighty four ORFs were annotated; including sixty four EHV-2 homologues and twenty seven HSV homologues. Eighteen ORFs were unique to EHV-5, some with potential immunomodulatory functions. EHV-5 ORF eg 2 and ORF eg 10 assigned with a putative viroceptor and apoptosis regulator function. Homologues of at least six other ORFs were also detected and identified with immunomodulatory effects during herpesviral infection. ORF 74 found as exclusive homologue to HVS genome whereas ORF E7 is a homologue from EBV genome with putative viroceptor role. In conclusion, the presence of host derived immunomodulatory putative genes may suggest a possible role for disease development by compromising host immunity or as co-factors for other organisms.

Workshop Sessions

VIR-WK216.03 - The anterior commissure is a pathway for contralateral spread of herpes simplex virus type 1 (HSV-1) after olfactory tract infection

Charlotta Eriksson[1], Eva Jennische[2], Stefan Lange[1], Edward Trybala[1], Tomas Bergström[1]
[1]Department of Infectious Diseases, University of Gothenburg, Gothenburg, Sweden, [2]Dept. of Medical Biochemistry & Cell Biology, University of Gothenburg, Sweden

Herpes simplex encephalitis (HSE), mainly induced by HSV-1, is the most common cause of sporadic viral encephalitis in the Western world and can occur both through primary and recurrent infections. Previous discussions whether infection resulting in HSE occurs via the olfactory or the trigeminal pathways in humans are not settled. Furthermore, studies of the viral spread between the two brain hemispheres are scarce also in animal systems, but such a spread might be of relevance to the location and development of clinical manifestations of HSE. We have developed a rodent model in which we have investigated the viral spread of HSV-1 in the central nervous system (CNS) of rats after intranasal instillation of virus in the right nostril. Rats were sacrificed after 1-6 days post-infection and tissues were analyzed by immunohistochemistry, and viral DNA load was quantified by TaqMan PCR. In this model HSV-1 penetrated lamina cribrosa to infect the mitral cells of the olfactory bulb on the right side only. Interestingly we found that the anterior commissure, a bundle of nerve fibers extending between the two brain hemispheres, contained clusters of HSV-1 positive cells in oligodendrocytes. The anterior commissure appeared to convey a rapid transmission of virus between the right and the left olfactory bulb. As the viral spread between the right and left trigeminal ganglia did not involve a similar rapid transmission but was instead associated with a delay, the anterior commissure appears to act as contralateral shortcut for transmission of HSV-1 between the olfactory bulbs and probably also between the olfactory cortices. Earlier studies have indicated an affinity of HSV-1 for the limbic system of the brain, based on MR findings and clinical manifestations in HSE, and our results indicate that the anterior commissure may assist in viral spread to these locations.

Workshop Sessions

VIR-WK216.04 - Mutation of the herpes simplex virus 1 gene UL24 impedes the spread of infection from corneal epithelial cells to neurons

Pierre-Alexandre Rochette[1], Amélie Bourget[2], Angela Pearson[1]
[1]INRS-IAF, Laval, Canada, [2]IRCM, Montréal, Canada

Herpes simplex virus 1 (HSV-1) is a neurotropic virus that, following infection of epithelial cells in the mucosa, goes on to infect and then persist in a latent state in trigeminal ganglia (TG). UL24 is a non-essential core herpesvirus gene. In a mouse model of ocular infection, a UL24-deficient virus (UL24X) exhibits a 10-fold reduction of viral titers in tear films, but a 4 log10 reduction of viral titers in TG. The goal of this study was to understand how UL24 affects viral titers in this neural tissue. We found that compared to KOS, UL24X exhibited a 5- to 10-fold reduction of viral yield in both a neuronal cell line and in primary neurons, which is similar to what is observed in epithelial-derived cell lines. Furthermore, by testing the impact of varying the amount of virus in the inocula, we determined that the 10-fold reduction of viral load in the eye observed in absence of UL24 was not sufficient to explain the 4 log10 reduction of viral titers in the TG. To test whether this reduction in the number of infectious viral particles reflected a reduction in the total number of acutely infected neurons, an in situ analysis of TG harvested from infected mice was conducted. Upon immunofluorescence staining of histological sections for viral proteins, we found that the average number of infected neurons detected per section of TG from UL24X-infected mice compared to TG from KOS-infected mice was significantly reduced. Moreover, infected neurons were detected in only 50% of the TG harvested from UL24X-infected mice, in contrast to 100% of TG for KOS-infected animals. Our results suggest that UL24 is important for the efficient dissemination of HSV-1 infection from the mucosa to the TG, and that UL24 promotes the productive infection of neurons in vivo.

Workshop Sessions

VIR-WK216.05 - Comparative functional analyses of herpesvirus regulatory proteins

Roger Everett[1]
[1]University of Glasgow, Glasgow, UK

After initial primary infection, herpesviruses establish latent infections that ensure that they are carried by infected individuals for life. Periodic episodes of reactivation of lytic infection enable transmission of the viruses amongst the population. The mechanisms that govern the balance between lytic and latent infection involve many factors, including cellular proteins that repress viral gene expression and viral proteins that overcome this repression. One group of inhibitory cellular factors includes constitutively expressed components of cellular structures known as PML nuclear bodies (PML NBs). Different herpesviruses express regulatory proteins that, despite being apparently unrelated, counteract the repressive effects of one or more PML NB components. This talk will summarise recent work on comparative analyses of several of these viral proteins, illustrating their functional interchangeability despite lack of sequence conservation and diverse mechanisms of action. The fact that essentially different viral proteins target the same group of cellular proteins with similar functional consequences strengthens the hypothesis that PML NB components are important factors in regulating the balance between lytic and latent herpesvirus infections.

Workshop Sessions

VIR-WK216.06 - Contribution of MEF2 family transcription factors to BZLF1 expression in EBV reactivation from latency

Takayuki Murata[1,2], Hiroshi Kimura[1], Tatsuya Tsurumi[2]
[1]Nagoya University, Nagoya, Japan, [2]Aichi Cancer Center, Nagoya, Japan

Reactivation of Epstein-Barr virus (EBV) from latency is dependent on expression of the viral transactivator BZLF1 protein, whose promoter (Zp) normally exhibits only low basal activity but is activated in response to chemical or biological inducers. Using a reporter assay system, we screened for factors that can activate Zp and isolated genes, including those encoding MEF2B, KLF4, and some cellular b-Zip family transcription factors. After confirming their importance and functional binding sites in reporter assays, we prepared recombinant EBV-BAC, in which the binding sites were mutated. Interestingly, the MEF2 mutant virus produced very low levels of BRLF1, another transactivator of EBV, in addition to BZLF1 in HEK293 cells. The virus failed to induce a subset of early genes, such as that encoding BALF5, upon lytic induction, and accordingly, could not replicate to produce progeny viruses in HEK293 cells, but this restriction could be completely lifted by exogenous supply of BRLF1, together with BZLF1. In B cells, induction of BZLF1 by chemical inducers was inhibited by point mutations in the ZII or the three SP1/KLF binding sites of EBV-BAC Zp, while leaky BZLF1 expression was less affected. Mutation of MEF2 sites severely impaired both spontaneous and induced expression of not only BZLF1, but also BRLF1 in comparison to wild-type or revertant virus cases. We also observed that MEF2 mutant EBV featured relatively high repressive histone methylation, such as H3K27me3, but CpG DNA methylation levels were comparable around Zp and the BRLF1 promoter (Rp). These findings shed light on BZLF1 expression and EBV reactivation from latency.

Workshop Sessions

VIR-WK216.07 - High-throughput sequencing of complete human cytomegalovirus genomes directly from clinical material reveals the common occurrence of mutants

Gavin Wilkie[1], Marylouisa Holton[1], Tina Ganzenmueller[2], Karen Kerr[1], Thomas Schulz[2], Maurizio Zavattoni[3], Andrew Davison[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK, [2]Institute of Virology, Hannover Medical School, Hannover, Germany, [3]Molecular Virology Unit, Virology and Microbiology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Human cytomegalovirus (HCMV) is the leading infectious cause of abnormalities in newborn babies and a serious risk to people with weakened immune systems. Our long-term objective is to discover whether particular viral alleles or combinations thereof in single or mixed strain infections contribute to the outcome of infection. We are approaching this by determining complete HCMV genome sequences (236 kbp) directly from clinical samples. Standard Illumina methods require relatively large amounts of DNA, but HCMV-infected clinical material typically contains very limited quantities, of which only a tiny proportion is viral. We have successfully combined modified library preparation techniques with target enrichment technology in order to sequence HCMV genomes from nanogram quantities of clinical material, thereby avoiding any need to grow the virus in cell culture or amplify viral DNA by error-prone PCR. Many samples may be analysed on a single Illumina MiSeq run, making the process both fast and cost-effective. We have also developed a bioinformatics pipeline to facilitate rapid reconstruction of complete genome sequences and assess variation. Our results thus far show that clinical HCMV strains, including those present in the amniotic fluid of infected foetuses, frequently bear potentially deleterious mutations in protein-coding regions. These mutations are evident as frameshifts caused by small deletions or insertions or as premature stop codons caused by single nucleotide substitutions. Members of the RL11 and US6 gene families are prominent among the range of genes affected. The occurrence of identical mutations in specimens from patients in different countries suggests that some of these mutants are circulating in human populations. Our observations indicate that the full range of HCMV gene functions is not required for the infectious cycle, at least under certain conditions, and that we may be observing in a human virus the natural operation of evolutionary pathways leading to gene loss.

Workshop Sessions

VIR-WK216.08 - Role of human Herpesvirus 6 U94 protein in viral genome integration within host chromosomal telomeric regions

Frédéric Trempe[1], Guillaume Morissette[1], Annie Gravel[1], Nina Wallaschek[2], Benedikt Kaufer[2], Louis Flamand[1]
[1]Université Laval, Québec, Canada, [2]Institute for Virology, Berlin, Germany

Objective HHV-6 is unique among human herpesviruses by its ability to integrate human chromosomes. The mechanism by which HHV-6 integrates its genome into the telomeric regions of human chromosomes remains undefined. Our objective was to determine whether the U94 protein possesses enzymatic and biological properties (such as DNA-binding, ATPase, helicase and exonuclease) expected from a protein playing a role in viral integration within the telomeres. Methods Recombinant MBP-U94 fusion proteins were expressed and purified. The following assays were conducted to determine the biological activities of MBP-U94 proteins; single (ss) and double-stranded (ds) DNA binding properties to telomeric repeat sequences (TRS) using gel shift assays and surface plasmon resonance (SPR) assays; the ability to hydrolyze ATP measured by HPLC; helicase and exonuclease assays using a variety of DNA substrates and analysis by gel electrophoresis. Results Our gel shift experiments indicate that U94 is capable of binding both dsDNA and ssDNA telomeric motifs (CCCTAA or TTAGGG). U94 binding to DNA could be competed with heterologous DNA suggesting non-specific affinity for a particular DNA sequence. However, using SPR we could demonstrate that U94 preferentially binds ssDNA CCCTAA motif and dsDNA TRS motifs over non-telomeric DNA. ATPase activity. U94 hydrolyzes ATP into ADP while the conversion to ADP into AMP is minimal. The U94 ATPase activity is independent of the presence of DNA. Exonuclease activity. U94 displays 3’ to 5’ exonuclease activity on dsDNA with a preference for 3’-recessed ends. This activity is independent of ATP but requires Mg^{2+}. Helicase activity. Helicase activity is currently being determined using a variety of DNA substrates. Conclusion The preferential DNA binding of U94 to TRS-related sequences, its ability to hydrolyze ATP and the fact that U94 possesses exonuclease activity are consistent with a role in HHV-6 genome integration in the telomeric region of human chromosomes.

Workshop Sessions

VIR-WK217.01 - Pathogenic mechanisms of emerging coronaviruses

Ralph Baric[1]
[1]University of North Carolina, Chapel Hill, USA

Coronaviruses are important emerging pathogens of mammals, birds and other species, as evidenced by the recent emergence of severe acute respiratory coronavirus (SARS-CoV), Middle East respiratory coronavirus (MERS-CoV) and porcine epidemic diarrhea virus (PEDV disease outbreaks in various locations throughout the world. .Using molecular, genetic and biochemical approaches, we investigate the common and unique mechanisms regulating coronavirus cross species transmission both in vitro and in vivo. In parallel, we use synthetic genome techniques to reconstruct select emerging animal and human coronaviruses and evaluate their cross species transmission and pandemic potential in humans and other species. To understand the mechanisms that regulate emerging coronavirus virulence potential, we apply a variety of systems biology and systems genetic platforms to identify host susceptibility alleles that regulate disease outcomes following MERS-CoV and SARS-CoV infection in in bred and outbred animal models of human disease. Our data supports a model of high pandemic disease potential for future emerging coronavirus disease outbreaks in human and animal populations.

Workshop Sessions

VIR-WK217.02 - Characterization of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) spike protein cleavage sites: role of the ubiquitously expressed protease furin during viral entry

Jean Millet[1], Gary Whittaker[1]
[1]Cornell University, Ithaca, USA

Middle East respiratory syndrome coronavirus (MERS-CoV) is a newly identified coronavirus causing high morbidity and mortality in humans. Many enveloped viruses, including coronaviruses, are activated for infection by proteolytic processing of their envelope or spike protein. Modulation of spike protein cleavage activation can have profound effects on viral pathogenesis. We have uncovered distinctive characteristics of MERS-CoV spike (S) cleavage site(s) that distinguish it from its closest relatives, as well as most other coronaviruses. We identify, by bioinformatics and peptide cleavage assays, two potential cleavage sites for furin, a ubiquitously expressed protease, located at the S1/S2 interface and at the S2' positions of S. Western blot cleavage and mutational analyses confirmed that the two identified sites could be proteolytically processed by furin, with the S1/S2 site cleaved during biosynthesis of S, and the S2' site cleaved after virus assembly. Use of MERS-CoV pseudoparticles, as well as infectious MERS-CoV, indicated a role for furin-mediated activation during viral entry. MERS-CoV entry was highly enhanced by overexpression of furin in target cells. Conversely, MERS-CoV entry was reduced by treating target cells with furin inhibitor I or siRNA silencing, and furin activity appeared to partially override the low pH-dependent nature of MERS-CoV entry, indicating a role for furin in endosomal compartments. Overall, we show that MERS-CoV has an unusual priming step for fusion activation suggestive of a role during the process of emergence into the human population. The ability of MERS-CoV to utilize furin in this manner, in addition to other reported proteases, may explain the polytropic nature of the virus.

Workshop Sessions

VIR-WK217.03 - The accessory proteins ns2 and ns5/ns12.9 of a neurotropic human coronavirus are dispensable for viral replication in cell culture but play a significant role in neuropathogenesis in vivo

Marc Desforges[1], Louis-Charles Levros Jr[2], Jessica Desjardins[1], Hélène Jacomy[1], Éric Rassart[2], Pierre J. Talbot[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada, [2]Université du Québec à Montréal, Montreal, Canada

Human coronaviruses (HCoV) are respiratory pathogens causing common colds, but also more severe pathologies like pneumonia and respiratory distress syndrome. Accumulating experimental evidence indicate that HCoV infect the mouse CNS and may also infect and persist in the human CNS, suggesting an association with neurological diseases. The HCoV RNA genome has 4 or 5 genes that encode structural proteins, including S and HE, two important virulence factors acting in viral attachment, entry and cell tropism, as well as several genes encoding non-structural accessory proteins. Even though accessory proteins of different coronaviruses have been attributed roles in cellular stress response and pathogenesis, their precise function remains poorly understood for most HCoV strains. Making use of a cDNA infectious clone (pBAC-OC43FL), we show that the accessory proteins ns2 and ns5 of the HCoV-OC43 strain are dispensable for viral replication in cell culture while being important factors during infection of the murine CNS. Indeed, a recombinant virus without the gene encoding the ns2 protein induced a mouse strain-specific transient increase in survival compared to wild-type virus. On the other hand, a recombinant virus deleted for the ns5 protein-encoding gene was completely devoid of its neurovirulent potential, which correlated with a significant decrease in replication and spread. Furthermore, our results suggest a new unexpected function for the ns2 protein as it transactivated the apolipoprotein D (ApoD) promoter and induced the expression of the related gene and protein in different portions of the mouse CNS. As overexpression of the ApoD protein was shown to partially protect against neurodegeneration after HCoV-OC43 infection. Further experiments are underway in order to decipher the underlying mechanism associated with an increased ApoD expression and a potential new function of the ns2 protein of HCoV-OC43. (Supported by an NSERC discovery grant and a Tier-1 Canada Research Chair to PJT)

Workshop Sessions

VIR-WK217.04 - Preliminary analysis of simian hemorrhagic fever virus (SHFV) minor structural protein function and comparison of infections in primary macaque and baboon cells.

Heather Vatter[1], Margo Brinton[1]
[1]Georgia State University, Atlanta, USA

The 15.7 kb SHFV genome is the largest among the known arteriviruses and uniquely encodes three nonstructural protein 1 (nsp1) proteins as well as two sets of the four minor structural protein open reading frames (ORFs), 2a', 2b', 3', 4' and 2b, 2a, 3, 4. The replication characteristics of virus produced by a stable, infectious, full-length SHFV cDNA clone (SHFVic) were similar to those of the parental SHFV-LVR virus. A series of mutant infectious clones, each with the start codon of a different minor structural protein ORF mutated, were generated. Analysis of extracellular virion production by MA104 cells transfected with a mutant viral RNA indicated that GP2', GP3', E' and E play a role in extracellular virus production, while GP4', GP2, GP3 and GP4 are required for virion infectivity but not for extracellular virus production. The results indicated that the two sets of SHFV minor structural proteins are not functionally redundant as previously suggested. SHFV causes a fatal hemorrhagic fever in macaques but asymptomatic, persistent infections in natural hosts such as baboons. The in vivo target cells of SHFV infection, macrophages (MΦs) and myeloid dendritic cells (mDCs), were differentiated from macaque and baboon peripheral blood monocytes and used to compare viral replication and cell responses. SHFV replicated in >90% of macaque MΦs but in only ~10% of baboon MΦs. In contrast, SHFV infected ~50% of macaque and baboon mDCs. However, virus replication was efficient in macaque but not in baboon mDCs. Macaque but not baboon cultures produced pro-inflammatory cytokines, including IL-1β, IL-6, IL-12/23(p40), TNF-α and MIP-1α, in response to SHFV infection suggesting less efficient counteraction of these responses by SHFV proteins in macaque cells.

Workshop Sessions

VIR-WK217.05 - Discovery of novel methyltransferases of coronaviruses and their use as antiviral drug targets

Deyin Guo[1], Yu Chen[1]
[1]*State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, PR. China*

Coronaviruses pose a significant threat to human health as exemplified by the severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and the newly emergent Middle East respiratory syndrome coronavirus (MERS-CoV). However, effective therapeutic means are still unavailable for coronavirus-induced diseases. Therefore, the screening and development of antiviral drugs that either specifically inhibit highly pathogenic CoVs or broadly inhibit CoV replication is a research priority. We have been focusing on the identification of RNA capping enzymes, including the N7-methyltransferase (N7-MTase) and the 2'-O-methyltransferase (2'O-MTase) which were previously unknown for coronaviruses, and making use of them as antiviral targets. We found that the SARS-CoV non-structural protein 14 (nsp14) acts as both cap N7-MTase and RNA 3'-to-5'exoribonuclease (ExoN). The discovery that the N7-MTase is physically and functionally linked with ExoN indicates that nsp14 represents a novel form of RNA-processing enzymes. We further found that the nsp10/nsp16 complex functions as 2'-O-MTase with nsp10 as a stimulatory factor. As both N7-MTase and 2'-O-MTase of coronaviruses are structurally and mechanistically different from cellular MTases, we developed strategies for drug designing and screening based on coronaviral MTases. First, we revealed the crystal structure of nsp10/nsp16 complex and showed that a short peptide derived from nsp10 possesses inhibitory effect on the 2'-O-MTase activity. Second, we analyzed the structure-functional relationship by alanine scanning and deletion mutations. We further replaced the yeast N7-MTase with SARS-CoV and human N7-MTase, and thus established a high throughput screening system. Until now we have screened more than 10,000 microbial natural product extracts and three of them showed specific inhibition on SARS-CoV over human N7-MTase.

Workshop Sessions

VIR-WK217.06 - Inhibition of TLR-7/8 signaling pathway by SARS coronavirus papain-like protease via K63-linked deubiquitination of TRAF3 and TRAF6

Cheng-Wen Lin[1], Shih-Wen Li[1], Li-Hsin Hsiao[1]
[1]China Medical University, Taichung, Taiwan

Severe acute respiratory syndrome coronavirus (SARS-CoV) papain-like protease (PLpro) has proteolytic and deubiquitinating enzymatic activities, showing interferon (IFN) antagonistic potential. Our previous report demonstrates SARS-CoV PLpro causing upregulation of UBC E2-25k and enhancing ubiquitin-proteasome degradation of ERK 1, being responsible for the resistance to IFN-α-induced signaling (J Gen Virol 2011, 1127-40). Recent, we find SARS-CoV PLpro suppressing innate immune responses induced by imiquimod (a Toll-like receptor (TLR) 7/8 ligand). The study further investigates the inhibitory mechanism(s) of SARS-CoV PLpro on TLR-7/8 signaling pathway. Immunoprecipitation analysis with anti-ubiquitin antibodies indicates SARS-CoV PLpro removing the ubiquitin moieties from TRAF6, but increasing TRAF3 and TAK1 ubiquitination. However, imiquimod treatment results in the removal of K63-polyubiquitin chains of TRAF3 and TRAF6 in PLpro-expressing, but not vector control cells. PLpro suppresses imiquimod-induced phosphorylation of NF-κB, p38 MAPK, CREB and IRF-3 that correlates with inhibiting imiquimod-induced mRNA expression of IL-6, IL-8, RIG-1, PKR and 2', 5'-OAS in PLpro-expressing cells. The results reveal the K63-linked deubiquitination of TRAF3 and TRAF6 by PLpro playing the key regulation affecting miquimod-induced NF-κB activation and antiviral responses.

Workshop Sessions

VIR-WK218.01 - Oncolytic Viruses: State of the Art

John Bell[1]
[1]*Ottawa Hospital Research Institute, Ottawa, Canada*

Replicating viruses that specifically infect and kill tumour cells are being developed as targeted agents for cancer therapy. It is now clear that these viruses have both an ability to kill tumour cells by direct lysis but also are now known to infect and destroy tumour vasculature as well as initiating or amplifying anti-tumour immune responses. The multi-mechanistic activity of these agents has made them attractive agents for clinical testing. Several viral platforms are in development and the clinical development of the most advanced oncolytic virus platforms will be discussed.

Workshop Sessions

VIR-WK218.02 - PVRL4 (Nectin-4) is the epithelial cell receptor for measles and canine distemper viruses and is a target for viral oncolytic therapy

Sebastien Delpeut[1], Ryan Noyce[1], Gary Sisson[1], Ricky Siu[1], Paola Marcato[1], Rong-Zong Liu[2], John Mackey[2], Roseline Godbout[2], Christopher Richardson[1]

[1]Dalhousie University, Halifax, Canada, [2]University of Alberta, Edmonton, Canada

Our laboratory recently discovered that PVRL4 (Nectin-4) is the epithelial cell receptor for measles and canine distemper viruses using a comparative microarray analysis [Noyce et al. (2011) PLoS Pathogens 7(8): e1002240; Noyce and Richardson (2012) Trends Micro. 20: 429-439; Noyce et al. (2013) Virology 436: 210-220]. This discovery established a new paradigm for the spread of virus from lymphocytes to airway epithelial cells and virus release into the environment. We have investigated the interactions of the V domain of human and canine PVRL4 with the viral attachment protein (H) using site-specific mutagenesis and chimeric molecules consisting of the V domain attached to a PVRL1 backbone. PVRL4 exists at low levels in the adherens junctions of primary airway epithelial and breast cells, but microarray and flow cytometry analysis revealed cultivation of cells with 2% fetal calf serum rapidly induced PVRL4 and other junction proteins through a process of mesenchymal-epithelial transition. In addition, microarray and tumor tissue arrays of patient samples revealed that PVRL4 is highly expressed over many metastatic breast, bladder and prostate tumors. As expected, cell lines from these tumors expressed PVRL4, and were highly susceptible to measles virus infections. Recombinant measles and related viruses that express green fluorescent protein, luciferase, or suicide gene products were shown to target patient derived tumors that express PVRL4. Virus spreads via membrane fusion throughout the tumor, causing cell death. Incorporation of nucleoside based suicide genes into the virus genomes, enhanced the tumor killing effect of these viruses. The oncolytic potential of these viruses has been evaluated in a variety of human xenograft mouse models.

Workshop Sessions

VIR-WK218.03 - Linking tumor-specific T cell responses induced by oncolytic VSV therapy to tumor control: a correlation hard to assess

Valérie Janelle[1,2], Marie-Pierre Langlois[1], Pascal Lapierre[1], Tania Charpentier[1], Laurent Poliquin[2], Alain Lamarre[1,2]
[1]Institut national de la recherche scientifique, INRS-Institut Armand-Frappier, Laval, Canada, [2]Biomed Research Center, Université du Québec à Montréal, Montréal, Canada

Cancer is a multifactorial disease difficult to treat. Indeed, tumor cells have acquired mutations allowing them, among other things, to evade from the immune system. This immune tolerance is then extremely hard to break. Thus, promoting solid tumor regression can be quite a challenge due to this tolerized microenvironment. In order to reverse this phenomenon, oncolytic viruses have been used as a way to induce a pro-inflammatory state at the tumor site. This allows for the recognition of tumor antigens in the presence of appropriate activation signals to mount an antitumor immune response. Vesicular stomatitis virus is a well-characterized candidate for this alternative cancer treatment. However, the relative contribution of the antitumoral immune response induced by virotherapy versus the intrinsic oncolytic activity of viruses to the treatment outcome has been difficult to assess. To enable the study of immune mechanisms involved in viral oncolysis, we compared glycoprotein and matrix mutants of VSV, showing different lytic potentials for B16gp33 melanoma tumor cells in vitro, in their ability to induce gp33-specific CD8+ T cell responses and control tumor progression in vivo. While every VSV strains showed a similar capacity to slow down tumor progression when compared to mock-treated animals, only wild-type and G mutants induced a significant immune response against the gp33 exogenous epitope. VSV matrix mutant seemed meanwhile to allow a wider tolerance break against tumor antigens by inducing upregulation of MHC-I at the tumor cell surface thus favoring recognition by CD8+ T cells. These results demonstrate that VSV mutants affect the induction of an antitumor immune response using different mechanisms. A better understanding of such mechanisms will prove useful for the rational design of viruses with improved therapeutic efficacy.

Workshop Sessions

VIR-WK218.04 - Ex Vivo Virotherapy with Oncolytic Myxoma Virus

Masmudur Rahman[1], Winnie Chan[1], Nancy Villa[1], Christopher Cogle[1], Grant McFadden[1]
[1]*University of Florida, College of Medicine, Gainesville, USA*

Myxoma virus (MYXV) is a host-restricted leporipoxvirus that causes an acute lethal infection specifically in European rabbits (Orcytolagus cuniculis). Although MYXV is pathogenic only in European rabbits, the virus can productively replicate in cultured mammalian cell lines derived from a variety other species, including humans. Our studies on MYXV tropism have shown that the virus is also fully permissive for a wide spectrum of human cancer cells in vitro and in vivo. We are currently developing MYXV as an oncolytic virotherapeutic to treat a spectrum of human cancers that exhibit defective cell signaling responses. We have recently shown that MYXV can selectively infect and kill primary human cancer cells that contaminate bone marrow samples from patients with acute myeloid leukemia or multiple myeloma but the virus spares the normal CD34+ hematopoietic stem and progenitor cells within the sample needed to reconstitute the immune system following autologous bone marrow transplantation. Using flourescently-tagged MYXV virions, we have shown that the virus fails to bind CD34+ hematopoetic stem and progenitor cells from primary human bone marrow samples, which explains why these cells are uniquely unable to be infected with this virus. Unexpectedly, the identical ex vivo treatment of allogeneic bone marrow transplant samples from normal cancer-free donors was found to suppress the development of graft-vs-host-disease (GVHD) in recipient NSG mice. Our recent studies indicate that tagged MYXV virions can efficiently bind naive human T cells ex vivo but the virus does not infect these T cells until after they have become activated, for example by treatment with anti-CD3/CD28 or by encountering “nonself” antigens following allogeneic stem cell transplantation. Thus, the fundamental study of a rabbit-specific poxvirus pathogen has revealed unexpected applications for improving the clinical outcomes of both autologous and allogeneic stem cell transplantation therapy for cancer.

Workshop Sessions

VIR-WK218.05 - WITHDRAWN - Squirrel poxvirus as a novel oncolytic agent

Manbok Kim[1], Dong Guk Park[1], Jong Seog Ahn[2], Chae Ok Yun[3], Bo Yeon Kim[2]
[1]Dankook University, Cheonan, South Korea, [2]KRIBB WCI, Ochang, South Korea, [3]Hanyang University, Seoul, South Korea

SQPV (Squirrel poxvirus) was identified in 1936 by Kilham et al. SQPV natural host tropism is highly restricted to gray squirrels or red squirrels. Due to a SQPV host range restriction, SQPV doesn’t infect and cause pathogenesis in other species such as sheep, wood mice or bank voles and there is no known human infection/pathogenesis caused by SQPV in nature. We found that non-human pathogenic SQPV can be used as a novel oncolytic viral agent in order to treat human cancers, such as lymphoma, leukemia, and various solid malignancies. SQPV didn't affect normal non-squirrel tissues. Our data showed that SQPV infected various types of human cancers such as brain, ovarian, liver cancers as well as human hematopoietic malignancies such as lymphoma and leukemia. Suppression of human tumor growth was observed following SQPV treatment in vivo. Therefore, SQPV can be used for a viral oncolytic agent with a high safety profile specifically targeting human cancers while sparing normal human tissues. SQPV viral safety can be derived from highly restricted poxviral tropism on non-squirrel hosts.

Workshop Sessions

VIR-WK218.05 - A novel orthoreovirus as a potential therapeutic for hepatocellular carcinoma

Robert Kozak[1], Larissa Hattin[1], Wing Au Yeung[1], Evan Lusty[1], David Leishman[1], Jordan Feld[2], Byram Bridle[1], Eva Nagy[1]

[1]University of Guelph, Guelph, Canada, [2]University of Toronto, Toronto, Canada

Hepatocellular carcinoma (HCC) is one of the most prevalent types of cancer in both men and women, and often is the result of infection with the hepatitis C virus (HCV). Despite studies into the pathogenesis of this disease, and advances in treatment, improvement in patient outcome has been marginal. Novel therapies are urgently needed. Oncolytic viruses (OVs) have shown potential against numerous types of cancer both in vitro and in clinical trials. As a therapy, they demonstrate exquisite specificity since viral replication is limited to cancerous cells, which are known to have defects in their antiviral response. Oncolytic viruses function as endogenous vaccines, inducing tumour-specific immune responses, which can be further enhanced by OV-mediated delivery of antigens. Furthermore, they can be paired with other therapies to enhance their effect. We isolated and sequenced a novel orthoreovirus (hereby referred to as isolate PB1) and investigated its oncolytic potential in vitro against a panel of 21 human, murine and canine cancer cell lines. The decrease in cell viability was measured using a resazurin dye-based assay, and viral titers were also determined. Additionally, cytopathic effect was observed by both DAPI, and crystal violet staining. Results indicate that PB1 is an efficient oncolytic for HCC, as it showed activity in Huh7, Huh7.5, Huh7.5.1 and HepG2 cells, at low multiplicities of infection. Due to the association of HCV infection with HCC, we also tested PB1 against an HCV-replicon containing cell line, and observed significant oncolytic activity using the aforementioned methods. As a next step we plan to further investigate mechanisms of oncolytic activity of PB1. Additionally, we will evaluate its potential to induce innate and tumor specific immunity, and its applicability in more clinically relevant models of HCV infection and HCC.

Workshop Sessions

VIR-WK218.06 - Rapid measles virus epithelial spread through nectin-4: implications for cancer therapy

Mathieu Mateo[1], Patrick L Sinn[2], Chanakha K Navaratnarajah[1], Crystal Mendoza[1], Ryan C Donohue[1], Roberto Cattaneo[1]

[1]Molecular Medicine Department, Mayo Clinic, Rochester, USA, [2]Pediatrics Department, University of Iowa, Iowa City, USA

Spread of several viruses used for cancer therapy is mainly cell-associated. Cell-cell spread without budding has three advantages: speed, immune evasion, and bypass of physical barriers. We discovered that measles virus (MeV) uses nectin-4, an adherens junction (AJ) protein as epithelial receptor. We now show that MeV takes advantage of the epithelium-organizing function of nectins in two ways. First, its hemagglutinin (H) binds nectin-4 through the adhesive surface located in the most membrane-distal immunoglobulin-like domain. We generated mutants of this surface and assessed their ability to function as receptors. We identified three amino acids crucial for functional interactions in the FG loop; in addition, residues in two other loops govern function. Using soluble nectin-4 and H ectodomains and surface plasmon resonance, we demonstrated that integrity of both FG and BC loops is required for binding. Thus, H binds to the nectin adhesive surface, and this interaction localizes the viral membrane fusion apparatus to the AJ, which connects the actin and myosin cytoskeletal belts of apposed columnar cells. Second, we documented rapid MeV spread in reconstituted human airways epithelial sheets without visible syncytia formation while trans-epithelial resistance was maintained. We observed that cytoplasmic contents flow from infected to contiguous cells from the expected location of the AJ. Thus fusion pores are formed. However they do not expand, possibly due to local tissue stabilization through the cytoskeletal belt. We postulate that constrained pores or “canals” form during viral infection. These canals would directly connect the cytoskeletal belts of adjacent columnar cells, creating an epithelial rapid transit system for the viral components. Herpes simplex, an enveloped oncolytic virus that fuses membranes after recognizing the adhesive surface of nectin-1, may also rely on canals to rapidly spread in tumor tissue. We are assessing on which cytoskeletal filaments the viral components move.

Workshop Sessions

VIR-WK219.01 - Proteomic responses to H1N1, H5N1 and H7N9 influenza virus infections in A549 cells

Philippe Simon[1,2], Pingzhao Hu[3], Stuart McCorrister[2], Patrick Chong[2], Garrett Westmacott[2], Kevin Coombs[1,4], Darwyn Kobasa[1,2], Darwyn Kobasa[1,2]

[1]University of Manitoba, Winnipeg, Canada, [2]Public Health Agency of Canada, Winnipeg, Canada, [3]Hospital for Sick Children, Toronto, Canada, [4]Manitoba Center for Proteomics and Systems Biology, Winnipeg, Canada

Influenza viruses cause significant mortality and morbidity every year and are a constantly re-emerging threat for outbreaks and pandemics. The different subtypes of influenza circulate amongst a very wide range of mammalian and avian species and exhibit a wide range of virulence and transmissibility. At one end of the spectrum, seasonal H1N1 (sH1N1) and 2009 pandemic H1N1 (pH1N1) viruses are easily transmissible between humans, infect hundreds of millions of people annually yet generally cause infections with low lethality in otherwise healthy individuals. At the other end of the spectrum, H5N1 viruses cause severe disease in humans, with lethality rates of up to 60%. They are transmitted mostly in avian species with no sustained human-human transmission being documented. H1N1 viruses have been present in human population since at least the deadly Spanish Flu pandemic of 1918. Highly pathogenic H5N1 viruses caused the first human infections in 1997. Recently, a novel avian H7N9-subtype virus emerged in China in humans and, as of October 25th, had killed 47 people out of 135 confirmed infections. The severity of H7N9 infections seems, so far, to be somewhere between that of H1N1 and H5N1 viruses. This provides us with a spectrum of viruses that are suitable for studying markers of virulence in infection in human cells. In the present project we used shotgun proteomics, with a 4-plex iTRAQ labeling technique, to compare the host response to infection with 4 strains of influenza. Using an MOI of 10, we infected A549 cells with sH1N1 and pH1N1, H5N1 and H7N9 strains, harvested cells at 1, 3 and 6h post-infection and compared host protein levels. Our preliminary results highlight similarities and differences in the host response to these viruses, contributing both to our understanding of viral biology and potentially providing new therapeutic targets.

Workshop Sessions

VIR-WK219.02 - Transcription regulatory network atlas for comprehensive downregulation of housekeeping genes induced by Morbillivirus infection

Hiroki Sato[1], Misako Yoneda[1], Toshiyuki Nakamura[1], Fusako Ikeda[1], Jun Kawai[2], Yoshihide Hayashizaki[2], Chieko Kai[1]

[1]Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]RIKEN Omics Science Center, Yokohama, Japan

Morbillivirus infection causes several severe and common syndromes including transient strong immunosuppression. In vitro morbillivirus-induced immunosuppression and immune responses are cell-type dependent. The various pathogenic forms and the different immune responses of morbillivirus are a result of the interaction between the host and the virus, but the full picture remains unclear. To clarify the infection-induced host responses, we previously performed microarray analysis after infection with measles virus and rinderpest virus, which belong to morbillivirus, and found that the infections induced comprehensive downregulation of housekeeping genes. In this host response, transcriptional regulatory networks for the downregulation are thought to be driven by a defined set of core transcription factors (TFs) which represent the most upstream of the network. Therefore, to focus on construction of the network after infection, we employed the CAGE (cap analysis of gene expression) with a next generation sequencer, which identifies active transcription starting site and quantifies their activities by sequencing mRNA 5'-ends in a high-throughput way. Promoter activities were quantified by counting the CAGE tags aligned to the reference genome, and alteration of the activities over the infection time course was analyzed with the MARA (motif activity response analysis) for prediction of transcriptional regulatory interactions. Of 195 TFs, only limited set of TFs was activated or inactivated at an early phase of infection. At late phage of infection, alterations of TF activities were comprehensively expanded. To reconstitute the transcriptional regulatory cascade, we set out to identify the core elements. As a result, we revealed that core TFs control and maintain the expression of downstream peripheral genes which make up the rest of the transcriptional regulatory network. These high-throughput experiments uncover a novel host response against virus infection, and will be useful for understanding the whole picture of virus pathogenesis.

Workshop Sessions

VIR-WK219.03 - The question of ”modularity” in the genome of multipartite viruses

Anne Sicard[1], Geoffrey Carpentier[2], Michel Yvon[2], Yannis Michalakis[3], Serafin Gutierrez[4], Stéphane Blanc[2]
[1]Montpellier SupAgro, Montpellier, France, [2]Institut National de la Recherche Agronomique, Montpellier, France, [3]Centre National de la Recherche Scientifique, Montpellier, France, [4]Centre de coopération internationale en recherche agronomique pour le développement, Montpellier France

Multipartite viruses are composed of two or more nucleic acid segments, each encapsidated individually. It is commonly assumed that the whole viral genome is replicated within individual cells and then reiterated in successively infected cells during host invasion. For multipartite viruses, this view implies that at least one copy of each of the genomic segments must enter each infected cells. The genome of the Faba bean necrotic stunt virus (FBNSV, Nanoviridae) is composed of 8 ssDNA circles of about 1000 bases, each encapsidated in an individual viral particle. We have previously shown that each of the eight segments reproducibly accumulates at a specific relative frequency, some representing around 30% of the total viral DNA within an infected plant and others not exceeding 2%. In this situation, it is difficult to conceive how FBNSV can actually transmit the whole genome information both from cell to cell and from host to host. If the segments enter cells indifferently, solely according to their relative frequency, the successful infection of 95% of susceptible cells would require the entry of nearly 200 particles per cell. This figure illustrates the enormous cost that FBNSV might bear at each cell-to-cell transmission step. Alternatively, this virus might infect individual cells with subgroups of genomic segments, partial genome information being replicated at distinct location within a host. This may alleviate the cost at cell-to-cell passage but would imply a sort of unknown viral communication or complementation in between these subgroups of segments to maintain the genome information integrity. In any case, the actual functioning of FBNSV is an enigma, because it is hard to conceive that a virus could force hundreds of particles in each newly colonized cells, or that the genome could function with separate subunits in distinct cells. We are currently developing tools to test the above alternatives.

Workshop Sessions

VIR-WK219.04 - Genome-wide RNAi screen reveals a novel role of spliceosomal proteins in virus-induced innate immune responses.

Nicolas Tremblay[1,2], Martin Baril[1], Daniel Lamarre[1,2]
[1]Centre de Recherche du CHUM (CRCHUM), Montreal, Canada, [2]Faculté de Médecine, Université de Montréal, Montreal, Canada

The type I interferon (IFN) response is the first line of defense of the cell when engaged by a virus. To identify new regulators of innate antiviral immunity, we completed the first genome-wide gene silencing screen assessing the transcriptional response at the IFNB1 promoter following Sendai virus (SeV) infection. In this study, we identified six genes encoding spliceosome components that significantly reduced IFNB1 transcription upon gene silencing. Four of these genes: splicing factor 3a, subunit 1, 120kDa (SF3A1), PHD finger protein 5A (PHF5A), small nuclear ribonucleoprotein 200 kDa (SNRNP200) and NHP2 non-histone chromosome protein 2-like 1 (NHP2L1) showed a silencing profile in secondary screens consistent with either a direct role in sensing cytoplasmic viral RNA or with an indirect role in expression of proteins in early RIG-I-Like Receptor (RLR) signaling pathway. The mRNA and protein knockdown of each gene were confirmed by qRT-PCR and western blot analysis, and correlated with the inhibition of inducible expression of ISG56 following SeV infection. More importantly, the strong inhibition in ISG56 expression correlated with decreased protein abundance of IRF3 and RIG-I upon knockdown of SNRNP200, SF3A1, PHF5A and to a lesser extent with NHP2L1. This is, to our knowledge, the first report of a protein affecting IRF3 regulation. Microarray experiments show that the knockdown of SNRNP200 induces changes in gene expression clustering to the RLR pathway. Although the exact mechanism of action remains unknown, our data shows that spliceosomal proteins can bind biotinylated-polyI/C and -HCVRNA genome in vitro, and might function as a novel sensor of cytosolic viral RNA. Our findings reveal spliceosome protein SNRNP200 to be a critical component of the virus-induced IRF3-mediated innate immune responses that functions as a cytosolic sensor of viral RNA and a key modulator of innate immune genes.

Workshop Sessions

VIR-WK220.01 - Novel roles of cytoplasmic ICP0 in HSV-1 immune evasion and replication

Kathryne Taylor[1], Karen Mossman[1]
[1]*McMaster University, Hamilton, Canada*

HSV-1 is a successful human pathogen that has evolved with its host over millions of years. The immediate early protein ICP0 is a multi-functional protein involved in immune evasion and virus replication. ICP0 contains a RING finger domain with E3 ubiquitin ligase activity. Almost all of the functions of ICP0 involve the RING finger domain and have been described to occur within the nucleus, where ICP0 localizes early in infection. However, within 4 hours post-infection, ICP0 localizes to the cytoplasm. We previously found that cytoplasmic ICP0 functions to block the activation and nuclear localization of the transcription factor Interferon Regulatory Factor 3 (IRF3), thus impeding the expression of interferon and interferon-stimulated genes. Further, we noted that proteasome activity was not required for ICP0 to mediate its activities within the cytoplasm. Here, we evaluate viruses encoding cytoplasmic-restricted ICP0 that retain, or are deleted for, the RING finger domain, both in vitro and in vivo. Moreover, we discuss binding partners identified by mass spectrometry following SILAC. These studies further our understanding of the intricate ways in which HSV-1 has evolved to evade host innate responses and ensure virus replication and spread.

Workshop Sessions

VIR-WK220.02 - Influenza Virus NS1 Protein Mimics Oncogenic PI3K Resulting in Isoform Specific Cellular Redistribution and Activation

Juan Ayllon Barasoain[1,4], Benjamin G. Hale[2], Maria T. Sánchez-Aparicio[1,4], Adolfo García-Sastre[1,2,3]
[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA, [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK, [3]Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, USA, [4]GHEPI, Icahn School of Medicine at Mount Sinai, New York, USA

The non-structural protein 1 (NS1) of influenza virus performs a broad variety of pro-viral activities in the infected cell, mostly mediating the evasion from the host innate immune response by being the main viral interferon antagonist. However, among the multiple interactions described for this small, multifunctional protein there are several whose biological relevance remains obscure, such as NS1 ability to bind to and activate class IA phoinositide-3-kinases (PI3K). PI3K are highly regulated lipid kinases that act as critical nodes in multiple cell signaling networks that regulate cellular physiology, including differentiation, growth, survival, trafficking and immune function. As such, PI3K are also important proto-oncogenes whose deregulation lies behind a great number of different human cancers. Structurally, class IA PI3K are heterodimers formed by a regulatory (p85) and catalytic (p110) subunits, of which there are several isotypes described, adding further layers of complexity to their activity. In order to unravel the cellular relevance of NS1-activated PI3K, we have developed a bimolecular fluorescence complementation (BiFC) assay to selectively track the different regulatory and catalytic isotypes of PI3K and their behavior upon activation. Using this system we found that NS1 induces an isotype-specific relocation and activation of the different PI3K heterodimers. However, the effects of other known activators of PI3K such as Ras, Src and receptor tyrosine kinases were different from those induced by NS. By contrast; clinically relevant, oncogenic hyper-activating mutations in both catalytic and regulatory subunits of PI3K recapitulate the effect caused by NS1. We postulate that by mimicking an oncogenic deregulation of the PI3K pathway influenza virus induces a transient, transformed-like status in the infected cell to stimulate virus replication.

Tuesday, 29 July 2014 **15:15 - 15:30 Room 513 C**

Workshop Sessions

VIR-WK220.03 - Human Rotavirus NSP1 antagonizes Interferon expression using a Phosphodegron-like motif to target and induce the degradation of β-TrCP

John Patton[1], Allison Dennis[1], Marco Morelli[1]
[1]*National Institutes of Health, Bethesda, Maryland, USA*

Rotavirus (RV), a major cause of pediatric gastroenteritis, antagonizes the innate immune response through its nonstructural protein NSP1. NSP1 is a putative viral E3 ubiquitin ligase that bridges a cellular E2 conjugating enzyme (through a conserved N-terminal RING domain) with a protein target (through a highly variable C-terminal domain). This complex directs the polyubiquitination of the target, leading to its proteasomal degradation. While the NSP1 targets of most animal RVs are interferon (IFN)-regulatory factors (IRFs), the NSP1 target of most human RVs is β-TrCP. β-TrCP, itself an E3 ubiquitin ligase, regulates the turnover of the NFkB-inhibitor IkBa, which associates with NFkB in an inactive cytosolic complex. Unless prevented, virus infection induces the phosphorylation of a conserved phosphodegron (DSGxxS) within IkBa. β-TrCP recognizes this motif and induces IkBa degradation, freeing NFkB to translocate to the nucleus and upregulate IFN-I expression. By comprehensive sequence analysis, we identified a phosphodegron-like motif (PDL; DSGxS) in the NSP1 target domain of nearly all human RVs. Transient expression assays performed with NSP1 truncation mutants indicate that the PDL is essential for recognition and degradation of β-TrCP. Serine-to-alanine, but not glutamic acid, mutagenesis inactivated NSP1, suggesting that these residues must undergo phosphorylation for NSP1 to recognize β-TrCP. β-TrCP deletion mutagenesis identified the WD40 domain, which binds the IkBa phosphodegron, as the target of NSP1 activity. Mutating the phosphodegron-binding pocket of β-TrCP also blocked NSP1 activity, providing evidence that the NSP1 PDL and IkBa phosphodegron interact with β-TrCP in a similar manner. The phosphodegron is sufficient for interaction of IkBa with β-TrCP, and substituting the PDL for the C-terminus of an NSP1 protein unable to degrade β-TrCP is sufficient to establish activity. These data support a critical role for the PDL in NSP1 function and reveal a primary mechanism used by human RVs to counter IFN expression.

Workshop Sessions

VIR-WK220.04 - Analysis of neutrophil extracellular traps formation by Dengue virus

Bertha Moreno-Altamirano[1], Misol Velasco-Cardenas[1], Oscar Rodríguez-Espinosa[1], Sergio Islas-Trujillo[1], Patricia Paredes-Arce[1], Oscar Rojas-Espinosa[1]
[1]Dep. Inmunología. Escuela Nacional de Ciencias Biológicas-IPN, México D.F., México

Abstract: Dengue is a mosquito-borne viral disease affecting tropical and subtropical countries. The more severe forms of this infection are dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) and the mechanisms that lead to these clinical outcomes are mostly unknown. However, there is evidence that the host immune response correlates with the clinical outcome. Neutrophils are the most abundant circulating leukocytes and play an important role in innate immunity through several mechanisms, including the formation of extracellular structures composed of chromatin fibers termed Neutrophil Extracellular Traps (or NETs). NETs are able to trap diverse types of pathogens, including viruses. However, NETs are not 100% effective in killing all pathogens, some viruses, such as HIV, have evolved mechanisms to evade their action. AIM: Analyze if dengue virus induce NETs formation in neutrophils and if so, whether these are able to trap the virus. Accordingly, neutrophils were obtained from peripheral blood from healthy donors and cultured under different conditions: medium plus Phorbol Myristate Acetate (PMA) as a positive control for NETs formation; dengue virus serotype-2 (DENV-2); pre-incubation with DENV-2 and then PMA. After six hours of incubation, cells were mounted in Vectashield-medium with DAPI and observed under a confocal microscopy. Results: NETs were observed in PMA-stimulated neutrophils, no NETs were observed when neutrophils were incubated with DENV-2, a 20 % reduction in NETS formation was observed when neutrophils were incubaterd with DENV-2 before PMA stimulation, as compared with PMA alone. These findings correlated with H2O2 and NBT reduction (as a measure of oxidative burst). Together, these findings suggest that DENV-2 do not induce NETs formation, but is able to partially inhibit PMA-induced NETs formation. MMMA is supported by SIP20141089 and COFAA.

Workshop Sessions

VIR-WK220.05 - Exploring the antigenic relatedness of influenza virus hemagglutinins with strain-specific polyclonal antisera

Blanca García-Barreno[1], Teresa Delgado[1], Sonia Benito[1], Inmaculada Casas[2], Francisco Pozo[2], José A. Melero[1]
[1]Biología Viral, Centro Nacional de Microbiología and CIBERES, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain,
[2]Virus respiratorios, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

Alternative methods to the standard hemagglutination inhibition (HI) and neutralization tests were developed to probe the antigenic properties of the influenza virus hemagglutinin. A reference HA molecule was expressed from vaccinia virus recombinants that additionally were used to immunize rabbits from which polyclonal antibodies were obtained. When tested in ELISA against heterologous HAs, these antibodies did not recognize HAs from subtypes different from the original reference strain or reacted only partially with distantly related HAs of the same subtype. However, the discriminatory potential of these antibodies was markedly increased after adsorption of the antibodies to cells infected with heterologous viruses. This step was intended to eliminate the antibodies that cross-reacted between the HAs of the reference strain and the virus that infected the cultures. The unadsorbed antibodies then reacted differentially in ELISA with highly related HAs which were otherwise undistinguishable by standard HI and neutralization tests. Furthermore, the unadsorbed antibodies could be used not only to discriminate viruses serologically but additionally to select escape mutants of the reference strain. Sequencing of the mutant HAs identified amino acid changes responsible of the antigenic differences between the reference strain and the virus used in the adsorption step. This methodology therefore can be used to explore the sequence changes that drive antigenic evolution of influenza HA. Several examples will be presented that illustrate these applications.

Workshop Sessions

VIR-WK220.06 - Comparison of vaccinia virus anti-apoptotic proteins in the context of viral infection: how to avoid functional redundancy

David Veyer[1], Carlos Maluquer de Motes[1,2], Rebecca Sumner[1,3], Luisa Ludwig[1], Ben Johnson[3], Geoffrey Smith[1,3]
[1]University of Cambridge, Cambridge, UK, [2]University of Surrey, Guildford, UK, [3]Imperial College, London, UK

Apoptosis can restrict the replication of intracellular pathogens. Vaccinia virus (VACV) is a large dsDNA virus that replicates in the cytoplasm and encodes about 200 genes, 4 of which have been reported to inhibit apoptosis (B13, F1, GAAP, & N1). Recombinant VACVs lacking each of these proteins individually show attenuated phenotypes in vivo, but replicate normally in cultured cells. A possible explanation for this normal replication is functional redundancy that complicates the analysis of the specific roles of VACV anti-apoptotic proteins. To avoid this problem, we have used a recombinant VACV v811 that lacks the genes coding for all described anti-apoptotic proteins and introduced each anti-apoptotic gene individually into this background. The aim of this work was to study the relative contribution of the 4 VACV proteins to inhibition of apoptosis. Methods. (i) Generation of transduced cell lines expressing anti-apoptotic proteins from VACV and analysis of apoptosis induced by drugs or v811 (expression in trans). (ii) Generation of knock-in recombinant v811 viruses (expression in cis) and analysis of apoptosis. Results. In the transduced cell lines, all 4 VACV proteins showed anti-apoptotic activity depending on the stimulus employed. B13 showed greatest inhibitory capacity of both intrinsic and extrinsic apoptosis, whereas GAAP and N1 had milder effects. Furthermore, infection with v811 was sufficient to induce caspase-3 activation, indicating that this virus can be used as a trigger of apoptosis by itself. In trans, both B13 and F1 were capable of blocking v811-induced apoptosis. In cis, only recombinant v811 expressing N1 did not blocked caspase-3 activation. Conclusions. Tools to assess the ability of VACV proteins to prevent apoptosis with and without viral infection have been developed. This platform can be used to identify novel anti-apoptotic proteins. Recombinant v811 can be used to infect a wide range of cell lines to study apoptosis.

Workshop Sessions

VIR-WK221.01 - The Cutting Edge of Virus Taxonomy

Eric Carstens[1]
[1]*Queen's University, Kingston, Canada*

The International Committee on Taxonomy of Viruses (ICTV), a committee of the Virology Division of the International Union of Microbiology Societies, is responsible for: developing an internationally agreed taxonomy for viruses; for establishing internationally agreed names for virus taxa; communicating decisions reached concerning the classification and nomenclature of viruses to virologists by holding meetings and publishing reports; and maintaining an official index of agree names of virus taxa. Meeting these obligations involves the continuous cooperation and efforts of hundreds of virologists from around the world, many of whom provide their expertise to specific Study Groups that develop the latest taxonomic proposals for consideration by ICTV. These are exciting times in virus taxonomy because advances in nucleic acid sequencing, environmental sampling, bioinformatics, and structural analysis technologies have dramatically increased our recognition of not only the tremendous biodiversity in the world of viruses but also our understanding of evolutionary relationships between viruses. Recognition and the study of 'viromes' in a multiplicity of host organisms as well as metagenomic analysis of environmental samples is leading to an explosion of data on newly discovered viruses (unknown viruses with possible known homologues) and potential unique virus genomes (unknown viruses with no homology to anything known in the virus world). The challenge to the ICTV is to integrate this vast amount of data into the current taxonomy. However, this ever expanding taxonomic framework is extremely important because it will lead to advances in applied areas of virus diagnosis, surveillance and epidemiology, which in turn, will lead to a better understanding of all ecosystems and their inter-relationships with the "virosphere".

Workshop Sessions

VIR-WK221.02 - Could genome-based classification provide the foundation for modern taxonomy of viruses?

Alexander Gorbalenya[1,2,3], Anastasia Gulyaeva[2], Erik Hoogendoorn[1], Alexander Kravchenko[3], Chris Lauber[1], Andrey Leontovich[3], Dmitry Samborskiy[3], Igor Sidorov[1]

[1]Dept. of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands, [2]Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia, [3]Belozersky Inst. of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia

Virus taxonomy is governed by experts of the International Committee on Taxonomy of Viruses who are under mounting pressure to accommodate viruses identified on the sole basis of their genome sequences. We have recently introduced a computational approach (DEmARC, DivErsity pArtitioning by hieRarchical Clustering) to classify viruses of a large monophyletic group (family or order). In DEmARC, virus clustering is achieved by applying decision functions within an evolutionary framework of pairwise genome comparisons (1). For the family Picornaviridae, the genetics-based classification closely approximated the taxonomy with few notable deviations affecting the composition of few taxa and the introduction of a new rank above genus (2). For the family Filoviridae it revealed the considerable uncertainty of its taxonomy associated with limited virus sampling (3). Additionally, application of DEmARC facilitated biological insight, such as the unbiased identification of molecular markers, the implication of recombination into species formation, and the linking of abrupt environmental changes with high-ranking virus clustering (2). In this presentation, we will describe the current state of the genetics-based classification of several RNA virus families. They were built with an advanced DEmARC version that uses weighting schemes to account for uneven virus sampling. We will explore consequences of increased sampling of virus genomes on the quality of classification, including the support for taxa and ranks, and biological implications. We will use the DEmARC-based taxonomy of the family Coronaviridae (4) as a case study for highlighting benefits and challenges for taxonomy founded on genome comparisons.

Workshop Sessions

VIR-WK221.03 - Progress and challenges in filovirus taxonomy

Jens H. Kuhn[1]
[1]*NIH/NIAID Integrated Research Facility at Fort Detrick, Frederick, USA*

Presented for the the ICTV _Filoviridae Study Group (A. A. Bukreyev, K. Chandran, O. Dolnik, J. M. Dye, H. Ebihara, J. H. Kuhn, E. M. Leroy, E. Mühlberger, S. V. Netesov, J. L. Patterson, J. T. Paweska1, E. O. Saphire, S. J. Smither, A. Takada, J. S. Towner, V. E. Volchkov, and Travis K. Warren). The International Committee on Taxonomy of Viruses (ICTV) _Filoviridae Study Group prepares proposals on the classification and nomenclature of filoviruses to reflect current knowledge or to correct disagreements with the International Code of Virus Classification and Nomenclature (ICVCN). In recent years, the Study Group has brought filovirus taxonomy in agreement with the Code, removed numerous (but not all) ambiguities among taxon and virus naming, and introduced novel taxa for recently discovered divergent filoviruses. The Study Group has also been part of a larger, community-wide effort to create a nomenclature for natural, laboratory-adapted, and cDNA clone-derived filoviruses on the strain, variant, and isolate level in accordance with evolving NCBI requirements for populating RefSeq, GenBank, and other databases. Here we present an overview of past taxonomic developments, discuss existing controversies, and clarify misconceptions. Furthermore, we provide an overview of ongoing efforts to consolidate taxonomy, virus isolate-associated metadata, and sequence information.

Workshop Sessions

VIR-WK221.04 - Rottboellia yellow mottle virus is a distinct species within the genus Sobemovirus

Merike Sõmera[1], Erkki Truve[1]
[1]*Tallinn University of Technology, Tallinn, Estonia*

Three species of sobemoviruses infecting graminaceous plants have been recorded in Africa. Besides the well-studied Rice yellow mottle virus (RYMV) which is a real threat for cultivated rice in Africa, there are two others collected from the weed plants in maize fields. In 1981, Rottboellia yellow mottle virus (RoMoV, formerly abbreviated as RoYMV) was isolated from itchgrass (Rottboellia cochinchinensis) in Ibadan, Nigeria. Experimentally, it was found to infect maize too. In 2000, a new sobemovirus species, Imperata yellow mottle virus (IYMV) was found in south-western Burkina Faso. IYMV was collected from cogongrass (Imperata cylindrica) and maize. Experimentally, it was also found to infect itchgrass. The biological and chemical properties of RoMoV resembled the viruses from sobemovirus group and it was recognized as a tentative species of the genus Sobemovirus by the ICTV although its genome was not sequenced. IYMV was sequenced and proposed to classify as a member of sobemoviruses. The phylogenetic studies showed a close relationship with RYMV. It had the symptoms and host range similar to RoMoV, but it was not possible to claim whether these two species are distinct or identical due to missing sequence data on RoMoV. Consequently, RoMoV was left out from the latest list of sobemovirus species accepted by the ICTV. We sequenced the complete genome of RoMoV. It showed a genome organization characteristic of sobemoviruses. Surprisingly, the genomic alignments displayed the highest identity between RoMoV and Ryegrass mottle virus (RGMoV), a sobemovirus with different host range and geographic record. The PSI-BLAST results for the majority of individual ORF products (P2a, P2a2b and CP) were in concordance with the full genome alignments. In phylogenetic tree, RoMoV clustered together with RGMoV and a dicot-infecting Artemisia virus A (ArtVA) lately isolated from Switzerland. Thus, the phylogenetic analysis confirmed that monocot-infecting sobemoviruses break into two different lineages.

Workshop Sessions

VIR-WK221.05 - Taxonomy and ecological peculiarities of the previously unclassified arboviruses, belonging to the Bunyaviridae, Reoviridae, Flaviviridae, Orthomyxoviridae and Picornaviridae families, isolated in Northern Eurasia

Dmitry Lvov[1], Sergey Alkhovsky[1], Michail Shchelkanov[1], Alexey Shchetinin[1], Petr Deryabin[1], Asay Gitelman[1], Andrey Botikov[1], Eugene Samokhvalov[1]

[1]D.I. Ivanovsky Institute of Virology, Moscow, Russia

Next-generation sequencing approach was used for genomic and phylogenetic analysis of 26 arboviruses (including unclassified) isolated in pasture (p), shelters (s), birds colonies (bc) biocenoses in the different ecosystems of Northern Eurasia - high latitudes (HL), Southern Far East (SFE), Central Asia (CA), Transcaucasia (T), Russian plain (RP). Bunyaviridae: Orthobunyavirus – Khurdun (KHURV) (RP–bc), Phlebovirus – Razdan (RAZV) (T–p), Khasan (KHAV) (SFE–p), Komandory (KOMV) (HL–bc), Zaliv Terpenia (ZTV) (HL–bc, T–bc), Gissar (GISV) (CA–p); Nairovirus – Issyk-Kul (ISKV) (CA–s), Tamdy (TAMV) (CA, T–p), Burana (BURV) (CA–p), Uzun-Agach (UZAV) (CA–s), Chim (CHIMV) (CA–s), Geran (GERV) (T–s), Sakhalin (SAKV) (HL–bc), Paramushir (PRMV) (HL–bc), Caspiy (CASV) (CA, T–bc), Artashat (ARTSV) (CA–s); Reoviridae: Orbivirus – Okhotskiy (OKHV) (HL–bc), Aniva (ANIV) (HL–bc), Baku (BAKV) (T, CA–bc); Flaviviridae: Flavivirus – Sokuluk (SOKV) (CA–s), Tyuleniy (TYUV) (HL–bc), Kama (KAMV) (RP–bc), Alma-Arasan (AAV) (CA-p); Orthomyxoviridae: Quaranjavirus – Tyulek (TLKV) (CA–s); Picornaviridae: Cardiovirus – Sikhote-Alin (SAV) (FE–p), Syr-Darya Valley fever (SDVFV) (CA–p). Most of studied viruses were isolated from Ixodidae and Argasidae ticks, but some viruses (ZTV, ISKV, TYUV) also can be transmitted by mosquitoes. The new phylogenetic group (Khurdun) in the Orthobunyavirus genus is formed by KHURV that has 30-40% of similarity with other orthobunyaviruses. M-segment of KHURV is shorter and does not encode NSm protein. Three of the new phylogenetic groups (Issyk-Kul, Tamdy, Artashat) is discovered in the Nairovirus genus. Genetic divergence between complete genome sequences of the nairoviruses of different groups reaches 65-70%. The research was conducted in the frame of study of the biodiversity in different ecosystems of Northern Eurasia and biodefense (emerging infection of humans and animals – ISKV, TAMV, SDVFV, TLKV and AAV fevers).

Workshop Sessions

IUMS10.01 - Black clouds, silver linings and fungal volatiles

Joan W. Bennett[1]
[1]*Department of Plant Biology and Pathology, Rutgers University, New Brunswick, USA*

After Hurricane Katrina in 2005, approximately 80% of New Orleans flooded, including the author's home. My house and its contents, along with other flooded buildings, became a rich substrate for mold growth. This huge fungal "bloom" released many volatile organic compounds (VOCs) and caused extremely unpleasant odors. Scandinavian researchers had postulated that mold VOCs contribute to the adverse health effects exhibited by some inhabitants of water damaged buildings but there were few experimental data to support this hypothesis. After moving to a new university in the aftermath of Hurricane Katrina, my laboratory initiated a pioneering research project in which we used genetic models for testing the bioactivity of fungal VOCs. Mixtures of VOCs emitted from living cultures of molds isolated after Hurricane Katrina and Super Storm Sandy, as well as low concentrations of chemical standards of some individual VOCs, were toxic in *Drosophila melanogaster*. Low concentrations (0.5-2.8ppm) of "mushroom alcohol" (1-octen-3-ol) showed potent neurotoxicity and caused Parkinsonian effects in this genetic model. When a yeast knock out library was screened for resistance to 1-octen-3-ol, ninety-one resistance genes were identified, the majority of which were involved in protein trafficking

Plenary Sessions

BAM-PL06.01 - Comparative analyses of the human- and animal-adapted strains of the *Mycobacterium tuberculosis* complex

Stephen Gordon[1]
[1]*University College Dublin, Ireland*

The *Mycobacterium tuberculosis* complex (MTBC) is a group of highly genetically related pathogens that cause tuberculosis (TB) in mammalian species. Our knowledge of these pathogens is dominated by studies on the human pathogen, *M. tuberculosis* a global pathogen for which new control tools are urgently needed. In the same way as *M. tuberculosis* is the best studied human tubercle bacillus, our knowledge of the animal-adapted strains has been dominated by studies with *Mycobacterium bovis*, the agent of bovine TB. Taking *M. tuberculosis* and *M. bovis* as the human- and animal-adapted 'poles' of the complex, what can comparative studies across these pathogens teach us about diversity, virulence and host adaptation within the MTBC. In this presentation I will discuss our current understanding of the make-up of the MTBC, focusing on comparisons of *M. tuberculosis* and *M. bovis* as the exemplar human- and animal-adapted strains, and look to what studies of these pathogens can teach us about virulence across the MTBC specifically and the emergence of host adaptation in general.

Plenary Sessions

BAM-PL06.02 - The sigma factors of Mycobacterium tuberculosis

Riccardo Manganelli[1]
[1]*Dept of Molecular Medicine, University of Padova, Padova, Italy*

Mycobacterium tuberculosis is a remarkable pathogen capable of adapting and surviving to various harsh conditions encountered during infection. It can also enter a dormant state, allowing asymptomatic infections that persist for decades. Any weakening of the immune system response, possibly due to malnutrition, debilitating diseases or age may result in the reactivation of latent bacilli. The sophisticated infection and adaptation mechanisms used by *M. tuberculosis* are expected to require complex genetic programs. The genome of *M. tuberculosis* is the largest among obligate human pathogens and intracellular bacteria, and encodes approximately 190 regulatory proteins, including 11 two-component systems, five unpaired response regulators, two unpaired histidine kinases, 11 protein kinases and over 140 other transcription regulators, including 13 sigma factors. Most of them have been investigated, and so far were all shown to be required for virulence. The better characterized alternative sigma factor of *M. tuberculosis* is SigE, belonging to the extra-cytoplasmic functions (ECF) sigma factors. It can be transcribed from three different translational start codons and is subjected to a very complex regulatory circuit including three positive forward loops involving the two component system MprAB, the antisigma factor RseA, the serin-threonine protein kinase PknB, the global regulator ClgR and the ECF sigma factor SigH. Beyond being responsible for controlling surface stability and composition following the exposure to damaging environmental conditions, its function confers ability to avoid the induction of the host response. A *sigE* mutant of *M. tuberculosis* is unable to grow inside macrophages and is severely attenuated in mice. Interestingly, this mutant confers better protection than *Mycobacterium bovid* BCG from infection with virulent *M. tuberculosis* and is more sensitive to several antimycobacterial drugs.

Plenary Sessions

BAM-PL07.01 - Salmonella typhi: pathogenesis and host specificity

Jorge Galán[1]
[1] *Yale University, School of Medicine, New Haven, USA*

“No abstract provided”

Plenary Sessions

BAM-PL07.02 - Attaching and effacing pathogens use type III secretion system effectors to conquest signalling in infected cells

Gad Frankel[1]
[1]*Centre for Molecular Bacteriology and Infection, Imperial College, London, UK*

Enteropathogenic and enterohaemorrhagic E. coli (EPEPC and EHEC) and the mouse pathogen Citrobacter rodentium, are enteric pathogens that colonize the gut mucosa via attaching and effacing lesion formation. Following cell attachment the bacteria use their type III secretion system to inject multiple effectors that subvert numerous signalling pathways including apoptosis, actin dynamics and innate immunity. Few effectors are now known to introduce novel posttranslational modifications. For example NleB has an N-acetylglucosamine transferase activity, which modifies R117 in the death domain of FADD and NleE has an S-adenosyl-l-methionine-dependent methyltransferase activity that modifies TAB2 and TAB3. Amongst the effectors Tir is integrated into the plasma membrane of the mammalian cell in a hairpin loop topology where it serves as a receptor for the outer membrane bacterial adhesin intimin. Clustering of Tir EPEC by intimin leads to phosphorylation of Y474 by redundant tyrosine kinases, including the Src and Abl. This leads to recruitment of Nck, activation of N-WASP and the Arp2/3 complex and actin polymerization underneath the attached bacteria. Similarly, Y112 in the Vaccinia virus membrane protein A36 is phosphorylated by Src and Abl, which triggers actin polymerization through Nck/N-WASP/Arp2/3 under the virions, enhancing cell-to-cell spread via actin-based motility. These signal transduction pathways resemble those triggered by the Fc gamma receptor FcγRIIa, which signal through immunoreceptor tyrosine-based activation motifs (ITAM) that are comprised of two adjacent YxxL/I motifs. Clustering of the receptors leads to phosphorylation of the ITAM tyrosines by Src and activation of downstream signalling pathways leading to actin rearrangement, membrane delivery and engulfment of the attached particles. Recently, we identified an E. coli effector that inhibits the activity of Src and Abl and hence cell signalling downstream of Tir, A36 and FcγRIIa. In this presentation I will describe the steps that led to its discovery and report its mechanism of action.

Plenary Sessions

MEM-PL06.01 - Systems Biology and Metabolic Engineering of Yeast

Jens Nielsen[1,2]

[1]Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden, [2]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark

Microbial fermentations are the core of biorefineries as this process ensures value addition when the raw material is converted to desired fuels and chemicals. The development of efficient cell factories that can be used as biocatalysts in microbial fermentations is often the most time consuming and R&D intensive part in the development of a biorefinery. Most current biorefineries involves ethanol production, but there is much interest to upgrade the production of ethanol to other more valuable fuels like butanol and diesel. This can be done through simple replacement of the biocatalyst, which in this case is the yeast Saccharomyces cerevisiae, and replacement of the biocatalyst can often be done in a plug-and-play fashion with little requirement for retrofitting of the production facility. Besides its classical application in the production of bread, beer, wine and bioethanol, the yeast Saccharomyces cerevisiae is a widely used cell factory for production of fine chemicals such as resveratrol, and for production of pharmaceutical proteins such as human insulin and vaccines. In connection with the development of biorefineries for sustainable production of fuels and chemicals, there is much interest to use yeast as a cell factory due to its general acceptance in the industry, its robustness towards contaminations, its high alcohol tolerance, and its low pH tolerance. Further advantages of using yeast are that a large number of molecular biology techniques are available for this organism, there is a large experimental database available and there is an excellent research infrastructure. In this lecture will be presented results from systems biology analysis of the central carbon metabolism, with the objective to unravel key regulatory structures. This knowledge can be used for designing novel metabolic engineering strategies, in particular for generating platform cell factories that can be used for production of a range of different products.

Plenary Sessions

MEM-PL06.02 - Experimental and model-based analyses of algal metabolism

Kourosh Salehi-Asthtiani[1]
[1]*Division of Science and Math, New York University Abu Dhabi, Abu Dhabi, UAE*

The increased demand and consumption of fossil fuels have raised interest in finding renewable energy sources throughout the globe. Biofuels have been presented as an alternative source of energy and much focus has been placed on optimizing microorganisms to efficiently produce compounds that can substitute for fossil fuels. Such approaches require a deep understanding of metabolic networks of the organisms and their genomic and proteomic profiles. The advent of next generation sequencing and other high throughput methods has lead to a major increase in availability of biological data. Integration of such disparate data can help define the emergent metabolic system properties, which is of crucial importance in addressing biofuel production optimization. In this talk, I will present our work on metabolic network model reconstruction of the model green alga *Chlamydomonas reinhardtii*, as well as our large-scale cloning efforts to generate resources for downstream phenotypic studies, and metabolic phenotyping of the species to characterize this and other algae in a systematic manner. The last part of the talk will include an in-depth analysis of evolutionary profiles of genes within the *Chlamydomonas* metabolic network and the relationships between their evolutionary profiles, expression, function, and network distances. Based on our results, we propose that the metabolic network of *Chlamydomonas* is assembled with an arrangement to minimize phylogenetic profile distances topologically, while expanding such distances for functionally interacting genes. This architecture may increase the buffering capacity of the network.

Plenary Sessions

MEM-PL07.01 - Fungal Genomes in Flux

Jason Slot[1], Antonis Rokas[2], Kriston McGary[2], John Gibbons[3], Jennifer Wisecaver[2], George Greene[2], Matthew Campbell[4]

[1]The Ohio State University, Columbus, USA, [2]Vanderbilt University, Nashville, USA, [3]Harvard School of Public Health, Boston, USA, [4]University of Montana, Missoula, USA

Fungal genomes are a dynamic assemblage of genetic information. The content and structure of these genomes has been shown over recent years to undergo remodeling by rearrangements, gene duplication, gene loss and horizontal gene transfer. As a consequence, fungal genomes are in a constant state of flux. As gaps in fungal genome sampling become narrower, we are beginning to model the processes of genomic change by reconstructing the histories of genes, both individually and in the context of their chromatin neighbors. Recent work suggests that the extent of fungal genome remodeling is influenced by both intracellular and extracellular environmental factors. Evolutionary links between fungal ecology, metabolism and genome structure will be illustrated by vignettes and trends revealed through comparative genomics.

Plenary Sessions

MEM-PL07.02 - Evolutionary genomics of yeasts

Bernard Dujon[1]
[1]*Institut Pasteur, Unité de Génétique moléculaire des levures, Paris, France*

The complete genome sequencing of *Saccharomyces cerevisiae*, back in 1996, pioneered very fruitful developments of yeast genomics whose significance remained unanticipated at the time. Next to the functional explorations of this genome that made this yeast the best described eukaryotic cell today, the sequencing of other yeast species revealed, by comparisons, a wealth of highly significant results about the evolutionary origin of yeasts, the major architectures of their genomes and the mechanisms underlying major evolutionary changes. Today, hundreds of yeast genomes have been sequenced - a rapidly rising number given present sequencing techniques – offering an unprecedented data set for extensive comparisons and evolutionary studies. Despite a biased taxon sampling owing to the considerable attractiveness of *S. cerevisiae* and related species, and to the preference given to species of biotechnological or biomedical interests, a number of studies have addressed the very broad evolutionary range covered by yeasts. The importance of gene and genome duplications, copy number variations, gene losses, horizontal gene acquisitions, *de novo* gene creations, introgressions, interspecific hybridizations will be summarized in the formation and diversification of various yeast lineages of distinct origins. Recent data will be provided about yet poorly explored branches and evolutionary gaps, and the power of yeast genome dynamics will be illustrated by experimental results.

Plenary Sessions

VIR-PL04.01 - Attachment strategies of glycan-binding viruses

Thilo Stehle[1]
[1]*University of Tübingen, Germany*

Virus attachment to cells initiates infection and is also a key determinant of host range, tissue tropism and pathogenesis. Carbohydrates such as sialic acids are prominently displayed on many cell surfaces, and they are frequently used by many viruses as their initial, and sometimes only, attachment receptors. Understanding how viruses engage sialic acid is essential for combating infection and designing improved therapeutic viral vectors. Recent advances in studies of virus-glycan interactions have made it possible to rapidly identify specific receptors using glycan array screening, define the atomic level structure of virus-glycan interactions using crystallography, and generate recombinant viruses or pseudoviruses to rationalize the effect of glycan binding in cell entry, tissue tropism, and disease pathogenesis. I will report on the current state of our ongoing effort to define the receptor binding properties of human polyomaviruses, adenoviruses and coxsackieviruses. All three pathogens use sialylated glycan receptors for their cell attachment. In combination with mutagenesis experiments and functional studies, structural analyses have enabled us to understand the determinants of specificity in each case. Exploitation of these determinants provides an excellent platform for the development of antiviral agents. We are also able to show that receptor specificities can be switched through subtle changes in the binding pockets, demonstrating the dynamic aspects of virus interactions with receptors.

Plenary Sessions

VIR-PL04.02 - Polymerase transcription and encapsidation by a small dsRNA virus

Yizhi Jane Tao[1]
[1]*Biochemistry and Cell Biology, Rice University, Houston, USA*

“No abstract provided”

Plenary Sessions

VIR-PL04.03 - Exploiting viruses as a system to understand and treat cancer

Clodagh O'Shea[1]
[1]*Molecular and Cell Biology, Salk Institute, La Jolla, USA*

"No abstract available at time of publication"

Plenary Sessions

VIR-PL04.04 - Studies of non-enveloped virus maturation and infection: Insights into elegantly programmed nano-machines

John Johnson[1]
[1]*The Scripps Research Institute, La Jolla, USA*

Virus particles with quasi-equivalent surface lattices (i.e. identical gene products in different quaternary structure environments) often assemble as a fragile, spherical shell in which subunits are properly positioned on the surface lattice with differences in the environments minimized. Quasi-equivalent subunit contacts then differentiate during particle maturation, creating a robust, faceted particle with subunits in dramatically different local environments. Nudaurelia Capensis w Virus (NwV) is a eukaryotic, quasi-equivalent virus, with a T=4 surface lattice, where maturation is dramatic (a change in particle size of 100Å) and is novel in that it can be investigated in vitro. We used X-ray crystallography, molecular genetics, biochemistry, computational chemistry, Small Angle X-ray Scattering, and electron cryo-microscopy and image reconstruction (CryoEM), to characterize the kinetics of morphological change, maturation intermediates, an associated auto-catalytic cleavage, and to demonstrate that regions of NwV subunit folding are maturation-dependent and occur at rates determined by their quasi-equivalent position in the capsid.
Supported by NIH R01 GM054076

Bridging Plenary Sessions

BR-03.01 - Hepatitis C: 25 years later

Charles Rice[1]
[1]*Rockefeller University, New York, USA*

“No abstract provided”

Bridging Plenary Sessions

BR-03.02 - C-type lectin receptors in infection and immunity

Teunis Geijtenbeek[1]
[1]*University of Amsterdam, Amsterdam, the Netherlands*

Pattern recognition receptors are crucial for the induction of innate and adaptive immune responses. C-type lectin receptors (CLRs) such as DC-SIGN and dectin-1 are an important family of PRRs that recognize carbohydrate structures expressed by viruses, fungi and (myco)bacteria. Recent studies have shown that CLRs are not only important in capture of pathogens but these receptors are also inducing signalling pathways that shape both innate and adaptive immunity. Notably, several pathogens target CLRs to escape immune surveillance, or to promote their transmission. Here I will discuss the importance of CLRs in sensing pathogens and shaping both type I IFN responses as well as adaptive immunity.

Bridging Plenary Sessions

BR-03.03 - Pathogenesis of Shigella infection, a model of immunosubversion

Philippe Sansonetti[1]
[1]*Institut Pasteur, Paris, France*

Shigella, a gram-negative enteropathogenic bacteria causes the rupture, invasion and inflammatory destruction of the human colonic epithelium. It is a major cause of mortality and morbidity among pediatric populations in the most impoverished areas of the planet. The invasive phenotype of Shigella is encoded by a large plasmid encoding a Type III Secretory System (TTSS) and cognate effector molecules that are injected into the membrane and cytoplasm of eukaryotic cell targets. The invasive phenotype is linked to the triggering of a strong inflammatory response elicited in part by activation of the Nod intracellular sensors by bacterial muropeptides. Injected effectors can be subdivided into two major categories (i) Ipa proteins that are mainly involved in the formation of the translocating structure of the TTSS, and triggering of the massive actin cytoskeletal rearrangements that carry out the entry process via macropinocytosis. (ii) Osp and IpaH proteins whose genes are transcribed when the TTSS is functional. These proteins are major regulators of the innate and adaptive immune responses of the mucosa to the invading pathogen. They act very specifically upon key steps of major signalling pathways such as NF-κB and MAPK, through specific enzymatic activities. OspG is a kinase that binds a subgroup of E2 ubiquitine-ligases, thereby blocking ubiquitination of I-κB thus activation of the pro-inflammatory genes, that are under the control of this pathway. OspF reaches the cell nucleus where it dephosphorylates active Erk1/2 and P38, thereby regulating histone phosphorylation, chromatin compaction and the transcription of a set of important pro-inflammatory genes including IL-8. IpaH molecules form a new family of 10 proteins that share a common of E3 ligase activity. We will show how these effectors collectively « carve » a particular profile of immune genes expression in their target cells.

Poster Session

BAM-PW1001 - Clade differentiation of Escherichia coli O157 using high resolution melting revealed the weak virulence of clade 12 and evolutionary discontinuity among lineage I/II strains

Yoshiki Etoh[2], Shinichiro Hirai[1], Sachiko Ichihara[2], Eriko Maeda[2], Eiji Yokoyama[1], Nobuyuki Sera[2], Kazumi Horikawa[2], Tomoko Yamamoto[3]
[1]Chiba Prefectural Institute of Public Health, Chiba, Japan, [2]Fukuoka Institute of Health and Environmental Sciences, Fukuoka, Japan, [3]Chiba University, Chiba, Japan

We have studied 167 epidemiologically unlinked strains of enterohemorrhagic Escherichia coli O157 (O157) isolated from patients with hemorrhagic colitis (HC patients), 87 strains from patients without hemorrhagic colitis and 35 asymptomatic carriers (NOT HC group), and differentiated these strains into clades using high resolution melting analysis. In addition, lineage analysis was carried out using lineage-specific polymorphism assay-6 and analysis of the distribution of IS629 insertion sites was carried out using IS-printing. Most strains were correctly clustered by minimum spanning tree analysis, and strains in the major clades showed linkage disequilibrium, confirming the clade differentiation in this study. The number of O157 strains in the different clades isolated from HC and NOT HC group was significantly different (Chi square test, $P < 0.05$), indicating that strains in different clades had different pathogenicities for hemorrhagic colitis. Pairwise comparison of the number of strains in different clades isolated from HC patients indicated that clade 12 strains were weakly pathogenic for hemorrhagic colitis. Stx2 titers and the number of strains with an stx2 gene were significantly different for different clades (Kruscal-Wallis test and Chi square test, $P < 0.05$). Pairwise comparison of the Stx2 titer and the number of strains with an stx2 gene in different clades revealed that the weak pathogenic for hemorrhagic colitis in clade 12 would be related to low Stx2 production and no stx2 gene possession. Interestingly, the Stx2 titer and the number of strains with an stx2 gene were significantly higher among clade 6 and 8 strains compared to clade 7 strains, although clades 6, 7, and 8 were all in lineage I/II. These results indicated a discontinuity in the O157 evolutionary model and suggested that insertion of an stx2 gene in lineage I/II strains may have occurred after divergence of each clade.

Poster Session

BAM-PW1003 - Sensitive detection of A1γ peptidoglycan in Gram-negative bacteria by a mass spectrometric approach

Peter Schumann[1], Christian Jogler[1], Stefan Spring[1]
[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*

Peptidoglycan layers in cell envelopes of Gram-negative bacteria are occasionally very thin and hardly detectable. It is assumed that cell walls of most Gram-negatives contain peptidoglycan of the type A1γ based on *meso*-diaminopimelic acid (*meso*-Dpm) (1). Therefore, *meso*-Dpm is a suitable marker for indicating peptidoglycan in Gram-negatives. Whole-cell hydrolysates have been commonly examined for *meso*-Dpm by TLC and amino acid analyzers (e.g., (2)). These methods are, however, not sensitive enough for reliable information about the presence of peptidoglycan when *meso*-Dpm occurs in traces. Therefore it has remained elusive whether or not representatives of some clades of Gram-negative bacteria (e.g. the PVC superphylum) contain peptidoglycan. Gas chromatography is suited for quantification of N-heptafluorobutyryl amino acid isobutyl esters from peptidoglycan (3);(4). Highly sensitive detection of the *meso*-Dpm derivative was achieved by selected ion monitoring of the fragmentions 380, 324, 306 and 278 m/z. This gas chromatographic/mass spectrometric approach was successfully applied for detection of low amounts of peptidoglycan in the model organisms *Planctomyces limnophilus* and the unclassified verrucomicrobial strain L21-Fru-AB. In contrast, the yet undescribed verrucomicrobial isolate 31 was shown to lack peptidoglycan. These findings are surprising as all *Planctomycetes* were proposed to lack peptidoglycan while the vast majority of verrucomicrobial strains contain a peptidoglycan sacculus. Thus the presented approach, consistent with recent findings (5, 6), might necessitate rethinking of the planctomycetal cell plan that might be less exceptional than previously predicted. 1. Schleifer KH & Kandler O. Bacteriol Rev 36 (1972), 407-477. 2. König E, Schlesner H, Hirsch P. Arch Microbiol 138 (1984), 200-205. 3. O'Donnell AG, Minnikin DE, Goodfellow M, Parlett JH. FEMS Microbiol Lett 15 (1982), 75E-78E. 4. Schumann P. Methods Microbiol 38 (2011), 101-129. 5. Speth DR, van Teeseling MC, Jetten MS. Front Microbiol 3 (2012), 304. 6. Devos DP. Trends Microbiol 22 (2014), 14-20.

Poster Session

BAM-PW1005 - Characterization and gene expression of Torulose Hyphae morphotype in *Frankia alni* ACN14a

Cynthia Lafond-Lambert[1], Audrey Bernèche-D'Amours[1], Vanessa Gagnon[1], Ryszard Brzezinski[1], Sébastien Roy[1]
[1]Centre d'étude et de valorisation en diversité microbienne, Université de Sherbrooke, Sherbrooke, Canada

Frankia are filamentous actinobacteria capable fixing nitrogen in symbiosis with over 220 plant species, or when free-living in soil. Different morphologies are observed in *Frankia*; vesicles, sporangia, regular hyphae, and reproductive torulose hyphae (RTH). The last forms were first described in strain Cj1-82 by Diem and Dommergues in 1985, and have seldom been studied since. We have developed a differentiation induction method using salts for *Frankia alni* ACN14a. RTH are the result of hyphae enlargement, a thickening of the cell wall, and a marked increase in septation. We characterized RTH by microscopy (epifluorescence; TEM) with different salts. Our previous transcriptomic study identified 77 genes involved in RTH differentiation. Ten genes (*kdpC*, *capD*, *thiC*, *sigH*, *ileS*, *atpD*, FRAAL3700, FRAAL2028, FRAAL2084, FRAAL2827) were selected for qPCR analysis and the results obtained confirmed the expression level fluctuations initially found in the transcriptomic study - all genes gave the same results except *thiC* and *atpD* during cell differentiation. The relative expression of many genes fluctuated over the 2-week period of cellular differentiation progressed. Based on cyclical fluctuations in expression levels, we hypothesized that Sigma H may control the expression of the other genes. Our results demonstrate that *Frankia alni* ACN14a can differentiate massively into RTH as a consequence of a particular gene expression patterns, different from regulars hyphae cells. Because of the dramatic shift in cellular resources required to undergo such a profound cellular differentiation, RTH are likely an important component of the *Frankia* life cycle. Our study opens the path to a better fundamental understanding of the *Frankia* life cycle, and a possible exploitation of RTH morphology for biotechnological purposes.

Poster Session

BAM-PW1007 - X-Ray cystallographic structure of ZapA from Escherichia coli and identification of key residues involved in the ZapA-FtsZ interaction

Elyse Roach[1], Matthew Kimber[1], Cezar Khursigara[1]
[1]University of Guelph, Guelph, Canada

FtsZ is an essential cell division protein in Escherichia coli. Its localization, filamentation and bundling is required for Z-ring formation and subsequent recruitment of the other proteins that comprise the bacterial divisome. Divisome proteins are recruited in a hierarchal manner and depend on the Z-ring for stability. Included in the divisome are the FtsZ associated proteins ZapA, ZapB, ZapC and ZapD. These act to stabilize the Z-ring by increasing lateral interactions between individual proto-filaments, thus bundling FtsZ to provide a scaffold for divisome assembly.We solved the X-ray crystallographic structure of E. coli ZapA and identified a charged α-helix on the globular domains of the ZapA tetramer. Using site-directed mutagenesis we modified key helix residues on ZapA and demonstrated that these ZapA variants significantly decreased FtsZ bundling in vitro by protein sedimentation assays when compared to wild-type ZapA proteins. Electron micrographs of ZapA-bundled FtsZ filaments showed that the modified ZapA variants altered the number of FtsZ filaments per bundle. These in vitro results were corroborated in vivo by expressing the ZapA variants in an E. coli ΔZapA knockout strain. The in vivo studies demonstrated that ZapA variants that altered FtsZ bundling showed an elongated phenotype, indicative of improper cell division. Taken together, our results help to further our understanding of the nature of the ZapA-FtsZ interactions and FtsZ bundling. Overall, this will aid in answering key questions about how the divisome assembles at midcell to drive bacterial cell division.

Poster Session

BAM-PW1009 - FtsEX interaction network in the bacterial divisome

Daniel Maturana[1], Alexis Ordenes[1], Rosalba Lagos[1], Octavio Monasterio[1]
[1]*Departamento de Biologia, Facultad de Ciencias, Universidad de Chile, Santiago, Chile*

Bacterial division is an important and complex process. The machinery (divisome) involved in this process includes more than 12 essential proteins, which are transcriptionally, spatially and temporally regulated. The divisome formation can be separated in 2 steps. The first step involves the formation of the proto-ring with the proteins ZipA, FtsA and FtsZ, and in the second step the rest of the proteins are recruited sequentially to the septal ring. The first complex recruited in the second step is FtsEX, which has structural and sequence similarity to an ABC transporter, being FtsE the ATP binding subunit and FtsX the transmembrane subunit. The recruitment of late proteins depends on FtsEX. Binding of ATP to FtsE is critical for the division, but not for the formation of the divisome. FtsE was purified and structurally characterized using circular dichroism and fluorescence spectroscopy. Additionally the dissociation constants for the interaction of FtsEX with the neighbor proteins in the divisome was determined using microscale thermophoresis. Thus, using this technique it was possible to quantify the role of FtsEX in the divisome. Our results show that FtsX interacts with high affinity with the "coiled-coil" domain of EnvC, a regulator of the divisome amidases. Also, FtsX interacts with lower affinity with FtsQ and with itself. On the other hand, refolded FtsE binds ATP and ADP and interacts with FtsZ. From these results we constructed a model of this sub-network of the divisome to explain how FtsEX is recruited and that its function could be a control point. Funded by FONDECYT #1130711

Poster Session

BAM-PW1011 - The C-terminal domain of the flagellar protein FlgJ from *Salmonella enterica* serovar *typhimurium* functions as a lytic transglycosylase

Francesca Herlihey[1], Anthony Clarke[1]
[1]University of Guelph, Guelph, Canada

Salmonella are Gram-negative, pathogenic bacteria, which are motile through the use of the flagella. This locomotive organelle is used as a virulence factor for many pathogenic bacteria. The flagellum is a heterooligomeric structure that crosses the cytoplasmic membrane, periplasm and outer membrane. To incorporate the flagellum into the cell wall, the peptidoglycan (PG) layer is locally degraded by enzymes such as muramidases. Depending on the catalytic mechanism, enzymes that cleave PG are either hydrolases or lytic transglycosylases (LT). Recently, the bimodular flagellar protein FlgJ of *Salmonella enterica* serovar *typhimurium* (*S. typhimurium*) was shown to contain a C-terminal domain with lytic activity. The characterization and enzyme kinetics of the C-terminal domain of FlgJ with its natural substrate *S. typhimurium* strain LT2 PG, is being investigated. At present, RP-HPLC and mass spectrometry data suggests that FlgJ is an endo-acting LT with a specific activity of 0.516 mmoles 1,6-anhydromuramic acid /min*mg. The proposed catalytic residue E223, has been substituted with glutamine and aspartic acid to determine its importance in lytic activity. Both E223Q and E223D proteins were shown to be inactive, confirming the importance of the proposed catalytic residue E223 for activity. By having a better understanding of the type of lytic activity of FlgJ, there is potential to use this enzyme as a new target for the development of novel antibacterials

Poster Session

BAM-PW1013 - Role of minor pilins in Pseudomonas aeruginosa type IV pilus assembly

Ylan Nguyen[1], Seiji Sugiman-Marangos[1], Stephanie Bell[1], Hanjeong Harvey[1], Carmen L. Giltner[1], Murray Junop[1], Lori L. Burrows[1]

[1]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada

Many bacterial pathogens including Pseudomonas aeruginosa use type IV pili (T4P) for attachment, motility and biofilm formation. T4P are primarily composed of monomers of the major pilin subunit, but low abundance minor pilins of unknown function are also incorporated into the pilus. Here we show the minor pilins prime pilus assembly and traffic the pilus-associated anti-retraction protein PilY1 to the cell surface. PilV, PilW, PilX and PilY1 are mutually dependent for pilus incorporation, forming a putative priming complex that recruits PilE. FimU is incorporated independently from the other minor pilins suggesting it might couple the priming complex to the major pilin subunit. The 1.4 Å crystal structure of $FimU_{\Delta 1\text{-}28}$ was solved and despite sharing only 13% sequence identity, strongly resembles the putative coupling minor pseudopilin, GspH, of type II secretion (T2S) system. In a strain lacking all minor pilins, a small amount of surface pili can be assembled in a retraction-deficient background. However, the additional deletion of the T2S minor pseudopilins resulted in the loss of surface piliation suggesting the T2S minor pseudopilins can prime pilus assembly in the absence of the minor pilins. In a minor pseudopilin mutant background, the loss of either FimU or PilE only modestly reduces surface piliation whereas the absence of both abolishes piliation, suggesting that they may function together for coupling. Together, these data lead to a model in which the minor pilins PilVWX and the adhesin PilY1 form a priming complex that recruits PilE. FimU can interact with PilE and couple the priming complex to the major subunit of the growing pilus fibre.

Poster Session

BAM-PW1015 - Role of a bacteriophage glucosylation operon in Campylobacter fetus N-linked protein glycosylation

Justin Duma[1], Harald Nothaft[1], Bernadette Beadle[1], Christine Szymanski[1]
[1]Alberta Glycomics Centre and Department of Biological Sciences, University of Alberta, Edmonton, Canada

The N-linked protein glycosylation (Pgl) pathway is conserved throughout the *Campylobacter* genus and involved in a multitude of cellular functions. In *Campylobacter fetus* , the pathway produces two hexasaccharides: GlcNAc-(Glc)-GlcNAc-GalNAc-GalNAc-diNAcBac and GlcNAc-(GlcNAc)-GlcNAc-GalNac-GalNAc-diNAcBac, in a ratio of 1:4 respectively. These hexasaccharides are assembled onto undecaprenylphosphate (UndP) on the cytoplasmic membrane, flipped into the periplasm and then transferred onto asparagine of the bacterial glycosylation sequon D/E-X-N-X-S/T by the oligosaccharyltransferase, PglB. Analysis of the *pgl* gene cluster identified the presence of a putative bacteriophage glucosylation operon, *gtr*, which encodes three putative enzymes: GtrA a flippase for the UndP-Glc precursor, GtrB a bactoprenol glucosyltransferase, and GtrC a serotype-specific glucosyltransferase. Typically this operon is involved in O-antigen modification and seroconversion, where the glucose branch prevents bacteriophage co-infection. We hypothesized that the *gtr* operon in *C. fetus* is responsible for the addition of Glc or GlcNAc to the pentasaccharide and utilizes a unique mechanism of saccharide transfer. The Gtr pathway first transfers the glucose residue from UDP-glucose to UndP resulting in a UndP-Glc intermediate from which the Glc then gets transferred by GtrC to the N-glycan backbone in the periplasm. We demonstrate that the Gtr enzymes preferentially transfer Glc from UDP-Glc to Und-P using an in vitro assay that allows the quantification of UDP release by GtrB. In addition, co-expression of the *C. fetus* gtr operon, the components of the “classical” *pgl* operon and an N-glycan acceptor protein in *E. coli* resulted in the formation of the *C. fetus* hexasaccharide with the Glc branch that could be readily detected by mass spectrometry. This strongly indicates that the *gtr* operon is responsible for the addition of a glucose residue to the pentasaccharide synthesized by the *pgl* operon and therefore represents a unique and unknown mechanism in N-glycan biosynthesis.

Poster Session

BAM-PW1017 - Quantitative analysis of VBNC Salmonella metabolism using multicolor flow cytometry

Yuta Morishige[1], Ko Fujimori[1,2], Fumio Amano[1,2]

[1]Laboratory of Biodefense and Regulation, Graduate School of Osaka University of Pharmaceutical Sciences, Takatsuki, Japan, [2]Laboratory of Biodefense and Regulation, Osaka University of Pharmaceutical Sciences, Takatsuki, Japan

Many microorganisms are considered to be in VBNC (viable but non-culturable) state in the natural environment. It is also considered that experimentally stressed microorganisms turn into VBNC state. The VBNC cells from pathogenic bacteria might be a problem on hygienic field because of its non-culturability on the colony-formation assay and its resuscitation to the culturable state. We previously reported that Salmonella Enteritidis, a gram-negative bacteria of food-poisoning, became VBNC state through incubation in LB medium with 3 mM hydrogen peroxide (H2O2). The H2O2-treated cells were not culturable, but they maintained high activities of respiration, DNA synthesis and protein synthesis. These metabolic activities were examined by the direct counting method with a confocal laser scanning microscope and by the incorporation of radioactive precursors*, although the methods take long time. In this study, we established an easier, more rapid detection method of VBNC Salmonella by using multicolor flow cytometry with fluorescent precursors for the macromolecule synthesis. We also assayed both respiratory activity and glucose uptake by the VBNC Salmonella by using CTC-reduction assay and NBD-glucose incorporation. Taken together, the methods in this study seem to be applicable for elucidation of the mechanisms underlying transition of bacteria to VBNC state and resuscitation of them from VBNC, and also for estimation and detection of VBNC bacteria in Food and natural environment. *Morishige et al., 2013. Microbes and Environments 28 (2) 180-6

Poster Session

BAM-PW1019 - Some technological properties of probiotic lactic acid bacteria isolated from spontaneously fermenting KUNU-ZAKI

Solakunmi Oluwajoba[1,2], Felix Akinyosoye[1], Victor Oyetayo[1]
[1]Department of Microbiology,Federal University of Technology,Akure,Nigeria., [2]Department of Biological Science,Yaba College of Technology,Lagos,Nigeria

Twenty-one (21) probiotic Lactic acid bacteria (LAB) earlier isolated in another published work from spontaneous fermenting kunu-zaki (a local beverage, processed using composite and non -composite, germinated and un-germinated Digitaria exilis, Sorghum bicolor and Pennisetum americanum cereal grains) were in this study assessed in vitro for preliminary suitability as starter cultures. Ability to produce CO2 from glucose, growth at 10°C, 15°C & 45°C, tolerance against 4% and 6.5% NaCl concentrations and inhibition of test reference pathogens were investigated. Ten out of the twenty-one strains were determined to be homo-fermentative whilst the others were hetero-fermentative. Hetero-fermentative strains were excluded from further study. Seven of the homo-fermentative strains were able to grow at temperatures of 10°C and 15°C but only six were able to grow at 45°C. Seven strains were able to grow at 4% NaCl concentration but only five showed growth at 6.5% NaCl. Eight strains showed zones of inhibition against the test pathogens E.coli25922, S.aureus25923, P. aeuriginosa27853 and E.faecalis29212 but P. damnosus and L.lactis ssp lactis 1 could not inhibit E.faecalis. Pediococcus pentosaceus 2 stood out from all the strains assessed with ability to grow at all temperatures, NaCl concentrations and inhibition of the test reference pathogens. The use of starter cultures in the production of kunu-zaki beverage in West Africa remains quite largely unexplored. These results would help in the selection of LAB strains with probiotic properties that may find further use as commercial starter cultures in the industrial production of kunu-zaki Keywords: Lactic acid bacteria, Starter culture, Probiotic, homo-fermentative, hetero-fermentative.

Poster Session

BAM-PW1021 - Comparative transcriptomic analysis of Lactococcus chungangensis sp. nov. with Lactococcus lactis

Maytiya Konkit[1], Jong-Hwa Kim[1], Hee Kuk Park[1], Wonyong Kim[1]
[1]*Department of Microbiology, Chung-Ang University College of Medicine, Seoul, Republic of Korea*

Lactococcus chungangensis CAU28, isolated from activated sludge foam is also characterized in phynotyping and gene sequencing is done to compare with other Lactococcus sp. However, for more deeply information in gene functions, the study of genotyping through the comparative genomics and transcriptomic based on wide genome microarray was used to determine and compare the genes of L. chungangensis CAU28 with L. lactis KCTC 3769T, reference strain. All the expression level of 1,915 probes, there has 396 genes that was up regulated and 1519 genes was down regulated in L. chungangensis when it was compared with L. lactis. Of these results, the interesting information found that L. chungangensis has the genes function in field of cheese making like L. lactis such as aminohydrolase and S-adenosylmethionine which are dominant in the cheese flavor production. Besides, stress response genes such as cold shock and heat shock protein that prevent the cell to survive in harmful condition also found in L. chungangensis. It is the new insight of the present gene function that can be the useful genes in part of fermentation of milk and cheese production. The study in the gene function level through the microarray analysis not only give the information in gene expression but also the knowledge to the forward experiment.

Poster Session

BAM-PW1023 - Assessment of potential spoilage and pathogenic organisms in Nigerian *ogi* during natural fermentation

Adanma Innocent-Ukachi[1], Christine Dodd[1], Gregory Tucker[1]
[1]*University of Nottingham, Nottingham, UK*

'Ogi' is a lactic fermented starch made from maize, sorghum or millet which serves as breakfast and weaning food in Nigeria. It is a naturally fermented product known to have reduced shelf life due to chance inoculation by spoilage organisms. Although fermented foods are known for improved nutritional value and safety against pathogens, this study is to investigate the possibility of spoilage and pathogenic organisms surviving during natural fermentation of this important weaning food and their safety implications. We assessed the microbial diversity of the fermenting population in maize and sorghum ogi. Genomic DNA was directly extracted from ogi, single colonies of pure isolates and bulk cells harvested from differential media, respectively. The V3, V6-V8 variable regions of 16S rRNA bacterial genes and the D1 domain of 26S rRNA fungal genes were PCR amplified. Amplicons were characterized by denaturing gradient gel electrophoresis (DGGE) and sequencing of band amplimers. Potential spoilage organisms were further isolated in selective media and characterized by cultural and molecular methods. Bacterial species identified were: Bacillus, Acetobacter, Enterococcus, Enterobacter, while fungal species were: Aspergillus, Penicillium and Fusarium. The potentials of the fungal isolates to produce aflatoxin, zearalenone and ochratoxin were evaluated by biosynthetic pathway gene expression. Expression of aflatoxin structural genes (polyketide synthase acetate and norsolonic acid-1) and ochratoxin (OCRA1/OCRA2 and CAR1 and CAR2) in Aspergillus and Penicillum isolates and polyketide synthase of zearalenone (PKS13 and PKS4) biosynthetic pathways in Fusarium were evaluated by polymerase chain reaction. Interestingly all the genes were not expressed suggesting the likelihood of the isolates being non-mycotoxigenic. However further confirmation of this result by testing the presence of the mycotoxins in 'ogi' is needed.

Poster Session

BAM-PW1025 - Occurrence of Escherichia coli O157: H7 in beef chain, dairy products, lettuce and diarrheic stools in Nigeria's capital, Abuja

Bolaji Akanbi[1], Lynda Utoh[1]
[1]University of Abuja, Abuja, Nigeria

A study of the ecology E. coli O157:H7 in the most populated area council of Nigeria's capital city was carried out by sampling nomadic herds, an abattoir, produce, beef products, fresh and homestead fermented cow milk and clinical diarrheic samples in a tertiary health center. The isolation of the pathogen from all but clinical samples was performed in a two-step procedure namely; enrichment with modified tryptone soy broth with novobiocin and subsequently plating on cefixime tellurite sorbitol MacConkey agar (CT-SMAC). For clinical samples, detection was by plating samples directly on CT-SMAC. Typical morphology of the organism on CT-SMAC, biochemical tests, antisera to somatic antigen and motility were used for identification. The sample size for each type of sample was 150. The production of shiga toxin 1 and 2 was determined by the use of enzyme immunoassay. The average prevalence rate of E. coli O157: H7 in the nomadic herds was 55%. Approximately 90% of cattle in the abattoir shed the pathogen in their feces. The pathogen was detected in 40% of fresh raw milk and 17 % of fermented milk products whereas 33% and 35% of lettuce and beef products were positive for the pathogen respectively. E. coli O157: H7 was the etiologic agent in approximately 18% of stool samples. In two outbreaks involving families, consumption of beef products and lettuce were implicated as the source of the pathogen. The importance of E. coli O157:H7 in the study area is largely under-recognized because of lack of surveillance and inclusion of protocols for detection in clinical laboratories. This study highlights the importance of this pathogen, its prevalence in cattle as well as its survival and persistence in milk products and produce. Further characterization of these isolates using pulsed field gel electrophoresis (PFGE) will reveal genetic relationships between them.

Poster Session

BAM-PW1027 - Elucidation of biological characteristics and emetic activities of newly identified staphylococcal enterotoxins

Dong-Liang Hu[1], Masashi Okamura[1], Hisaya K Ono[2], Akio Nakane[2], Ken'ichi Imanishi[3]
[1]Department of Zoonoses, Kitasato University School of Veterinary Medicine, Towada, Japan, [2]Hirosaki University Graduate School of Medicine, Hirosaki, Japan, [3]Tokyo Women's Medical University School of Medicine, Tokyo, Japan

Staphylococcal enterotoxins (SEs) produced by Staphylococcus aureus are the most recognizable bacterial toxins causing food poisoning and toxic shock throughout the world. SEs are toxins of molecular weight of about 20 to 29 kDa and have strong resistance against heat and digestive enzymes. So far, five types of SEs, SEA to SEE, have been known from the differences in the antigenicity of the toxins. However, in 2000s, the presence of a variety of new SEs were discovered one after another. To date, SEA to SEE, SEG to SEI, and SER to SET with demonstrated emetic activity, and many new SE-like (SEl) toxins have been reported, although the role of SEls in food poisoning remains unclear. SEs are a major cause of food poisoning, which typically occurs after ingestion of foods contaminated with SEs. The primary symptoms are of rapid onset and include nausea and violent vomiting. However, how SE induces emesis and its emetic signal pathway remains unclear. We investigated the behavior of SEs in the gastrointestinal (GI) tract using the house musk shrew. Immunofluorescence of GI showed that administered SEs translocated from the lumen to the interior tissues of the GI tract and rapidly accumulated in certain submucosa cells. We further found that SE-induced emesis were inhibited by 5-hydroxytryptamine (5-HT) synthesis inhibitor and 5-HT3 receptor antagonist. Pre-treatment with 5,7-dihydroxytryptamine markedly inhibited SE-induced emesis. These results demonstrate that SE induces 5-HT release and the 5-HT3 receptors on vagal afferent neurons in intestine are essential for SE-stimulated emesis. The emetic potentials of newly identified toxins, SElK, SElL, SElM, SElN, SElO, SElP, and SElQ were also assessed using a monkey-feeding assay. All the SEls that were tested induced emetic reactions in monkeys. This result suggests that these new SEs may play some role in staphylococcal food poisoning.

Poster Session

BAM-PW1029 - Single laboratory validation of two vibrio TaqMan qPCR assays for identification of vibrio isolates

Willis Fedio[1], Ruben Zapata[1], Paul Browning[1], Lyssa White[1], Juan Olea[1], Ruiqing Pamboukian[2]
[1]New Mexico State University Food Safety Laboratory, Las Cruces, USA, [2]Food and Drug Administration, Office of Regulatory Affairs, Rockville, USA

Introduction: *Vibrio cholerae* (Vc), *V. parahaemolyticus* (Vp), and *V. vulnificus* (Vv) are well-documented human pathogens associated with seafood consumption. For detection of vibrios, the FDA BAM recommends enrichment in alkaline peptone water (APW) followed by isolation on selective/differential agar. Biochemical testing or conventional PCR procedures are recommended for identification of suspect vibrio isolates. This study compares the Life Technologies *Vibrio* qPCR and the BAX qPCR assay with the BAM procedure for identification of *Vibrio* isolates. Methods: *Vibrio* cultures were grown in APW overnight at 35°C, streaked onto selective agars and confirmed biochemically as Vc, Vv, or Vp using methods described in the BAM. For the BAX *Vibrio* assay, 5 µl of APW enrichment was used for multiplex qPCR detection of Vc, Vv, and Vp as described by the manufacturer. For the Life Technologies *Vibrio* Assay, *Vibrio* isolates were grown on T1N0 agar (Vc) or T1N3 agar (Vp and Vv). A well isolated colony was suspended in saline, boiled for 10 min., centrifuged 15,000 x g for 3 min. and the supernatant was used for vibrio detection. Template was diluted (1:100) for multiplex qPCR detection of Vc, Vv, and Vp as described by the manufacturer. Results: The BAX ® *Vibrio* assay correctly identified 51/52 Vc isolates while the *Vibrio* TaqMan assay correctly identified 50/52. The BAM procedure identified all 52. For Vv, 51/51 isolates were positive by the BAX ® *Vibrio* assay, 50/51 by the *Vibrio* qPCR assay and 50/51 by the FDA BAM PCR. For Vp, both the *Vibrio* qPCR assay and the BAM PCR confirmed 50/50 isolates tested while the FDA method identified 49/50 Vp isolates correctly. Of the 45 near-neighbor *Vibrio* isolates, none were detected by either *Vibrio* qPCR assay. Significance: The results presented here demonstrate that assays are reliable and rapid alternatives to the BAM methods for identification of Vc, Vv, and Vp isolates.

Poster Session

BAM-PW1031 - Microbial inactivation during microwave cooking

Pascale Gadonna-Widehem[1], Jean-Claude Laguerre[1], Cynthia Helou[1], Celine Jouquand[1]
[1]Institut Polytechnique Lasalle Beauvais, Beauvais, France

Microwaves are used increasingly in the catering sector for time and energy saving matters. The microwave heating differs from conventional heating in how heat is provided to the product. In a microwave oven the heating is generated inside the product by molecular friction while in conventional ovens the heating starts at the surface of the product. Regardless of the chosen cooking method, food safety must be ensured and pathogens must be destroyed. During the European project Hotpot, the cooking of different dishes was tested and the impact of microwave treatments on microorganisms was determined after addition of target germs or by following the evolution of in situ microorganisms. Two long cooking dishes (Beef Burgundy and Hotpot) and one fast cooking product (minced beef burger) were cooked using a microwave catering oven. The evolution of temperature during cooking was followed with optical fibers or by thermography at the end of the heating. Bacterial populations were determined after plating on agar media or enrichment in broth. Minced beef burgers were cooked according to the recommendations of the French Agency for Food, Environmental and Occupational Health & Safety during 2 min at 70°C (158 °F). This time/temperature combination was chosen in order to destroy pathogenic E. coli. Total destruction was obtained and pasteurization was ensured following the microwave cooking. However, the consumer test revealed the product to be unacceptable due to its texture and dried state. However, for the long cooking dishes, the complete destruction of the target germs was also obtained with microwave cooking but the final organoleptic qualities were found to be acceptable by the consumers. Regarding these results, microwave cooking can ensure food safety. However, consumers' acceptance of the product is essential and has to be taken into account while developing microwave applications.

Poster Session

BAM-PW1033 - Prevalence and antibiotic resistance of Methicillin-resistant Staphylococcus aureus from chicken meat

Jocelyn Bernier-Lachance[1], Josée Labrie[1], Mario Jacques[1], François Malouin[2], Marie Archambault[1]
[1]Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Canada, [2]Département de Biologie, Université de Sherbrooke, Sherbrooke, Canada

Methicillin-resistant Staphylococcus aureus (MRSA) can cause a wide range of diseases from skin and soft tissue infections to septicaemia. Methicillin resistance is due to a modified penicillin binding protein (PBP2' or PBP2a) encoded by the mecA gene. The presence of PBP2a confers resistance towards all β-lactam antibiotics, apart from ceftobiprole. In the past few years, MRSA have been found in food animals and in their meat and are referred to as Livestock-Associated MRSA (LA-MRSA). Isolates of animal origin constitute a risk for transmission to humans through the food chain. No information is available on the prevalence and antibiotic resistance of MRSA in poultry in the province of Quebec. One of the objectives of this project is to determine the prevalence of LA-MRSA from chicken meat from the province of Quebec and to characterize these strains in regards to their antibiotic susceptibilities. MRSA isolates from chicken meat were recovered using an enrichment media followed by a selective agar. The prevalence of LA-MRSA in chicken meat was 1.29 %. In addition to being resistant to all β-lactam antibiotics, most of these isolates were also resistant to tetracycline. To conclude, the prevalence of LA-MRSA in chicken meat is low and few antibiotic resistances have been observed.

Poster Session

BAM-PW1035 - The effect of some antibiotics on Streptococcus mutans in dental caries in Jos

Olubukola Mary Oyawoye[1], Ciciliya Caleb[1], Mary Istifanus[2]
[1]ATBU, Bauchi, Nigeria, [2]Platuea State University, Bokkos, Jos, Nigeria

Antibiotic sensitivity test was carried out on streptococcus mutans isolated from patients with tooth decay at the dental clinic of Plateau state Hospital Jos, to established their antimicrobial susceptibility. Samples where collected from 100 patients with tooth decay by scraping the sulcus part of the decayed tooth, out of which 74% were identified to have streptococcus mutans. The molar teeth were more affected with 56% compared to the premolars and the roots with 35% and 9% respectively, and the number of cases with dental caries was found to be highest between ages of 21-40 with more females 54% than male 46%. Obtained results shows streptococcus mutans to be susceptible to ampicilin 84%, amoxyl 90%, ciprofloxacin 85%, penicillin 78%, ampiclox 55%, and streptomycin 30%, but resistant to erythromycin, gentamycin and cefuroxine. The level of dental health in a community depends on the extend to which people seek dental care and apply preventive measures.

Poster Session

BAM-PW1037 - Cytotoxic effects of pedioccoccus pentosaceus on caco-2 cells

Sevda Er[1], Emine Dinçer[2], A. Tansu Koparal[3], Merih Kıvanç[3]
[1]Department of Microbiology, Faculty of Pharmacy, İstanbul Medipol University, İstanbul, Turkey, [2]Department of Nutrition and Diet, Faculty of Health Science, Cumhuriyet University, Sivas, Turkey, [3]Department of Biology, Faculty of Science, Anadolu University, Eskişehir, Turkey

Probiotics are defined as "live micro-organisms which confer a health benefit on the host when administered in adequate amounts." Characteristics of probiotics have been reviewed including survival in gastric conditions and colonization of the intestine, reduction of lactose intolerance, prevention of antibiotic-induced diarrhoea, prevention of colon cancer, and stimulation of the immune system. The term probiotics, usually refers to highly selected lactic acid bacteria such as Lactobacillus , Bifidobacterium, Leuconostoc, Pediococcus and Streptococcus spp. LAB are described as Gram positive, nonsporing, anaerobic cocci or rods. The aim of this study was to investigate the cytotoxic effects of increasing concentrations of lyophilized form of Pedioccoccus pentosaceus cytoplasm on human colon adenocarcinoma cells (Caco-2 cells). In this study, 5x104 Caco-2 cells/mL were incubated in 96-well microtiter cell culture plates. 24 h after different concentrations of lyophilized form of bacterial supernatants (1.000-100-10-1-0,1µg/mL) were added to the culture medium. Caco-2 cells were further incubated for 24. After incubation, the cell viability was determined using the MTT-test. The results showed that the significant dose-dependent cytotoxic effects of lyophilized form of P. pentosaceus cytoplasm were not observed on Caco-2 cells for 5x104cells/mL after 24 h incubation. We continue investigating effects of different concentrations of this bacterium supernatant on Caco-2 cells at different incubation times. Therefore, this study may be valuable in the evaluation of this probiotic. Key words: Probiotics, Pediococcus pentosaceus, Caco-2, cytotoxicity, MTT assay.

Poster Session

BAM-PW1039 - In-vitro study of the digestion of bread melanoidins: impact on intestinal microbiota

Cynthia Helou[1], Sylvain Denis[2], David Marier[3], Véronique Rame[3], Monique Alric[2], Frédéric J. Tessier[1], Pascale Gadonna-Widehem[1]

[1]EGEAL UP 2012-10.101 - Institut Polytechnique LaSalle Beauvais, Beauvais, France, [2]EA 4678 CIDAM - Université d'Auvergne - Faculté de Pharmacie, Clermont-Ferrand, France, [3]Institut Polytechnique LaSalle Beauvais, Beauvais France

The Maillard reaction (MR) in foods occurs between the carboxyl function of reducing sugars and the amine function of amino acids or proteins during heat treatments. At its final stage, the MR generates brown polymers with high molecular weights known as melanoidins. While MR products from the early and advanced stages have been widely documented, few studies have focused on melanoidins, particularly bread melanoidins, due to their complex and mostly unknown structure. The aim of this study was to assess the impact of digestive enzymes on bread melanoidins and the consequences on the intestinal microbiota. Bread crust rich in melanoidins, bread crumb containing no melanoidins and model melanoidins based on a heated starch/gluten model were subjected to in-vitro gastro-intestinal digestion using a TIM 1 system. The soluble fraction of melanoidins was measured using an innovative technique coupling size-exclusion chromatography and fluorescence detection. In order to study the impact of these products on the intestinal microbiota, batch fermentations under anaerobic conditions were performed using fecal matters from healthy adults. Different bacterial populations were followed during the 8-day fermentation period using plate counting techniques and qPCR. An increase of fluorescence was observed after gastric and jejunum (amylase) digestion of bread crust and model melanoidins. These findings indicate that part of the insoluble melanoidins is digested and solubilized under acidic and enzymatic activity. However, the relatively high molecular weights of the solubilized melanoidins suggests that they will not cross the intestinal barrier and remain in contact with the intestinal microbiota. Batch fermentations showed increases in lactic flora and bifidobacteria for all melanoidin-containing fractions and inulin (prebiotic control) but not for bread crumb. Therefore, melanoidins present in bread crust have a prebiotic effect and intervene in the modulation of intestinal microbiota. Further in-vivo investigations are to be performed to support these findings.

Poster Session

BAM-PW1041 - Biological control of postharvest disease in two varieties of British Columbia apples using Pseudomonas fluorescens isolates

Rhiannon Wallace[1], Danielle Hirkala[2], Louise Nelson[1]
[1]University of British Columbia Okanagan campus, Kelowna, Canada, [2]BC Tree Fruits Cooperative, Kelowna, Canada

Postharvest disease is a serious issue faced by apple producers and packinghouses worldwide. Three major postharvest fungal pathogens, *Penicillium expansum*, *Botrytis cinerea*, and *Mucor piriformis*, commonly infect and rot apples in storage. Synthetic fungicides such as thiabendazole and more recently, fludioxonil have been applied extensively to reduce postharvest loss, but pathogen resistance is emerging. In addition, public pressure to reduce fungicide application and public demand for produce free of synthetic fungicides has led to increased research for safer alternatives such as biocontrols. Three strains of *Pseudomonas fluorescens* 4-6, 1-112 and 2-28, isolated from the rhizosphere of pulse crops in Western Canada were studied as potential biocontrol agents under commercial cold storage conditions with two varieties of apple, Gala and McIntosh. All three isolates inhibited the growth of *B. cinerea*, *P. expansum*, and *M. piriformis* in vitro. Lesion diameters of apples inoculated with each of the three pathogens and biocontrol strains were determined after fifteen weeks in commercial cold storage and compared with the fungicide Scholar® (fludioxonil), the biocontrol agent Bio-Save® (P. syringae) and Bio-Save® in combination with Mertect® (thiabendazole). Efficacy of the *P. fluorescens* strains varied with pathogen and apple variety. McIntosh apples, which had higher titratable acidity than Gala, exhibited greater disease severity. Strain 4-6 showed the most consistent efficacy against all three pathogens and on both apple varieties. The disease control was comparable to Bio-Save® but less effective than Scholar®. These results suggest that *P. fluorescens*, has potential for control of common postharvest fungal pathogens during cold storage.

Poster Session

BAM-PW1043 - Comparison of primary and secondary enrichment broth for 7 different Salmonella species study using ground black pepper as a food matrix

Waraporn Mahlman[1], Shirley Ele[1]

[1]State of Nebraska Agriculture Department, Food and Dairy Laboratory Division, Lincoln, USA

This study is to compare the efficiency of primary enrichment, MP broth, to secondary enrichment, TT and RV broth, to detect the present of Salmonella in ground black pepper. The method of detection of salmonella was direct plating on XLD, BS, HE and R&F agar and BAX Q7 for PCR screening method. Samples number 1, 2, 3, 5, 6, 7 and 8 had been blind spiked by a third party laboratory in 7 different Salmonella species at 100-1000 cfu/g while sample number 4 was not inoculated. Aseptic weigh each sample 25 g and added 225 ml of primary enrichment, MP Broth. Incubate the enrichment for 18-24 hours at 37 ºC. Perform samples analysis from primary enrichment by using BAX PCR and plating to 4 different kinds of agars as indicated. Transfer 1 ml and 0.1 ml of primary enrichment to 10 ml TT and RV broth. Incubate TT broth at 43 ºC and RV broth at 42 ºC for 18-24 hours. Perform samples analysis from secondary enrichment by using BAX PCR and plating to 4 different kinds of agars. The BAX Q7 PCR successfully detected Salmonella in all 7 spiked samples from both primary and secondary enrichment on sample number 1, 2, 3, 5, 6, 7 and 8 respectively. The result of plating on XLD, BS, HE and R&F agars found Salmonella had typical growth colonies from both primary and secondary enrichment. There is no difference using primary and secondary enrichment broth to either plating or BAX PCR method of Salmonella spp. detection at the 100-1000 cfu/g concentration.

Poster Session

BAM-PW1045 - Advances in Ugba production-using genetically characterized Bacillus species, their safety and impact on aroma compounds

Ihuoma Ahaotu[1], Henry Njoku[1], Ngozi Odu[1], Amara Anyogu[2], Jane Sutherland[1], Irene Ouoba[1], Steve Elmore[3]
[1]University of Port Harcourt, Nigeria, [2]London Metropolitan University, London, UK, [3]University of Reading, Reading, UK

"Ugba" is produced from fermenting seeds of African Oil beans tree (Pentradethra marcophylla Benth). Forty-nine bacteria were isolated from Ugba produced in three different states of eastern Nigeria and identified by phenotyping and sequencing of 16S rRNA, gyrB and rpoB genes. Genotypic diversities at interspecies and intraspecies level of the isolates were screened by PCR amplification of the 16S-23S rDNA intergenic transcribed spacer (ITS-PCR) and repetitive sequence-based PCR (rep-PCR). The ability of the bacteria to produce toxins was investigated by detection of genes encoding production of haemolysin BL (HblA, HblC, HblD), non-hemolytic enterotoxin (NheA, NheB, NheC), cytotoxin K (CytK) and emetic toxin (EM1) using PCR with specific primers. The aroma profile of "Ugba" produced in the lab using the identified isolates as starter culture was investigated using Head space Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry. Sampling was done at different time during the fermentation process and result for 72 hour was compared with commercial sample from the market. The isolates were characterized as motile, rod-shaped, endospore forming, catalase positive, Gram-positive bacteria. They were identified as Bacillus cereus (42), Lysinibacillus xylanilyticus (3), B. clausii (1), B. licheniformis (1), B. subtilis (1), and B. safensis (1). Genes encoding production of non-hemolytic enterotoxin (NheA, NheB, NheC) and cytotoxin K (CytK) genes were detected in all B. cereus isolates, while Hbl genes (HblA, HblC, HblD) were detected in one isolate. The emetic specific gene fragment was not detected in any of the isolates studied. None of the toxin genes screened was detected in the isolates belonging to the other Bacillus spp. A wide range volatile flavor compounds were identified and they include alcohols, aldehydes, organic acids, ketones, esters, pyrazines, furan, sulfur compound, limonene, antioxidant BHA and acetamide. The aroma profile of samples produced with starter culture compared favourably with the commercial sample.

Poster Session

BAM-PW1047 - Correlation study of physicochemical analysis of dongchimi, a Korean watery kimchi, and assessing its sensory attributes

Joo-Hyoung Cho[1], Jin-joo Choi[1], Seung-Joo Lee[1], Chang-Ho Chung[1], Sri Lee[2], Kyu Kyung[2]
[1]Department of Culinary Sci. and FoodService Mgt., Sejong University, Seoul, S. Korea, [2]Department of Food Science, Sejong University, Seoul, S. Korea

Dongchimi is a representative watery kimchi consumed with watery content and vegetables among a wide range of kimchies, fermented vegetable foods by lactic acid bacteria, of Korea. This study aimed to examine the correlation with the physicochemical analysis data of Dongchimi by assessing its sensory attributes by executing generalized description analysis for the 9 types of commercially available Dongchimi. Dongchimi sample was collected and presented when the level of fermentation maturity was appropriate with a pH range of 3.9~4.2, and the panel executed description analysis 6 times through total of 4 sessions of training (360 hours). For the physicochemical data, sugar content, salinity, pH and acidity were measured, and organic acid, free sugar, ethanol and amino acid contents were measured by using HPLC. The physicochemical characteristics displayed correlation between the acetic acid, lactic acid, acidity, ethyl alcohol and mannitol with correlation between salinity, pH, °Brix, fructose, glucose, malic acid and citric acid. In addition, positive correlation was found between valine, proline, hydroxyproline and gamma-amino butyric acid (GABA), and sweet taste, and positive correlation also between asparagine and proline, and savory taste.

Poster Session

BAM-PW1049 - Gene trait matching identifies a mobile genetic element responsible for dramatically increased heat resistance of Bacillus subtilis spores

Erwin M. Berendsen[1,2,3], Oscar P. Kuipers[1,2], Marjon H.J. Wells-Bennik[1,3]
[1]Top Institute Food and Nutrition (TIFN), Wageningen, The Netherlands, [2]Laboratory of Molecular Genetics, University of Groningen, Groningen, The Netherlands, [3]NIZO Food Research B.V., Ede, The Netherlands

Spores of Bacillus subtilis are able to survive various harsh environmental conditions, including different heat preservation treatments used in food processing. Eighteen food and environmental isolates of B. subtilis could be divided into two distinct groups with different spore heat resistances. To obtain the same level of spore inactivation, spores belonging to the group with high heat resistance required 100 times longer heating times at 120°C than those with low heat resistance. Whole genome sequencing was performed for these isolates of B. subtilis, followed by correlating the genome content to the corresponding spore heat resistance phenotype. A set of genes were uniquely present in the strains producing spores with high heat resistance. All these genes were located on one mobile genetic element. The integration site of the mobile genetic element was determined, and the insertion was found in the same gene for all strains that produced high heat resistant spores (these strains had diverse backgrounds). We demonstrated that the genetic element was directly responsible for the very high heat resistance of spores; transfer of the element to a strain with significantly lower heat resistance (i.e. B. subtilis 168) rendered a phenotype of high spore heat resistance. The roles of the genes on the mobile genetic element and the exact molecular mechanisms that mediate the increase of spore heat resistance are currently subject of investigation and will be discussed.

Poster Session

BAM-PW1051 - *Clostridium perfringens* and *Salmonella* spp. as contamination of sanitary conveyor in poultry processing slaughterhouse before and after sanitization with water spray

Mariana Casagrande[1], Marita Cardozo[1], Livia Boarini[1], Mariana Beraldo-Massoli[1], Ricardo Cavani[1], Marcio Butrico[1], Andressa Souza[1], Ruben Pablo Schocken-Iturrino[1]
[1]*São Paulo State University, Jaboticabal, Brazil*

The Brazilian poultry industry, in 2012, was the third in global ranking with 12.65 million tonnes of meat production. Due to this production, there is concern about the transmission of pathogens to human health and to be under the Brazilian legal patterns. Nevertheless, proper hygiene in the conveyors could inhibit this transmission. This study aimed to evaluate the pre-operational and operational hygiene in the microbiological control, through the quantification of Clostridium spp. and Enterobacteria, in addition to the PCR detection of Salmonella spp. and Clostridium perfringens present in sanitary conveyor in Brazilian slaughterhouses. In order to quantify Clostridium spp. and Enterobacteriaceae, 250 samples were collected using sterile swabs, in four different periods, before and after the operational and pre-operational hygiene. The swabs were placed in tubes containing 10 mL of 0.1% peptone, plated by the pour plate method in specific culture medium for each type of bacteria. Clostridia cells counting were performed after incubation in agar RCA anaerobically at 37ºC for 48 hours and for Enterobacteriaceae cells counting it was used MacConkey agar with incubation at 37°C for 24h. Counts were made by the colony-forming units per mL (CFU/mL) method. All samples were submitted to DNA extraction by boiling method and subsequently subjected to PCR with specific primers. Clostridium perfringens was detected by amplification of specific band for cpa gene as well as for Salmonella spp. that was identified by using specific primers for invA gene. The average counts, expressed in log, for Clostridia was 2.61 and for Enterobacteriaceae was 3.09. Bacterial identification by PCR resulted in one positive sample for Clostridium perfringens and two for Salmonella spp. This results showed that slaughterhouse hygiene performed with only water spray is not sufficient to contain contamination. Thus, under this hygiene condition there is a high chance of transmission pathogens to humans.

Poster Session

BAM-PW1053 - Biosynthesis of unusually long chain polyunsaturated fatty acids by uncontrolled expression of pfa genes responsible for the biosynthesis of docosahexaenoic acid in Escherichia coli recombinants

Mikako Hashimoto[1,2], Hidetoshi Okuyama[1], Kiyohito Yoshida[1], Yoshitake Orikasa[2]
[1]Hokkaido University, Sapporo, Japan, [2]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

Bacterial biosynthesis of long-chain polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is well known in some γ-proteobacteria isolated from marine, deep sea, or cold environments, and five discrete enzymes (PfaA−E) encoded by pfaA−E genes are responsible for the biosynthesis of PUFAs. However, it has not yet been elucidated how the chain length of bacterial PUFAs is determined in their biosynthesizing systems. Two possibilities are present. One is an involvement of the PfaC-intrinsic thioesterase (TE) domain, which is speculated to have specificity to DHA in the DHA-biosynthesis by the Pfa enzyme complex. In the other possibility a TE activity determining the chain length of PUFAs is in PfaA. However, no direct evidence is available. To aim this challenge in this study we transformed Escherichia coli DH5α with pfaA-E genes (pDHA4) derived from a DHA-producing bacterium Moritella marina MP-1. E. coli DH5α/pDHA4 was cultivated by shaking at 150 rpm for three days at 15°C. Its total fatty acid methyl ester (FAME) fraction included, in addition to DHA, at least two types of minor PUFAs, whose retention time was much longer than that of DHA in gas-liquid chromatography. Gas-chromatography-mass spectrometry analysis of these FAMEs and their pyrrolydide and completely hydrogenated derivatives showed that one of them is a tetracosaheptadecenoic acid [24:7(Δ3,6,9,12,15,18,21)]. These results suggest that the chain length of final products (PLUFs) in the bacterial systems cannot be determined by the Pfa enzyme complex itself and that PUFA-producing bacteria should have a factor(s) stopping the biosynthesis of PUFAs out of the known Pfa system. This is supported by the findings that M. marina MP-1 and other PUFA-producing bacteria have no 24:7(Δ3,6,9,12,15,18,21).

Poster Session

BAM-PW1055 - Protective effect of inositol on survivability of freeze-dried vibrio pathogens in long-term preservation

Hyun Bae Kang[1], Na-Ri Shin[1], Mi-Hee Kim[1], Soonyoung Han[2], Kwang-Jun Lee[1]
[1]Pathogen Resource TF, Center for Infectious Diseases, National Institute of Health, Korea Centers for Disease Control & Prevention, [2]Center for Infectious Diseases, National Institute of Health, Korea Centers for Disease Control & Prevention

A wide range of microorganisms is routinely preserved with skim milk as a protectant in culture collection. However, Vibrio often fails to long-term preservation in this way, resulting in loss of them. The aim of this study is therefore to search an effective protectant for Vibrio preservation and investigate its protective mechanism. The respective pure culture of V. vulnificus, V. parahaemolyticus, and V. cholerae at the early exponential stage was suspended in 10% skim milk and/or 5% inositol solution as a protectant, and aliquoted in sterilized ampoules. After frozen and dried at -80℃ under vacuum, the ampoules were stored for 2 weeks at 37℃ for accelerated test. Freeze-dried bacterium was observed under the electronic microscope and their survivability was determined by flow cytometry and colony count methods. The data from three species commonly showed the greatest viability (108 CFU ml-1) when cultured in 3% NaCl-LB broth after stored with 5% inositol alone and this phenomenon was consistently observed to three non-pathogenic Vibrio species in the same condition. These protective effects of inositol to Vibrio pathogens was also supported by SEM and TEM micrographs showing the individual bacterial cell stably embedded in the inositol matrix and maintenance of the intact bacterial structure including flagella when the cell was reactivated, respectively. In addition, we are currently conducting proteomic analysis of long-term preserved Vibrio cells to find out a protective mechanism of inositol to their flagella and outer membrane proteins as the further study. Taken together, our results suggest that 5% inositol could be a good candidate as a cryo- and lyo-protectant for Vibrio pathogens, resulting from the ability of inositol to stably maintain bacterial structure. Keyword : Inositol, Vibrio pathogens, long-term preservation, protectant

Poster Session

BAM-PW1057 - Antibacterial activity of blood components

Mariam Taha[1, 2], Yuntong Kou[2], Heather Perkins[2], Sandra Ramirez[2]
[1]University of Ottawa, Ottawa, Canada, [2]Canadian Blood Services, Ottawa, Canada

BACKGROUND: Despite the bactericidal activities of plasma proteins, white blood cells and platelets present in blood, bacterial contamination of blood products used for transfusion is the major source of septic transfusion reactions. This indicates that some bacteria are resistant to killing by blood. AIM: To evaluate bacterial susceptibility to different blood components. METHODS: Twenty-one bacteria were screened for their susceptibility to the bactericidal activity of soluble factors of different blood components using a diffusion sensitivity assay on blood agar. Blood components tested here included plasma, buffy coat fractions, and platelet concentrates. Ten-µl drops of the blood components were placed on bacterial cultures and after incubation under ideal growth conditions, clear zones of inhibition surrounding the blood components were measured. Eight bacteria were selected and further tested for their ability to grow in blood components where not only the soluble factors but also the cellular fractions were present. This was done in 96-well plates with four replicates and bacterial growth was evaluated at 24h and 48h post-incubation. RESULTS: Bacteria displayed different degrees of sensitivity to blood components. Commercial isolates of Serratia marcescens and Yersinia enterocolitica showed the greatest susceptibility while clinical isolates of Serratia liquefaciens and S. marcescens resisted elimination by all components. Although other bacteria including Streptococcus agalactiae, Klebsiella pneumoniae and Pseudomonas aeruginosa had growth inhibition in the diffusion assay, they were able to proliferate in the blood components. CONCLUSIONS: In this study, we demonstrate that not all bacterial species have the same sensitivity to the bactericidal action of blood components. Differential sensitivity to soluble (diffusion assay) versus cellular (growth assay) components of blood was also observed. These data merit further investigation of the blood factors involved in bactericidal activity that can be used to increase the safety of transfusion patients.

Poster Session

BAM-PW1059 - Effects of Cordyceps militaris on the growth of rumen microorganisms and in vitro rumen fermentation with respect to methane emissions

Sungsill Lee[1], Shinja Lee[1]
[1]Division of Applied Life Science(BK21), Graduate School of Gyeongsang National University, Jinju, Korea

This experiment was designed to investigate the effects of different concentrations (0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 g/L) of dried Cordyceps militaris on in vitro anaerobic ruminal microbe fermentation and methane production using soluble starch as a substrate. Ruminal fluids were collected from Korean native cattle, mixed with phosphate buffer (1:2), and incubated anaerobically at 38 oC for 3, 6, 9, 12, 24, 36, 48 and 72 h. The addition of C. militaris significantly increased total volatile fatty acid and total gas production. Molar proportion of acetate was decreased and propionate was increased with a corresponding decrease in the acetate:propionate ratio. As the concentration of C. militaris increased from 1.0 to 3.0 g/L, methane and hydrogen production decreased. The decrease in methane accumulation relative to the control was 14.1, 22.0, 24.9, 39.7 and 40.9 % at 1.0, 1.5, 2.0, 2.5 and 3.0 g/L treatments, respectively. Ammonia-N concentration and numbers of live protozoa were decreased linearly with increasing concentrations of C. militaris. The pH of the medium significantly decreased at the highest level of C. militaris addition compared with the control. In conclusion, these results indicate that C. militaris stimulated mixed ruminal microorganism fermentation as well as inhibited methane production in vitro. Therefore, C. militaris could be developed as a novel compound for antimethanogenesis. Key words: Cordyceps militaris, methane, rumen anaerobes, protozoa, rumen fermentation

Poster Session

BAM-PW1061 - Microbial resource research infrastructure: A large-scale research infrastructure for microbiological services

Raquel Hurtado-Ortiz[1], Dominique Clermont[1], Manuela Schüngel[2], Chantal Bizet[1], David Smith[3], Erko Stackebrandt[2]
[1]Centre de Ressources Biologiques, Institut Pasteur, France, [2]Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany, [3]CAB International, UK

Microbiological resources and their derivatives are the essential raw material for the advancement of human health, food security, biotechnology, research and development in all life sciences. Microbial resources, and their genetic and metabolic products, are utilised in many areas such as production of healthy and functional food, identification of new antimicrobials against emerging and resistant pathogens, fighting agricultural disease, identifying novel energy sources on the basis of microbial biomass and screening for new active molecules for the bio-industries. The complexity of public collections, distribution and use of living biological material and service offer, demands the coordination and sharing of policies, processes and procedures. The Microbial Resource Research Infrastructure (MIRRI) is an initiative within the European Strategy Forum Infrastructures (ESFRI), bring together 16 partners including European public microbial culture collections and biological resource centres (BRCs), supported by several European and non-European associated partners. The objective of MIRRI is to support innovation in microbiology by provision of a one-stop shop for well-characterized microbial resources and high quality services on a not-for-profit basis for biotechnology in support of microbiological research. In addition, MIRRI contributes to the structuring of microbial resources capacity both at the national and European levels. This will facilitate access to microorganisms for biotechnology for the enhancement of the bio-economy in Europe. MIRRI will overcome the fragmentation of access to current resources and services, develop harmonised strategies for delivery of associated information, ensure bio-security and other regulatory conditions to bring access and promote the uptake of these resources into European research. Data mining of the landscape of current information is needed to discover potential and drive innovation, to ensure the uptake of high quality microbial resources into research. MIRRI will help the BRCs to work more closely with policy makers, stakeholders, funders and researchers, to deliver resources and services needed for innovation.

Poster Session

BAM-PW1063 - A DNA phosphorothioate-dependent restriction system in Salmonella

Delin You[1], Bo Cao[1], Qiuxiang Cheng[1], Chen Gu[2], Fen Yao[1], Michael DeMott[2], Xiaoqing Zheng[1], Zixin Deng[1], Peter Dedon[2]

[1]State Key Laboratory of Microbial Metabolism and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China., [2]Department of Biological Engineering and Center for Environmental Health Science, Massachusetts Institute of Technology, Cambridge, USA

Incorporation of phosphorothioate (PT) modifications into the DNA backbone is widespread among prokaryotes and is mediated by homologs of a five-gene dndA-E cluster, yet the function of PT modifications is poorly understood. As one physiological role for PT, we recently demonstrated that Salmonella enterica serovar Cerro 87 possesses dndB-E homologs, dpt-B-E, that function with a three-gene cluster, dptF-H, to restrict foreign unmodified DNA during transformation. We have performed a series of studies to define the restriction activity of dptF-H, and found that, unlike traditional restriction-modification (R-M) systems, the presence of restriction genes dptF-H in the PT-deficient mutant (ΔdptB-E) was not lethal, but instead resulted in several pathological phenotypes that suggested genomic instability, including impaired cell separation, slow growth rate, altered colony morphology, and loss of membrane integrity. Subsequent full-genome microarray analysis revealed that loss of dptB-E altered the expression of >600 genes, with the most prominently upregulated being SOS response and DNA repair-related genes. Consistent with a DNA damage phenotype, cells lacking dptB-E, and thus PT, showed significant increases in DNA strand breaks compared to wild-type cells. In vitro studies with purified proteins revealed that DptF and DptH possessed ATPase activity, though nuclease activity was not detected for individual enzymes or combinations. Surprisingly, overexpression of dptF-H was lethal to the host strain in spite of the presence of dptB-E and PT modifications. Along with our previous studies, these results suggest that DptF-H proteins function in an unusual DNA cleavage-based, PT-dependent restriction system and provided new insights into restriction-modification systems.

Poster Session

BAM-PW1065 - RNases and their roles as global regulators: the effects of RNA processing on antibiotic production in Streptomyces bacteria

Stephanie Jones[1], Vivian Leong[2], Joaquin Ortega[3], Marie Elliot[4]
[1]McMaster University, Hamilton, Canada

Streptomyces are soil-dwelling bacteria best known for their ability to produce an impressive array of antibiotics. They have a complex multicellular life cycle, and an improved understanding of the mechanisms governing their development may elucidate novel mechanisms to maximize antibiotic production. Bacterial ribonucleases (RNases) govern development by cleaving RNA, and little is known regarding RNA processing in Streptomyces. Here, we describe how RNase III and RNase J affect Streptomyces development and antibiotic production. We created knockouts of genes encoding both RNases, and both mutant strains have defective phenotypes that are restored to wild type following complementation. Growth assays and microscopy experiments revealed these mutant strains have defective growth at several life cycle stages. Antibiotic assays revealed the RNase J mutant strain produces half as much jadomycin as wild type, while jadomycin production is abolished in the RNase III mutant strain. To determine how these RNases lead to defective phenotypes and reduced antibiotic production, we examined their effects on ribosomal RNA (rRNA) processing. We found RNase III is essential for cleaving rRNA into mature species that are incorporated into ribosomes. We measured whether the mutant strains display increased cold sensitivity, as this phenotype is a hallmark of strains with defective ribosomes, and found the RNase J mutant strain is 17.4% more cold sensitive than wild type, and the RNase III strain is 54% more cold sensitive than wild type. Additionally, we used sucrose density gradients to measure free ribosomal subunits, and found the RNase III mutant strain has two to three times more small and large subunits than wild type, indicating this enzyme is essential for normal ribosome assembly. These results demonstrate a previously unrecognized link between Streptomyces antibiotic production and ribosome activity, suggesting it may be possible to alter RNase or ribosome activity to maximize antibiotic production.

Poster Session

BAM-PW1067 - Whole genome mapping of AtsR/AtsT regulon in Burkholderia cenocepacia by Microarray and ChIP-seq

Maryam Khodai-Kalaki[1], Daniel Aubert[1], Miguel Valvano[1,2]

[1]Department of Microbiology and Immunology, Centre for Human Immunology, Schulich school of Medicine, University of Western Ontario, London, Canada, [2]Center for Infection and Immunity, Queen's University Belfast, Belfast, UK

Burkholderia cenocepacia is a Gram-negative opportunistic bacterium that causes chronic, often serious infections in patients with cystic fibrosis (CF). This bacterium survives in different environments and in a wide range of hosts. Adaptation of B. cenocepacia to the environment of the CF airways probably plays an important role in the establishment of a chronic infection. Sensing and adaptation to new environmental conditions by bacteria is commonly governed by two component regulatory systems leading to modification of gene expression patterns required for bacterial survival. AtsR (Adhesion and Type Six secretion system Regulator) is a global virulence regulator modulating quorum sensing (QS) signalling and the expression of the QS regulated virulence factors including biofilm formation and type 6 secretion system (T6SS) expression in B. cenocepacia K56-2. We previously elucidated the mechanism of AtsR phosphorelay pathway and identified the cytosolic response regulator AtsT (BCAM0381) as a key component of the AtsR phosphorelay pathway. Here, we mapped the whole-genome of B. cenocepacia by performing a microarray analysis of atsR mutant and a ChIP assay coupled with next-generation sequencing (ChIP-seq) of atsT mutant to find genes targeted by this regulatory pathway. We report an applicable ChIP-seq method and compare transcriptional responses of atsR mutants with genomic binding-location to clarify direct and indirect AtsR/AtsT regulon. We validate a subset of AtsT-binding sites in vivo and show a common motif present in AtsT ChIP-seq peaks. Our results reveal a significant number of genes that may be further studied to increase our understanding of the virulence behaviour in B. cenocepacia.

Poster Session

BAM-PW1069 - Examination of the global regulatory role of the CpxR/A two-component system in *Legionella pneumophila*

Jennifer Tanner[1], Laam Li[2], Sebastien Faucher[2], Ann Karen Brassinga[1]
[1]Department of Microbiology, University of Manitoba, Winnipeg, Canada, [2]Department of Natural Resources Sciences, McGill University, Montreal, Canada

Legionella pneumophila is the major causative agent of the atypical pneumonia, Legionnaires Disease. This bacterium is readily found in fresh water environments that include both natural and man-made sources. In these aquatic environments *L. pneumophila* associates with its natural host, protozoa, as an intracellular parasite. Within the protozoan host *L. pneumophila* is able to carry out a dimorphic lifecycle in which it transitions from a replicative form (RF) to a highly infectious cyst-like form (CLF). Inhalation of water droplets from *Legionella*-laden water sources leads to disease in susceptible individuals via its replication within alveolar macrophages. The ability of *L. pneumophila* to navigate these diverse aquatic and eukaryotic environments relies on regulatory networks that include systems able to sense changes within these surroundings. One sensing system identified in *L. pneumophila* is the CpxR/A two-component system composed of a transmembrane sensor kinase, CpxA, and the cognate cytoplasmic response regulator, CpxR. Reports have associated this system with virulence gene regulation; however, its global regulatory role in *L. pneumophila* has not been examined. To this end, an in-frame null mutant of the *cpxR/A* system was generated and transcriptional differences between the mutant and wild-type *L. pneumophila* were assessed at exponential and post-exponential growth phases via microarray analysis. With a two-fold change in expression set as a minimum threshold, it was found that 63 genes were up regulated and 717 were down regulated in the exponential phase, while 35 were up regulated and 792 were down regulated in the post-exponential phase of the mutant. Cluster of Orthologous Genes (COG) analysis shows these transcriptional changes affect several cellular pathways that include motility, metabolism, replication, and translation. Therefore, the CpxR/A system seems to have a broader regulatory role in *L. pneumophila* warranting further investigation to determine how the CpxR/A system is directly regulating these pathways.

Poster Session

BAM-PW1071 - Development of ultrarapid assay system for multiplex detection of air-borne fungi by cyclone air sampler and multiplex PCR-based DNA microarray

Takayoshi Ootsu[1], Atsunori Isshiki[1]
[1]*Corporate Research & Development, Toyo Seikan Group Holdings, Ltd., Yokohama, Japan*

We developed an ultrarapid assay system for multiplex detection of air-borne fungi. The ultrarapid assay system is as follows: Coriolis µ cyclone air sampler and multiplex PCR-based DNA microarray with simultaneous use of internal transcribed spacer region (ITS) of ribosomal RNA gene probe and beta-tubulin gene probe. Both of first and second multiplex PCR was performed with the ITS primer pair ITS1-Fw / ITS1-Rv and the beta-tubulin primer pair Bt2a / Bt2b. We tested detection sensitivities for Aspergillus versicolor DNA with dilution assay (100 pg - 0.05 pg range). The sensitivity of the DNA microarray with first multiplex PCR and second multiplex PCR were 6.25 pg and 0.4 pg (equivalent to 9 spores), respectively. The sensitivity and specificity of this assay was evaluated in comparison with the results of ITS region sequence of isolates collected by surface air system sampler. 1,500 L of air was collected using Coriolis µ cyclone air sampler in 10 mL of sterile water including 0.005% Triton X100 at a flow rate of 300 L / min. DNA samples were extracted from the water containing fungal spores (approximately 750 spores) using a Water DNA Purification Kit (0.22 µm pore size filter). For the combination of first multiplex PCR and DNA microarray, any target fungal species were not detected. However, that of second multiplex PCR, Aspergillus penicilloides, Aspergillus versicolor, Aspergillus vitricola, Aspergillus restrictus and Cladosporium sp. were detected. These results were concordant with the results of ITS region sequence analysis. In conclusion, this assay is ultrarapid technique for simultaneous detection of target air-borne fungi present in indoor environments because it can omit a culture process, and a powerful tool to assess mycoflora and supports to prevent the biological deterioration caused by fungi.

Poster Session

BAM-PW1073 - Molecular diversity analysis of culturable bacterial flora of Diamondback moth, Plutella xylostella from India and its possible role in degradation of Indoxocarb

Ramya Shanivarsanthe Leelesh[1], Venkatesan Thiruvengadam[1], Srinivasa Murthy Kotilingam[1], Jalali Sushil Kumar[1]
[1]*National Bureau of Agriculturally Important Insects, Bangalore, India*

Geographic populations of DBM were screened and analyzed for bacterial diversity. Diverse bacterial species and phylotypes obtained are employed to elucidate the phylogenic relationship between the isolates and their possible role in insecticide degradation. Molecular characterization showed 25 bacterial isolates, out of which 13 from larval gut viz., Enterobacter asburiae, Pseudomonas stutzeri, Enterobacteriaceae bacterium, Leclercia adecarboxylate, Enterobacter cancerogenus, Enterococcus mundtii, Enterobacter sp., Pseudomonas sp., Bacillus cereus and 12 from adult gut i.e. Chryseobacterium sp., Pantoea agglomerans, Enterobacter cloacae, Pseudomonas stutzeri, Pseudomonas putida, Morganella morgani, Pseudomonas aeraginosa, Enterobacter mori, Weissella confuse and Bacillus cereus. All the larval gut bacterial strains belonged to the class Gammaproteobacteria (76%) and Bacilli (15.38%) and belong to the genus Enterobacter (31%), Enterococcus (23%), Pseudomonas (15%), Bacillus (15%) and Leclercia (8%). Adult gut bacterial strains fall into 3 major classes i.e. Gammaproteobacteria (66%), Bacilli (16.6%) and Flavobacteria (16.6%) and most of the strain belongs to the genus Enterobacter (25%) and Pseudomonas (25%), Chryseobacterium (17%), Weissella (9%) and Bacillus, Pantoea and Morganella with 8% each of the total isolates. Enterococcus mundtii (KC985226) and Bacillus cereus (KC985225) showed positive result for degradation in minimal salt medium. Spectrophotometer, colony counting and clear zone studies revealed that the bacterial strains were able to grow in a medium containing indoxocarb as sole carbon source. Further, GC-MS studies are necessary for detection and identification of final metabolite. Bioassay results revealed that the host insect (DBM) of these two strains exhibited moderate level of resistance to indoxocarb i.e. 27.7fold & 11.5fold high compared to susceptible. Also, identification of carboxylesterase in KC985226 (Est-1) and KC985225 (Est-1 & Est-2) revealed its possible role in insecticide degradation. The findings suggest that the bacteria associated with DBM may play role in the degradation of indoxocarb and this could be of interest for developing suitable pest management strategy.

Poster Session

BAM-PW1075 - Application of a single-colony coculture technique for the isolation of previously uncultured gut bacteria

Yoshiki Tanaka[1], Yoshimi Benno[2]
[1]Biofermin Kobe Research Institute, Kobe, Japan, [2]Benno Laboratory, RIKEN, Wako, Japan

Culture independent molecular researches have suggested the relations between gut microbiota and various diseases. On the other hand, cultivation of not-yet-cultured bacteria is essential to complete the extensive studies (bioactivity of individual species, genome analysis, gnotobiotic animal and so on). These detailed researches will provide the more detailed information about the relationships between gut microbiota and human diseases. One of the suggested reasons for uncultivability of bacteria is the lack of the bacteria-bacteria communications in the conventional culture method. To clarify the bacteria-bacteria communications in human intestine, we designed the ring of which bottom consisted of the membrane filter and devised the restricted nutrition medium compared with those of the conventional media. Then gut bacteria were co-cultivated in soft solid agar separated by the membrane filter to simulate the cell-cell communications in vitro without large-scale device. By using the membrane filter co-culture and soft agar, our findings indicate that 1) gut microbiota has multistage bacteria-bacteria communications, 2) we established the isolation method for some symbiotic organisms, 3) and succeeded in cultivating unidentified strains which have so far been difficult to sub-culture. Cultivation and isolation the organisms of which growth is depending on other strains as single colonies lead to handling these bacteria as living cells. Hence, this technique may have great significance. The gut bacteria live densely in narrow enteric canal. There- fore, it is unlikely that their survival strategies are completed without the relations to other strains. The symbiosis observed in this study might be ‘the tip of the iceberg’, and gut bacteria might live keeping complex positive/negative relationships.

Poster Session

BAM-PW1077 - Direct interspecies electron transfer investigation in granular sludge

Charles-David Dubé[1,2], Serge R. Guiot[1,2]
[1]National Research Council Canada, Montreal, Canada, [2]University of Montreal, Montreal, Canada

Direct interspecies electron transfer (DIET) is now an accepted pathway of interspecies electron transfer (IET) for some syntrophic microorganisms. Despite that most studies focused on Geobacter species, evidence suggests that DIET would be occurring in natural environments or in engineered ecosystems as anaerobic digesters. To evaluate the possibility that fermentative and methanogenic bacteria from granular sludge could achieve DIET, a small anaerobic reactor made of two chambers separated by a 100 µm stainless steel sheet, was inoculated on one side by fermentative bacteria, while the other side was either kept abiotic or inoculated with a methanogenic consortium kept starving. The architecture of biofilm that developed on glucose fed to the fermentative chamber on one side of the conductive surface was quite different from that of the biofilm that developed when the methanogenic chamber was kept abiotic. After several days, methane was produced in the chamber on the methanogenic side of the stainless steel sheet; this could mean that electrons were conveyed across the conductive surface and taken by the otherwise starving methanogenic consortium to reduce bicarbonate ions. We demonstrated also that the addition of particles made of conductive material, such as stainless steel and activated carbon, restored at 73±10% and 67±13% respectively the methanogenic activity of disrupted granules as compared to the intact original sludge granules, when using ethanol as a substrate. Non-conductive material such as porcelain restored the methanogenic activity at only 25±4%. Those results suggest that syntrophic bacteria from anaerobic sludge to achieve DIET could use conductive intermediates. As in granules exopolymeric substances (EPS) surround those bacteria, EPS could be therefore implied in DIET process. We therefore proceeded with the characterization of the proteinic fraction of EPS by mass spectrometry to identify candidates, as redox molecules, conductive appendages or enzymes, which could play a role in DIET.

Poster Session

BAM-PW1079 - Bacterial flora diversity associated with different developmental stages of the eastern larch beetle

Audrey-Anne Durand[1], Amélie Bergeron[1], Eric Déziel[1], Claude Guertin[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada

The eastern larch beetle (Dendroctonus simplex LeConte) is an important forest pest in Canada and the United States. This insect is described as a secondary pest because it normally attacks freshly dead or weakened trees. However, under epidemic conditions, D. simplex can also attack healthy trees, causing extended damages to plantations and natural larch stands. Symbiosis between insects belonging to the Dendroctonus genus and microbes is crucial for the organismal health. These microorganisms form a symbiotic complex that helps the insect colonize the subcortical environment of the host tree. These bacteria are involved in the nutritional process and the defence mechanisms, such as terpene resistance or protection against antagonistic microorganisms. However, little is known about the eastern larch beetle microbiome. The goal of the study was to perform a survey of the D. simplex microbial diversity over time, by testing the null hypothesis that the primary bacterial profile remains stable along various development stages of the insect. Samples were taken from the external surface and the interior of the insect body at different developmental stages and bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed. Differences in diversity and abundance between bacterial genera were observed across developmental stages. Phylogenetic analyses were carried out to confirm these dissimilarities. This is the first study revealing bacterial genera that could be associated with a symbiotic complex of the eastern larch beetle.

Poster Session

BAM-PW1081 - Bacterial dominant species and the species richness at the rhizosphere soil of red pepper for the growth stage

do-young ko[1], Geon-hyoung Lee[2]
[1]Jeollabukdo-do Agricultural Research &Extention Services, Gunsan, Korea, [2]Department of Biology, Kunsan National University, Korea

To investigate bacterial dominant species and the species richness at the rhizosphere soil of red pepper during growth stage by pyrosequencing analysis from May to November 2012, the samples were investigated at Agricultural Research & Extention Services Iksan-City, Jeollabuk-Do, Korea. The total bacterial DNA from samples were directly extracted and then PCR anmplication was performed by using primers targeting from V1 ~ V3 regions of the 16S rRNA gene with the extracted DNA. Bacterial species richness was estimated by Chao1 methods. The bacterial species richness, which mesns the number of species, was 6016 on May, 1816 on June, 3007 on July, 1322 on September and 2310 on November at the rhizosphere soi. Compared with Eztaxon database, dominant genera of bacteria on rhizosphere soil were identified as Anaerolinaceae Q500701 on May, Pseudomonas on June, Blastocatella AY28135 on July and November, and Flavobacterium on September. Dominant species of bacteria on rhizosphere were identified as Anaerolinaceae EU134112 on May, Pseudomonas mediterranea on June, Cytophagacea on July, Methylophilaceae EU589321 on September, and Blastocatella FJ478802 on November.

Poster Session

BAM-PW1083 - Microbial diversity in input and output samples of 15 German biogas plants

Stefanie Glaeser[1], Thorsten Schauss[1], Tina Wings[2], Wolfgang Dott[2], Peter Kaempfer[1]
[1]Institute of Applied Microbiology, Justus-Liebig-University Giessen, Germany, [2]Institute of Hygiene and Environmental Medicine, RWTH Aachen, Germany

Input (manure from livestock husbandry or food residues) and output samples of 14 mesophilic and one thermophilic German biogas plants were investigated with respect to their microbial community composition. Microbial fingerprint analysis using denaturing gradient gelelectrophoresis (DGGE) and universal 16S rRNA gene sequence targeting primers showed microbial communities of a high diversity in input and output samples that strongly differed among each other. Cluster analysis of DGGE pattern indicated that microbial communities of input samples differed among each other, but most of the output samples clustered together indicating that the biogas plant processes in general strongly affect microbial community composition in a specific manner. The composition of the microbial community of input and output samples were investigated in more detail by 16S rRNA Gen amplicon sequencing using univseral bacterial primers and the 454 Technology. A total of 6969 to 13852 reads with a mean sequence length of approximately 650 nucleotides were obtained for each individual sample. At the level of phyla, input samples dominated by Firmicutes (22-97%), followed by Actinobacteria (up to 59%), Proteobacteria (up to 26%), and Bacteroidetes (up to 20%). Microbial communities of output samples were also dominated by Firmicutes (35-89%), followed by Bacteroidetes (up to 27%) and Chloroflexi (up to 18%). In contract to input samples, Actinobacteria and Proteobacteria accounted for approximately 5 to 6% in output samples. At the family and genus level output samples were characterized by the high abundance of so far uncultured bacterial taxa, e.g. of the OPB54 cluster of the Firmicutes. First analysis of our data indicated that bacterial communities released with output samples from biogas plants are still unexplored containing mainly uncultured bacteria.

Poster Session

BAM-PW1085 - Stabilization of culturable and non-culturable bacterial species in a consortium as determined by using RTPCR-DGGE and CLPP.

Angelito Abaoag[1], Eng Vui Lai[1]
[1]*IMO Laboratories Pte Ltd, Singapore*

The stability of viable culturable and non-culturable bacterial species in a consortium can be achieved by combining repetitive exposure of the consortium on directed environmental stress, gradual changes in nutrient gradients and fluctuating growth conditions. A stable consortium was developed from environmental samples to develop a probiotic feed additive for the grower and finisher stage of pigs with the following properties: multiple enzyme production, acid and antimicrobial tolerance, and growth factor generations. The initial consortia from different samples were subjected with diverse growth and environmental factors such as carbohydrate types, pH changes, temperature fluctuations and introduction of compounds such as hormones and antibiotics. Initial microbial community and community changes were determined using DGGE-sequencing. Species identification was done by sequencing the cloned, excised, DGGE DNA fragments. Changes in the community physiological profile and growth factor production were determined using phenotype microarray. Stable communities were determined when the community diversity and the physiological profile falls below 10% from the previous stage.

Poster Session

BAM-PW1087 - Selectivity of membrane vesicle attachment to bacterial cells

Yosuke Tashiro[1], Yusuke Hasegawa[1], Masaki Shintani[1], Kazuhide Kimbara[1], Hiroyuki Futamata[1]
[1]Shizuoka University, Hamamatsu, Japan

Many bacteria including both Gram-negative and Gram-positive bacteria secrete membrane vesicles (MVs) in the size of 20-250 nm-diameter. MVs play a role in the transfer of various compounds such as DNA, proteins and low-molecular signal substances to both prokaryotic and eukaryotic cells. As far, MV production mechanism has been studied well and several models have been proposed. On the other hand, there is a little knowledge about a direction of MVs after secretion from bacterial cells. It has been known that Pseudomonas aeruginosa MVs fuse to not only own cells for intraspecies communication but also other bacterial cells for bacteriolysis. We hypothesized that MVs derived from several bacterial species attach specifically to certain cells and they use MVs for a targeting delivery system. Here we analyzed the selectivity of MV-attachment to bacterial cells. MVs were extracted from cultures of several bacterial strains, stained with fluorescent reagent and reacted with bacterial cells. The attachment of MVs to bacterial cells was detected by a fluorescent intensity and confirmed by transmitted electron microscopic observation. In the result of screening analyses, we found that MVs derived from Buttiauxella sp. bound specifically to its own cells. This result suggests that MVs secreted from Buttiauxella sp. may have a mechanism to select recipient cells and a role in intraspecies communication.

Poster Session

BAM-PW1089 - Bacterial and fungal species involved in the resistance of the eastern larch beetle against host tree defenses

Jean-Philippe Buffet[1], Audrey-Anne Durand[2], Narin Srei[3], Eric Déziel[4], Claude Guertin[5]
[1]INRS-Institut Armand-Frappier, Laval, Canada

The eastern larch beetle, Dendroctonus simplex LeConte, is a subcortical herbivorous insect that causes important damages to tamarack (Larix laricina) in North America. This beetle is mainly considered as a secondary pest attacking freshly dead or weakened trees but, under epidemic conditions, it can also kill healthy conifers by overcoming host tree defenses, which consist largely in the production of high terpene concentrations. Resistance mechanisms allowing these insects to cope with these toxic compounds are not well understood. Beetle-associated bacterial and fungal symbionts seem to confer the ability to insects belonging to the genus Dendroctonus to overcome tree defense compounds by insuring terpene detoxification. Moreover, these symbiotic microorganisms are proposed to contribute to the production of pheromones by the transformation of terpene molecules. This study was therefore undertaken to identify and isolate bacterial and fungal species associated with the eastern larch beetle and involved in terpene detoxification. We compared the microbial populations (bacteria and fungi) of insects that have been axenically exposed to saturated concentrations of a volatile monoterpenes mixture versus insects axenically reared in a terpene-free atmosphere. Furthermore, we also investigated the difference in microbiome between male and female individuals reared under the two above conditions. Analysis of microbial communities were performed by the use of culture techniques and bacterial and fungal tag-encoded FLX amplicon pyrosequencing. By visualizing morphological aspect of colonies using culture techniques, we obtained more diversified isolates from insects reared with saturated concentrations of terpenes than from insects reared in a terpene-free atmosphere, but we did not observed clear differences in the diversity of colonies between males and females. Analysis of the diversity using 16S (bacteria) and ITS (fungi) pyrosequencing are currently in process and will be presented. These results will lead to a better understanding of the implication of the beetle microbiome in terpene detoxification.

Poster Session

BAM-PW1091 - Characterization of an adapted microbial population to the bioconversion of carbon monoxide into methane using next-generation sequencing technology

Guillaume Bruant[1], Ruxandra Cimpoia[1], Sylvia Navarro[1], Marie-Josée Lévesque[1], Serge Guiot[1]
[1]National Research Council, Montreal, Canada

Microbial conversion of syngas into higher-value fuels, namely methane, has received an increased attention in the past decades. However, to date, only a small number of microorganisms possessing this capacity have been characterized, and a deeper understanding of the biochemical pathways involved is still needed. Investigating existing methanogenic consortia capable of using syngas as feedstock is thus of great interest. This presentation will focus on the identification of microbial species particularly adapted to the bioconversion of carbon monoxide (CO), a major component of syngas, into methane, and will investigate CO conversion routes to methane under mesophilic temperatures (35˚C). Microcosms containing an anaerobic sludge were incubated for two months at 35˚C and under an atmosphere of 100% CO in the headspace. Sludge samples were taken every two weeks for further analyses. The carboxidotrophic methanogenic potential of the CO-adapted sludge was assessed through kinetic activity tests, and microbial community analyses were performed using next-generation sequencing technology. The non-adapted sludge presented an interesting carboxidotrophic methanogenic potential and it was possible to achieve a higher methanogenic potential by acclimation of the sludge to high CO concentrations. A shift in the microbial population was then observed. At the end of test the bacterial population was dominated by members of the order Clostridiales, notably the genera Acetobacterium, Oscillibacter and Clostridium. In addition, fermentative acetate, H2/CO2 producing and acetate oxidizing bacteria, were detected suggesting a possible shift of substrate conversion routes to methane. The archaeal population, first dominated by acetoclastic methanogens evolved into a mix of acetate and hydrogen-utilizing methanogens dominated by hydrogenophilic methanogens such as the genus Methanobacterium. The identification of microbial species adapted to the bioconversion of CO into methane is presently extended to the study of microbial diversity evolution in a bubble column reactor run under mesophilic conditions.

Poster Session

BAM-PW1093 - Off-site impacts of agricultural composting: role of terrestrially derived organic matter in structuring aquatic microbial communities and their niche breadth

Thomas Pommier[1], Asmaa Merroune[2], Yvan Bettarel[3], Patrice Got[3], Jean-Louis Janeau[2], Pascal Jouquet[2], Thuy Doan Thu[4], Tran Duc Toan[4], Emma Rochelle-Newall[2]

[1]Microbial Ecology Center, Villeurbanne, France, [2]Institute of Ecology and Environmental Sciences, Paris, France, [3]Ecology of coastal marine system, Montpellier, France, [4]Soils and Fertilisers Research Institute, Hanoi, Viet Nam

While considered as sustainable and low-cost agricultural amendments, the impacts of organic fertilizers on downstream aquatic microbial communities remain poorly documented. We investigated the quantity and quality of the dissolved organic matter leaching from agricultural soil amended with compost, vermicompost or biochar and assessed their effects on lake microbial communities, in terms of viral and bacterial abundances, community structure and niche breadth. The addition of compost and vermicompost significantly increased the amount of dissolved organic carbon in the leachate compared to soil alone. Leachates from these additions, either with or without biochar were highly bioavailable to aquatic microbial communities, though reducing their niche breadth to more specific communities. Although not affecting bacterial richness or taxonomic distributions, the specific addition of biochar strongly affected the original lake bacterial communities resulting in a strongly different community. These could be partly explained by viral burst and converging bacterial abundances throughout the samples. These results underline the necessity to include off-site impacts of agricultural amendments when considering their cascading effect on downstream aquatic ecosystem.

Poster Session

BAM-PW1095 - The effect of oxygen on metabolism of Streptococcus intermedius

Michelle Mendonca[1], Michael Surette[1]
[1]*McMaster University, Hamilton, Canada*

The Streptococcus Anginosus Group (SAG) are considered commensals, found in about 30% of the population, but are also one of the most common pathogens in pyogenic infections including empyemas and abscesses. They are often isolated along with obligate anaerobes, suggesting an anaerobic host environment. Streptococci, in general, are facultative anaerobes that use glycolysis, anaerobic respiration and lactic acid fermentation for energy production. They lack components of the Krebs cycle. Oxygen presence during growth conditions affects which carbon source is used as well as the kind of byproduct produced (by anaerobic respiration or lactic acid fermentation). The aerobic vs. anaerobic metabolism was studied in strain *S. intermedius* B196, a hip abscess isolate from a cystic fibrosis patient. SAG growth kinetics differed with faster growth in conditions with lower levels of oxygen. In addition, it was found that SAG are capable of depletion of oxygen in a closed environment. Differential RNA-Seq analysis in late log phase detected induction of genes involved in oxidative stress under aerobic conditions. The ahpC/nox-1 operon, which is involved in NADH oxidation and alleviating stress from hydrogen peroxide, were upregulated 25 fold. Under anaerobic conditions, there was an induction of genes involved in carbohydrate uptake, lactic acid fermentation and glycogen synthesis. The ability of the SAG to rapidly deplete oxygen may be a mechanism to facilitate anaerobe growth in polymicrobial infections.

Poster Session

BAM-PW1097 - Characterization of a facultative anaerobic bacterium, representing novel class- or phylum-level taxon

Miho Watanabe[1], Hisaya Kojima[1], Manabu Fukui[1]
[1]Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

Culture-independent analyses of microbial community structure have revealed vast diversity of uncultured microbes in the natural environment. There are numbers of candidate divisions/phyla of bacteria that include no cultivated representatives. In this study, we isolated and characterized a novel strain HC45 representing the novel class- or phylum- level taxon. Strain HC45 was obtained from enrichment culture established under sulfate-reducing conditions at 45°C. The inoculum was sediment of Lake Harutori, a meromictic lake containing high concentration of sulfide in hypoxia. Isolation of the strain HC45 was carried out by repeated dilution-extinction method. Phylogenetic analysis based on 16S rRNA gene indicated that Moorella humiferrea shows the highest sequence similarity to the strain HC45. The novel strain had exceedingly low similarity with related strains (85% or less). Phylogenetic analysis based on several housekeeping genes showed that the strain HC45 and a few bacteria make independent cluster separated from known lineage. The taxonomic status of these bacteria has not been clarified. The strain HC45 could utilize glucose as carbon and energy source. The addition of yeast extract enhanced growth of the strain HC45. Facultative anaerobic growth on 2% NaCl-R2A medium was observed. Gram-staining test was negative. Morphologically, the cells were unbranched slender filaments, 10 µm and more in length. All of the environmental sequences related to the novel strain have been collected from compost soil, activated sludge and sewage.

Poster Session

BAM-PW1101 - Taxonomic evaluation of Streptomyces hirsutus and related species using multi-locus sequence analysis

David Labeda[1], Xiaoying Rong[2], Ying Huang[2], James Doroghazi[3], Ku-San Ju[3], William Metcalf[3]
[1]National Center for Agricutural Utilization Research - USDA/ARS, Peoria, USA, [2]Institute of Microbiology, Chinese Academy of Sciences, Beijing, PR China, [3]University of Illinois, Institute for Genomic Biology, Urbana, USA

Phylogenetic analyses of species of Streptomyces based on 16S rRNA gene sequences resulted in a statistically well-supported clade (100% bootstrap value) containing 8 species having very similar gross morphology. These species, including Streptomyces bambergiensis, Streptomyces chlorus, Streptomyces cyanoalbus, Streptomyces emeiensis, Streptomyces hirsutus, Streptomyces prasinopilosus, Streptomyces prasinus and Streptomyces viridis, all produce open looped (Retinaculum-Apertum) to spiral (Spira) chains of spiny to hairy surfaced, dark green spores on the aerial mycelium. These strains were subjected to multilocus sequence analysis (MLSA) utilizing partial sequences of the house-keeping genes atpD, gyrB, recA, rpoB, and trpB to clarify their taxonomic status. Phylogenetic trees were constructed using three different algorithms using alignments resulting from head to tail concatenation of the house-keeping loci. MLSA distances (Kimura 2-parameter distance) were calculated between the species pairs in this clade to determine if any were related at the species level (i.e., MLSA distance < 0.007). The MLSA data suggest that several pairs of the species under study are likely be con-specific, more specifically: S. cyanoalbus NRRL B-3040T represents a later synonym of S. hirsutus NRRL B-2173T (MLSA distance is 0.002) with identical alleles of 3 loci and only 2 bp differences over the 2519 bp alignment; S. bambergiensis NRRL B-12521T is a probable later synonym of S. prasinus NRRL B-2712T (MLSA distance is 0.003) with identical alleles for two loci and 7 bp differences over the 2519 bp alignment; S. eimeiensis NRRL B-24621T is a probable later synonym of S. prasinopilous NRRL B-2711T (MLSA distance is 0.003) with identical alleles of the rpoB locus and 7 bp differences over the 2519 bp alignment, predominantly in the recA locus. Streptomyces chlorus KACC 20902T and Streptomyces viridis KACC 21003T were observed to exhibit MLSA distances of > 0.007 to the other strains studied and therefore represent unique species.

Poster Session

BAM-PW1103 - Nostoc (Cyanobacteria): a polyphyletic genus with a primitive morphology within heterocytous cyanobacteria

Pavel Hrouzek[1], Jan Mares[2], Stefano Ventura[3]
[1]Institute of Microbiology, Czech Academy of Science, Trebon, Czech Republic, [2]Institute of Botany, Czech Academy of Science, Trebon, Czech Republic, [3]Institute of Ecosystem Study, CNR, Sesto Fiorentino, Italy

Although true prokaryotes, very few taxa of Cyanobacteria have been validly described and named under the Bacteriological Code; instead they have been and still are described under the Botanical Code as Cyanophyta. Even the genus name Nostoc, in spite of the widespread diffusion and ecological significance of its species and of their relevance for society, has no standing under the Bacteriological Code. Nostoc presents the typical morphological characters for the Nostocales: isopolar filaments which differentiate akinetes and heterocytes. Its morphological description is based on the formation of uniseriate isopolar filaments. Chains of akinetes differentiate following an apoheterocytic scheme, while heterocytes develop in both terminal and intercalar positions. The filaments are non-branched and always embedded in mucilage. Primary diagnostic characters of the genus are the production of mucilaginous colonies, and a complex life cycle. Both these characters are highly heterogeneous among Nostoc species. On the basis of results obtained applying molecular approaches on large strain selections, the genus Nostoc was again found to be genetically heterogeneous and having a likely polyphyletic origin. However, a large and well supported cluster including strains of the type species of the genus, Nostoc commune Vaucher ex Bornet et Flahault, the widely used strain Nostoc punctiforme PCC 73102, and several other well-defined Nostoc species with a firm sheath (N. desertorum, N. lichenoides, N. indistinguendum, N. edaphicum) was repeatedly found. This clade, also referred to as Nostoc sensu stricto, had the unifying feature of origin in soils, with many isolates well characterized by the production of massive laminar mucilaginous colonies. At least five other groups of cyanobacterial strains exhibiting Nostoc morphology, but falling outside the main cluster have been identified. A well-supported group of Nostoc muscorum strains located outside Nostoc sensu stricto required description as a new genus Desmonostoc to make the genus Nostoc monophyletic.

Poster Session

BAM-PW1105 - Development of a single nucleotide variant (SNV) assay for detection and surveillance of Salmonella Enteritidis

Muhammad Rehman[1], Kim Ziebell[1], Chad Laing[1], Patrick Boerlin[2], Linda Chui[3], John Devenish[4], Matthew Gilmour[5], Sadjia Bekal[6], John Nash[1], Roger Johnson[1]

[1]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Canada, [2]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada, [3]Provincial Laboratory for Public Health, Edmonton, Canada, [4]Canadian Food Inspection Agency, London, Canada, [5]Diagnostic Services of Manitoba, Winnipeg, Canada, [6]Labarotoire de santé public du Québec, Sainte-Anne-de-Bellevue, Canada, [7]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada

Salmonella Enteritidis (SE) is a frequent cause of human salmonellosis linked to consumption of a wide variety of contaminated foods. Because SE is genetically homogenous, current typing methods such as phage typing and pulsed-field gel electrophoresis (PFGE) often fail to discriminate epidemiologically related from unrelated isolates, limiting outbreak detection and source attribution. We propose a Single Nucleotide Polymorphism (SNP) or more appropriately Single Nucleotide Variant (SNV) analysis as most reliable approach for subtyping SE. Whole genome sequences (WGS) of 200 epidemiologically related and unrelated Canadian SE isolates representing diverse phage types and PFGE patterns were obtained by two platforms: Roche 454 at ~40X coverage and Illumina at ~70X coverage. Using reference guided alignment of SE genomes we have identified a panel of 15 informative SNVs validated by amplicon sequencing. Phylogenetic analyses were used to evaluate the ability of these SNVs to predict the relatedness among our 200 strains, and among 96 publicly available draft genomes from the 2010 US shell egg outbreak. These 15 SNV resolved our 200 isolates into 21 SNV profiles representing 6 major phylogenetic clusters, appropriately distinguished among isolates with the same PT and PFGE patterns, and clinical isolates with epidemiologic linkages accurately clustered together. Applied to the genomes of 96 US SE strains, mostly of the same PFGE type, our 15 SNVs resolved these isolates into eight clades. The 15 SNV based assay correlated with PT and PFGE patterns and additionally discriminated epidemiologically related from unrelated isolates that were indistinguishable by PT and PFGE. Our data suggests that SNV subtyping approach have a substantial discriminatory power, over other genetic typing methods, with potential for clustering cases with common exposures not resolved well by phage typing or pulsed-field gel electrophoresis.

Poster Session

BAM-PW1107 - Molecular genotyping of multi drug resistant Mycobacterium tuberculosis strains in Morocco

Fathiah Zakham[1,5], Imane Chaoui[1], Amina Hadbae Echchaoui[2], Fouad Chetioui[3], My Driss Messaoudi[3], My Mustapha Ennaji[4], Mohammed Abid[2], Mohammed El Mzibri[1]

[1]Unité de Biologie et recherché Médicale, CNESTEN, Rabat, Morocco, [2]Laboratoire de génétique mycobacterienne. Institut Pasteur, Tangier, Morocco, [3]Laboratoire de Tuberculose. Institut Pasteur, Casablanca, Morocco, [4]Laboratoire de Microbiologie, Hygiène et virologie, Faculté des Sciences et Techniques, Mohammedia, Morocco, [5]Laboratory of Microbiology, Faculty of Medicine and Health Sciences, Hodeidah, Yemen

Tuberculosis (TB) is a major health public problem with high mortality and morbidity rates especially in low income countries. This study is an attempt to establish a rapid diagnosis of MDR TB by sequencing the target fragments of rpoB gene which linked to resistance against Rifampicin and KatG gene and inhA promoter region, which are associated to resistance to Isoniazid. For this purpose, 133 sputum samples of TB patients from Morocco, where enrolled in this study. 100 samples were collected from new cases, the rest 33 were previously treated patients (drug relapse or failure, chronic) and did not respond to anti TB drugs after a sufficient duration of treatment. All samples were subject to rpoB katG and pinhA mutations analysis by PCR and DNA sequencing. Molecular analysis showed that 7 strains were isoniazid monoresistant and 17 were rifampicin monoresistant. MDR TB strains were identified in 9 cases (6.8%). Among them, 8 were traditionally diagnosed as critical cases: four chronic and four drug-relapse cases. The last strain was isolated from a new case. The most recorded mutation in the rpoB gene is the substitution TCG > TTG at codon 531 (Ser531Leu) accounting for 46.15%. Significantly, the only mutation found in katG gene was at the codon 315 (AGC to ACC) with Ser315Thr amino acid change. Only one sample harbored mutation in the inhA promoter region and was a point mutation at -15p position (C > T). The PCR-sequencing approach is an accurate and rapid method for the detection of drug-resistant TB in clinical specimens, and could be of great interest in the management of TB in critical cases to adjust the treatment regimen and limit the emergence of MDR/XDR strains.

Poster Session

BAM-PW1109 - Meningococcal serogrouping method of loop-mediated isothermal amplification

Mitsuko Seki[1], Paul Kilgore[2], Do Kyoung Lee[3], Eun Jin Kim[3], Hideyuki Takahashi[4], Makoto Ohnishi[4], Soon Ae Kim[5], Dong Wook Kim[3]

[1]Nihon University, Tokyo, Japan, [2]Wayne State University, Detroit, USA, [3]Hanyang University, Ansan, Republic of Korea, [4]National Institute of Infectious Diseases, Tokyo, Japan, [5]International Vaccine Institute, Seoul, Korea

We established the meningococcal serogrouping method of loop-mediated isothermal amplification (LAMP) and assessed its suitability for detecting meningococcal serogroups in cerebrospinal fluid (CSF) The LAMP assays targeting ctrA and serogroup-specific genes were developed to detect of Neisseria meningitidis and identify six major serogroups (A, B, C, X, Y, and W-135), respectively. The LAMP assays were evaluated using a set of 1,574 randomly selected CSF specimens from children with suspected meningitis collected between 1998 and 2002 in Vietnam, China and Korea. The specificity of the primer sets were validated using 14 N. meningitidis (serogroup A, B, C, D, 29-E, W-135, X, Y and Z) strains and 15 non- N. meningitidis species. Within 60 minutes, the assays could detect ten to 100 copies of purified N. meningitidis DNA with sensitivity more than 1,000 times that of conventional polymerase chain reaction (PCR) assays. The LAMP method targeting ctrA proved to be more sensitive than previously described PCR methods when using CSF samples (31 CSF were positive by LAMP vs. 25 by PCR). The detection rate of the LAMP methods was substantially higher than that of the PCR method. In comparative analysis of PCR and LAMP assay, the clinical sensitivity and specificity of LAMP assay was found to be 100% and 99.6%, respectively. The thirty one ctrA LAMP positive specimens were analyzed by serogrouping LAMP. The LAMP assay identified serogroups of 29 of the N. meningitidis in CSF specimens. In conclusion, highly accurate LAMP assays have been developed for identification of N. meningitidis infection including specific meningococcal serogroup.

Poster Session

BAM-PW1111 - Phenotypic and genotypic characterisation of Legionella pneumophila clinical isolates in Slovenia

Darja Kese[1], Rok Kogoj[1], Misa Korva[1]
[1]*Institute of Microbiology and Immunology, UL MF, Ljubljana, Slovenia*

Legionella pneumophila is a respiratory pathogen that can be ubiquitously found in aquatic environments. It is separated into 15 serogroups, however. L. pneumophila sg. 1 is most frequently implicated in human disease and can be further phenotyped using monoclonal antibodies of the Dresden panel or genotyped by SBT. As Legionella colonizes large man-made aquatic systems such as hospitals, hotels, whirlpools, cooling towers, etc., it is important for public health purposes to monitor patients and environmental sources. From January 2006 to August 2013 a total of 1085 lower respiratory tract samples of pneumonia patients were investigated for the presence of Legionella DNA by using real-time PCR. Infection with L. pneumophila was confirmed in 41 (3,78 %) patients. All 41 patients had also L. pneumophila urinary antigen positive by ELISA. Using the remainder of the respiratory sample from all PCR positive samples Legionella cultivation was performed. Isolates of L. pneumophila were successfully obtained and identified by MALDI-TOF from 23 (53.5%) patient samples, and were further used for serogroup determination, phenotypic and genotypic investigation. Serogrouping results show that 21 (91.3%) isolates belong to serogroup 1 and only 2 (8,7%) to serogroup 2-15. Phenotyping by MAb of L. pneumophila sg 1 isolates showed 6 different subgroups. The most frequent phenotype was Knoxville (55%) followed by Bellingham (15%), Oxford (10%), Benidorm (10%), Philadelphia (5%) and Allentown/France (5%). Genotyping results show even greater diversity as 14 sequence types (ST) were found. The most common ST was ST1 (26.9%) followed by ST23 (11.5%) and ST728 (7.7%). All other ST namely 37, 62, 203, 334, 421, 435, 762, 1299, 1424 and two not yet described ST's were found each in only one case. These data shows that L. pneumophila is greatly diverse in Slovenian patients which make epidemiological investigation of L. pneumophila cases very important.

Poster Session

BAM-PW1113 - Assessment of collagen-like genes as biomarkers for the detection of Burkholderia pseudomallei and Burkholderia mallei species

Beth Bachert[1], Slawomir Lukomski[1]
[1]*West Virginia University Health Sciences Center, Morgantown, USA*

Burkholderia pseudomallei, the causative agent of melioidosis, and *Burkholderia mallei*, the causative agent of glanders, are highly infectious and broad-spectrum resistant organisms that have been classified as category B bioterrorism agents. PCR based assays are a promising alternative to current detection methods for *Burkholderia*; however, identification of reliable biomarkers is difficult due to high genomic plasticity reported in these organisms. Here, we investigated the usefulness of collagen-like genes as potential targets for *Burkholderia* spp. detection. Using the Pfam database, we initially identified 69 collagen-like proteins annotated to 31 *Burkholderia* strains, which were categorized into 13 Bucl (*Burkholderia* collagen-like) protein types. Nucleotide BLAST searches revealed the presence of all 13 *bucl* genes in eight completed genomes of *B. pseudomallei*. Several *bucl* genes were absent in the genomes of four *B. mallei* strains, consistent with the evolution of *B. mallei* by genome reduction, whereas *bucl* genes were largely missing in the two sequenced genomes of non-pathogenic *B. thailandensis*. Mapping of *bucl* markers in 12 sequenced *B. pseudomallei* and *B. mallei* genomes showed considerable genomic rearrangements, and multiple sequence alignments for all *bucl* alleles showed length variation in the collagen-like regions, while the non-collagenous regions were conserved. Primers were designed targeting the conserved *bucl* sequences and amplification conditions were optimized with DNA from *B. pseudomallei* K96243. Two *bucl* genes, designated 1 and 10, were further tested against genomic DNA from 23 *B. pseudomallei* and 17 *B. mallei* strains, as well as 16 control DNA from *B. thailandensis*, *B. cepacia*, *B. cenocepacia*, and *B. multivorans* strains. PCR yielded *bucl1*-specific amplicons from DNA of all *B. pseudomallei* and *B. mallei* strains, while *bucl10* was detected in most of these strains and no amplification was recorded for negative controls. In summary, *bucl* genes represent promising targets for the identification of *B. pseudomallei* and *B. mallei* species.

Poster Session

BAM-PW1115 - Typing of Environmental Legionella via MALDI-TOF MS

Otto Schwake[1], Todd Sandrin[2], Lin Zhang[2], Morteza Abbaszadegan[1]
[1]Arizona State University, Tempe, United States, [2]Arizona State University West Campus, Glendale, USA

As a waterborne pathogen of increasing concern, *Legionella* are responsible for more drinking water outbreaks than all other microbes combined, thus demonstrating the relevancy of research aimed at developing and improving methodology for the characterization of these microbes. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has potential for use as a rapid and cost efficient method for protein-based molecular typing of bacterial strains. While progress has been made in the application of this technique for the characterization of *Legionella* at the genus and species levels, differentiation of environmental isolates of *Legionella* strains has not previously been described at length. The goals of this study were twofold: 1) to optimize sample preparation methods for the analysis of environmental *Legionella* isolates via MALDI-TOF MS, and 2) to implement these methods to perform typing on environmental *Legionella* isolates from Central Arizona. A MALDI-TOF MS sample preparation method involving agar based culturing and protein extraction was able to reliably produce high quality mass spectra, which were used to compare 28 *Legionella* isolates from two separate drinking water distribution systems via cluster analysis. Generated spectra were used to construct a dendrogram, with similarity between isolates of 90% (determined through analysis of strains previously determined through molecular analysis) used as a benchmark for strain differentiation. PCR analysis confirmed that multiple species were detected, including *L. pneumophila*, and strain level characterization was achieved, with 12 unique prospective strains identified. Isolates of *L. pneumophila*, the most common species observed in the study, were able to be correctly typed to specific sampling sites. These results demonstrate the ability for this technology to be utilized in the sub-species characterization of bacteria with significant benefits over established methodologies.

Poster Session

BAM-PW1117 - Rapid identification of Mycobacterium using mycolic acids by MALDI spiral-TOFMS with ultra high mass-resolving power

Kanae Teramoto[1], Nagatoshi Fujiwara[2]
[1]JEOL Ltd., Akishima, Japan, [2]Tezukayama University, Nara, Japan

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) has been applied to the rapid identification of bacteria. The mass spectra of ribosomal proteins are specific to the genus and species. However, Mycobacterium tuberculosis has a robust cell wall and low ribosomal protein density, making MALDI-TOFMS unsuitable to detect the ribosomal proteins of mycobacterial cells. On the other hand, mycolic acids (MAs), which are long carbon-chain fatty acids (FAs), have been traditionally used as chemotaxonomic markers of Corynebacterineae including Mycobacterium. MAs are heterogeneous in carbon-chain length, functional groups, and number of double bonds. MAs are detected on thin-layer chromatography (TLC), and analyzed by gas chromatography and high performance liquid chromatography. Recently, MALDI-TOFMS has been applied to analyze the carbon-chain lengths of MAs purified by TLC. These traditional methods require cumbersome procedures and specialized knowledge for data analysis. In our previous study, MALDI spiral-TOFMS with high mass-resolving power was used to analyze short-carbon chain MAs from the total FA fraction directly. In this study, direct analysis of the FA fraction was applied to identify Mycobacterium. Due to the high mass-resolving power and high mass accuracy, MAs were successfully analyzed without pre-purification. The relative peak intensities of the total MAs of each Mycobacterium species, for example M. tuberculosis and M. bovis, were compared. MALDI spiral-TOFMS enabled direct assignment of MAs from the total FA fraction and easy identification of Mycobacterium. This technique is applicable not only to rapid identification but also to verification of the effects for anti-tubercular drugs targeting the MA synthesis pathway.

Poster Session

BAM-PW1119 - 454 pyrosequencing – modern method for prediction of chronic periodontitis patients

Stepan Podzimek[1], Tatjana Janatova[1], Jaroslav Mysak[1], Lenka Sabova[2], Zdenek Broukal[1], Jana Duskova[1]
[1]The Institute of Clinical and Experimental Dental Medicine, First Faculty of Medicine and GUH, Prague, Czech Republic,
[2]Institute of Microbiology, Academy of Sciences, Prague, Czech Republic

Considerable body of evidence shows bacterial community and especially its anaerobic compartment residing periodontal environment as the causative agents of periodontal diseases. The cultivation of these fastidious microorganisms is very demanding and many of them are not cultivable at all. Microbiological detection and diagnostic techniques so far employed in periodontal microbiology detected several keystone bacteria as putative pathogens worth to be used as markers of progression risk in periodontal pathology. Aim: The aim of this study was test the relevance of the 454 pyrosequencing technique for the simplified identification of the broad spectrum of periodontal microbiome including keystone bacteria Tannerella forsynthia, Porphyromonas gingivalis, Treponema denticola, etc. for the prediction of risk of follow-up periodontal breakdown. Material and methods: 15 patients with adult periodontitis (AP) cared in periodontal maintenance regime, 15 patients with AP without previous therapy and 18 periodontally healthy individuals were involved in the study. All subjects were sampled for the contents of periodontal pockets/gingival sulci and the material was processed in the 454 pyrosequencing technology. An attempt has been made to define, based on results of individual samples analyses, the risk bacterial pattern (RBP). Results: Both groups of periodontally involved patients showed the RBP while in periodontally healthy individuals this RBP was found only occasionally. Conclusion: Screening periodontal bacterial community by means of the 454 pyrosequencing technology can thus provide significant data for the risk assessment in patients scheduled for surgery of heart or big vessels. Acknowledgement: The study was supported by research project PRVOUK-P28/LF1/6 of the Ministry of Education, Youth and Sports, Czech Republic and by projects NT 13087-3 and NT 14164-3 of the Internal Grant Agency, Ministry of Health, Czech Republic.

Poster Session

BAM-PW1121 - MS-H, a mass spectrometry-based rapid E. coli flagella typing at molecular level

Keding Cheng[1], Angela Solan[1], Lorea AM Peterson[1], Celine Nadon[1], J David Knox[1], Gehua Wang[1]
[1]*National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada*

Escherichia coli (E. coli) bacteria are major sources of food contamination that have significantly impacted both health and economics worldwide. The ability to identify particularly virulent strains and to rapidly determine sources of contamination are required to mitigate the adverse effects that these pathogens cause. The current gold standard of identifying E. coli is known as serotyping and is based on agglutination reactions between bacteria cultures and antisera raised against bacterial surface molecules, either O or H antigens. H antigens are related to bacterial flagella, the filament structures involved in motility. Major drawbacks to serotyping, especially on H antigens, are its time consuming nature, typically requiring 2-12 days to type a bacterial strain, due to motility induction and multiple agglutination reactions required to get a final serotype. Consequently, serotyping is a constraint when rapid diagnosis is of paramount importance during E. coli outbreaks. Recently, we developed a novel approach called MS-H for fast, sensitive, and accurate H antigen typing. Instead of using antisera, H antigens are identified by liquid chromatography and tandem mass spectrometry (LC-MS/MS) of purified, digested flagella. The method is straightforward and sensitive, typing H antigens at the protein sequence level straight from plate culture within a few hours without the need for motility induction. We have demonstrated that all 53 E. coli H antigens can be typed with this novel platform, and a one year study of 127 clinical E. coli isolates from three Canadian provinces showed MS-H typing to surpass traditional serotyping in both speed and accuracy. This new platform will be very useful for rapid and accurate typing of E. coli H antigens, especially in outbreak situation.

Poster Session

BAM-PW1123 - Effect of parasite dose on immune response of Leishmania tropica infection of BALB/c mice

Hamid M. Niknam[1], Davood Jafari[2], Maryam Abolghazi[3], Farzin Seyfpoor[2], Firoozeh Abrishami[1], Mosayeb Rostamian[1]

[1]Immunology Department, Pasteur Institute of Iran, Tehran, Iran, [2]Department of Veterinary Parasitology, Science and Research branch, Islamic Azad University, Boroujed, Iran, [3]Faculty of Basic Science, Qom Islamic Azad University, Qom, Iran

Leishmania tropica (L. tropica) is a causative agent of cutaneous leishmaniasis. The dose of infective parasite is an important variable in the experimental model of this infection. We studied the effect of parasite dose on the immune response in murine model. High dose was compared with low dose with regard to their effect on cytokine responses in BALB/c model of L. tropica infection. Aim of this study is to move towards a suitable experimental model for L. tropica infection. L. tropica was cultured and infective parasite was prepared. Two different doses (high dose and low dose) of the parasite were used to infect BALB/c mice. Mice were inspected clinically at different intervals and lesion development was assayed. Some mice were killed and cells were prepared from lymph nodes draining the infected tissue at one week, one month, and four months after infection. Cells were stimulated with soluble leishmania antigen of L. tropica and Interferon-gamma and Interleukin-10 were assayed in culture supernatant. Our results showed that: 1) Low dose as well as high dose did not result in production of Interleukin-10 at any interval studied. 2) Low dose resulted in no Interferon-gamma production at any interval studied. 3) High dose resulted in no Interferon-gamma production at one week and one month after infection, but Interferon-gamma was produced at four months after infection. Our study showed that up to 4 months after infection, the dose of L. tropica does not affect Interleukin-10 response, but the dose affects Interferon-gamma response. These findings suggest that in L. tropica murine model, Interferon-gamma may be used for studying the immune response in "high dose" infection model. However, in "low dose" infection model longer time periods may be necessary for studying Interferon-gamma or Interleukin-10 responses.

Poster Session

BAM-PW1125 - Alterations in peptidoglycan structure suppress defects in secretin assembly caused by mutation of the type two secretion system components ExeA and ExeB in Aeromonas hydrophila

Elizabeth Vanderlinde[1], S. Peter Howard[1]
[1]*University of Saskatchewan, Saskatoon, Canada*

The secretion of exoproteins by the type two secretion system (T2SS) is a widespread virulence mechanism of Gram negative pathogens. The secretin, known as GspD, is an outer membrane protein that forms the pore through which folded proteins are exported. The size of the GspD monomer and its similarity to other secretins that appear to completely cross the periplasm, suggest that it would require chaperoning to cross the cell wall or even to enable assembly directly within the peptidoglycan matrix. In Aeromonas hydrophila, assembly of the secretin ExeD requires a complex of two proteins, ExeA and ExeB. ExeA has been shown to bind peptidoglycan and form multimers. The ExeA-peptidoglycan association is also required for assembly of ExeD in the outer membrane. The objective of this research is to determine whether the ExeAB complex is involved in chaperoning ExeD through the peptidoglycan sacculus. Growth in glycine-rich media has been shown to affect peptidoglycan structure by reducing the number of peptide crosslinks. In A. hydrophila growth in buffered LB (bLB) medium with 2 % glycine decreased the MIC for carbenicillin and ampicillin by 10-fold and 4-fold, respectively, relative to bLB without glycine, suggesting it is affecting the structure of the cell wall. We also found that growth in bLB + 2% glycine suppressed the secretin assembly defects of strains with point mutations in ExeAB, and restored secretion of lipase to wild-type levels. Furthermore, the observed suppression was not dependent on the amount of ExeAB, suggesting that the main function of the complex is to alter the peptidoglycan structure to allow assembly of ExeD. Additional experiments to analyze the structure of the glycine-modified peptidoglycan, to determine the affect of penicillin-binding protein mutations on ExeD assembly in an ExeAB mutant, and to assess the affect of glycine on assembly of other secretins are on-going.

Poster Session

BAM-PW1127 - Analysis of the role of the interaction of VirB6 with VirB8 and VirB10 in Type IV secretion systems

Charline Mary[1], Ana Maria Villamil Giraldo[1], Christian Baron[1]
[1]*Département de biochimie et médecine moléculaire, Université de Montréal, Montréal, Canada*

Infectious diseases are a major problem worldwide in developed and developing countries. In order to promote infection, many bacterial pathogens employ multicomponent protein complexes to deliver macromolecules directly into their eukaryotic host cell, like type IV secretion systems (T4SS). T4SS are important for two reasons: genetic exchange (conjugation and DNA export or import) and the release of effectors (proteins, macromolecular complexes or DNA-protein complexes) into the target cell. These functions allow adaptation of pathogens to environmental change or disruption of host defense mechanisms. T4SSs are typically composed of 12 components (VirB1-VirB11 and VirD4) organized into ATP-powered cell envelope-spanning complexes. T4SSs consist of three groups of proteins: ATPases (VirB4, VirB11, and VirD4), core components (VirB6-VirB10) and surface-exposed components (VirB2 and VirB5). In our research we analyze the role of the interaction of VirB6 with VirB8 and VirB10 in T4SS using a three-pronged approach to gain biochemical and structural information on these interactions and their functional consequences. First, an Ala-scanning mutagenesis of the 24 amino acids region of the interacting domains was conducted to identify functionally important residues. Second, the effects of changes on VirB6 function will be tested using *in vivo* assays of T4SS function in *Agrobacterium tumefaciens*. Third, to characterize the interactions of VirB6 with both VirB8 and VirB10 these proteins will be overexpressed, purified and incorporated into lipid vesicles and nanodiscs. Past research has provided information on the structures and functions of T4SS, on the effectors and on the mechanisms by which secreted proteins hijack the functions of cells during infection. However, many details, like the different interactions between proteins in these systems are still unknown. Understanding how these systems contribute to virulence is critical for the development of new antimicrobial therapies.

Poster Session

BAM-PW1129 - Bacterial isolation and characterization from citrus phylloplane, of antagonistic genera able to disrupt quorum sensing by Xanthomonas axonopodis pv. citri.

Juan Carlos Caicedo[1], Katia Kuper[2], Maria Ines Tirabocshi Ferro[1], Sonia Villamizar[1], Jesus Ferro[1]
[1]UNESP.FCAV. departamento de tecnologia. Jaboticabal. Brasil, [2]Centro de Citricultura Sylvio Moreira IAC. SP Brasil.

The bacteria Xanthomonas axonopodis pv citri (Xac) is the causal agent of citrus canker (CC), a disease that affects almost all types of citrus crops. Xac production of pathogenicity factors is controlled by a group of genes known as rpfABFGCH, these genes are regulated by a signalling molecule of a cellular communication system called Quorum Sensing (QS). This molecule is known as Diffusible Signal Factor (DSF). The synthesis of DSF is not restricted to Xanthomonas genus, several bacterial genera produce structurally and functionally related molecules DSF, suggesting the possibility of interference of QS signaling between them. The objectives of this study are: Isolate from citrus phylloplane with different patterns of susceptibility to CC, bacteria able to disrupt DSF signalling in Xac and characterize the genes responsible for the modification and / or degradation DSF molecule produced by Xac. Epiphytic and endophytic bacteria were isolated from the phylloplane of tangerine, lime orange and grapefruit, subsequently these bacteria were tested in its ability to interfere with the DSF signalling in Xac, confronting them with a biosensor of the DSF (Xac 306 with the plasmid pKLN55 harboring the gene fusion eng: gfp inducible by DSF). The identification the isolated antagonistic bacteria was determined by molecular and biochemical methods. To characterize the responsible genes for the modification or degradation of DSF in Xac, a random mutagenesis was employed using the transponson Tn5. Is currently being evaluated in vivo the ability for biological control of antagonistic infiltrating and spraying in the same citrus species where was isolated the antagonistic bacteria. the most active degrading DSF bacteria belonging to the genera Pseudomonas spp. and Paenibacillus spp. The ability of microorganisms to interfere with QS signalling, could significantly increase their power in the ecosystem and providing a tool for biological control.

Poster Session

BAM-PW1131 - Sigma factor K signalling in *Mycobacterium tuberculosis*-host interaction

Louise H Lefrancois[1], Frederic J Veyrier[2], Alexander Dufort[1], Marcel A Behr[1,2,3]
[1]Research Institute of McGill University Health Center, Department of Medicine, Montréal, Canada, [2]Institut Pasteur, Infection Bactériennes Invasives, Département Infection et Epidémiologie, Paris, France, [3]McGill International TB Centre, Montréal, Canada

Evolution analyses of the *Mycobacterium tuberculosis* complex reveal that the antigenic proteins MPT70/MPT83 present 3 forms of expression. In *Mycobacterium tuberculosis*, the genes are 'OFF' *in vitro*, but induced upon infection of macrophages. In *Mycobacterium bovis* and early *Mycobacterium bovis* BCG strains, the genes are 'ON', such that MPT70 is the number 1 protein secreted by the bacteria. In late *Mycobacterium bovis* BCG strains, the genes are 'OFF". Previous work in the lab has shown that the extracytoplasmic function sigma factor K (SigK) controls expression of these genes. In late *Mycobacterium bovis* BCG strains, SigK is mutated (explaining OFF). In *Mycobacterium bovis* and early *Mycobacterium bovis* BCG strains, the anti-sigma factor of SigK, called RskA is mutated (explaining ON). While these mutations explain abnormal expression of MPT70/MPT83, we still do not understand how and why the SigK regulon is induced in *Mycobacterium tuberculosis* within minutes of infection. To understand the basis of SigK signalling, we have re-creating this signalling system in the harmless, rapid-growing specie *Mycobacterium smegmatis* using as a read-out of *mpt70* expression by luciferase assays. Using this model, we focused on the molecular description of a new partner and any additional members involved in SigK system. We demonstrated that RskA is able to play a role of either activator or inhibitor of SigK. Through a transposon mutant screen in *Mycobacterium smegmatis* that looked for mutants where activity was blocked, we uncovered an antigenic lipoprotein named Lipoprotein Z (LppZ) which directly interact with RskA to activate SigK regulon. Ongoing investigations aim to identify the nature of the host signal at the origin of this signalling cascade using macrophage-like cells as well as understand the function of LppZ in SigK activation in *Mycobacterium tuberculosis*.

Poster Session

BAM-PW1133 - Interaction analysis between quorum sensing receptor SpnR and Its signaling molecule on a quartz crystal microbalance

Yuriko Takayama[1,2], Eri Nasuno[1,2], Takuto Umemura[1], Chigusa Okano[1], Yuki Saito[1], Satoshi Ito[1,2], Ken-ichi Iimura[1,2], Tomohiro Morohoshi[1,2], Tsukasa Ikeda[1,2], Norihiro Kato[1,2]
[1]Utsunomiya University, Utsunomiya, Japan, [2]CREST, JST, Japan

Intermolecular interaction between a quorum sensing (QS) receptor SpnR and a signaling molecule, N-acylhomoserine lactone (AHL) in Serratia marcescens AS-1 could be successfully investigated by quartz crystal microbalance based on admittance analysis (QCM-A). The QS is one of the hierarchical processes of cell-cell communication that allows bacteria to share information about cell density as increasing concentration of the signaling molecule. S. marcescens is an emerging human pathogen that is sometimes involved in nosocomial infections and its QS system is governed by luxI and luxR homologues, spnI and spnR, respectively. The LuxR-type proteins bind their cognate AHL signals and regulate transcription of target genes. Expression of pig clusters is regulated by a stable complex of SpnR with N-hexanoylhomoserine lactone (C6HSL) in S. marcescens AS-1, while the complex probably becomes stable above a threshold concentration of C6HSL due to increasing cell density. A 27-MHz quartz crystal microgravimetry can detect 30 pg of adsorbed mass as a frequency change of one Hz. Amino-terminated C6HSL was coupled with carboxylic acid groups of the self-assembly membrane formed on the QCM gold electrode using water soluble carbodiimide. After stabilizing the sensor signal, desired volume of maltose binding protein (MBP)-tagged SpnR aqueous solution was gently mixed to form complex with immobilized C6HSL, where the MBP-SpnR was prepared by genetic engineering techniques. Time evolution of resonance frequency change could be separated into two specific frequency changes due to the mass load and the viscous load because the conductance change was identified by network analyzer. Just after adding MBP-SpnR into the buffer solution immersed in the C6HSL-modified electrode at 25ºC, the immediate decrease was detected in the mass load frequency as well as the resonance frequency. This means that the immobilized C6HSL could specifically form the complex with the non-immobilized SpnR domain in vitro.

Poster Session

BAM-PW1135 - A novel mediator of colistin-induced HAQ biosynthesis in Pseudomonas aeruginosa

James Schafhauser[1], Shuya Zhai[1], Geoffrey McKay[2], Dao Nguyen[1]
[1]McGill University, Montreal, Canada, [2]MUHC, Montreal, Canada

P. aeruginosa (PA) is a widespread opportunistic pathogen causing significant morbidity and mortality in Cystic fibrosis patients. PA produces 4-hydroxy-alkyl-quinolines (HAQs), a family of molecules with diverse biological functions, including quorum-sensing, virulence regulation, and antibiotic tolerance. We recently reported that the stringent response modulates HAQ biosynthesis, indicating that environmental signals can influence HAQ biosynthesis. PA encounters cationic antimicrobial peptides (CAMPs) that are produced by host cells or used as anti-pseudomonas antibiotic treatments (colistin). Interestingly, subinhibitory concentrations of colistin can induce HAQ biosynthesis and up-regulate expression of *pqsA* and *pqsH*. In this study, we report the novel role of PA5003 in mediating the colistin-induction of HAQ biosynthesis. PA5003, a Mig-14 like protein, is involved in colistin recognition and required for colistin-inducible tolerance. We identified PA5003 in a random mutagenesis screen of the *ΔrelAspoTpqsR* mutant where we searched for mutants with reduced *pqsA* expression using a *pqsA-lacZ* promoter fusion. The *ΔrelAspoTpqsRPA5003* mutant showed a ~50% reduction in *pqsA-lacZ* activity, and the PA5003 single mutant had a ~75% reduction in HHQ levels compared to wild-type. We hypothesized that PA5003 may mediate colistin induced HAQ overproduction. In wild-type, subinhibitory colistin (0.30ug/mL) leads to a 2-3 fold increase in *pqsA-lacZ* activity, and a 50% increase in the dominant HAQs, HHQ, PQS and HQNO (measured by LC/MS). In contrast, sub-inhibitory colistin had no effect on HAQ levels in the PA5003 mutant strain. We demonstrate that PA5003 mediates colistin induced up-regulation of HAQ biosynthesis. How PA5003 recognizes CAMPs and coordinates the downstream effect on HAQ biosynthesis remains unknown, but our results show that this gene provides an important mechanism for PA to sense its environment and modulate HAQ biosynthesis. This may have significant implications to the pathogenicity and antibiotic tolerance of PA infections during colistin treatment in cystic fibrosis patients.

Poster Session

BAM-PW1137 - Application of RNA sequencing for characterization of small RNAs in Rhodobacter capsulatus

Marc Gruell[1], Loudes Peña-Castillo[1], Martin Mulligan[1], Andrew Lang[1]
[1]Memorial University of Newfoundland, St. John's, Canada

Rhodobacter capsulatus is a purple nonsulfur bacterium that is studied for different aspects of physiology, such as anoxygenic photosynthesis, and also its ability to produce a bacteriophage-like particle called a gene transfer agent, RcGTA. The production of RcGTA is regulated by several different cellular signaling systems, one of which is a two-component phosphorelay involving the response regulator CtrA. Although the involvement of CtrA in controlling RcGTA gene expression is well established, the mechanism by which it does this is still unknown. We have performed RNA sequencing with size-fractionated RNAs to compare the transcriptome of a ctrA mutant to that of the wild type strain. The large RNA fraction (>200 bases) was used for validation of our RNA sequencing approach by comparison to a previously published microarray-based transcriptomic study of the same strains. The small RNA (sRNA) fraction (<200 bases) was used to discover sRNAs produced by R. capsulatus and to search for CtrA-dependent sRNAs. Involvement of identified sRNAs in regulation of RcGTA gene expression is under investigation.

Poster Session

BAM-PW1139 - Engineering of synthetic protein scaffolds in Saccharomyces cerevisiae for increased flux to geraniol production

Alex Campbell[1], Vince Martin[1], Philippe Bauchart[1], Nick Gould[1], Yun Zhu[1]
[1]*Concordia University, Montreal, Canada*

Terpenoid biosynthesis is a crucial metabolic process that produces a wide variety of valuable compounds that have applications in everything from anti-cancer indole alkaloids to cosmetics and biofuels. Engineering this pathway in Saccharomyces cerevisiae is somewhat challenging due the to the fact that several of the metabolites in the process strongly inhibit upstream enzymes or participate in side reactions. To address this problem we are working to scaffold several enzymes in this pathway to a synthetic peptide backbone using modular binding domains. Protein scaffolds have been used previously in E.coli to improve product yields up to 70 fold. The substrate channeling effect provided by scaffolding these reactions is intended to reduce the impact of feedback inhibition and side reactions in order to maximize production of geraniol, a simple monoterpenoid. This case should serve as an example for the viability of improving metabolic flux through pathways in Saccharomyces cerevisiae with synthetic protein scaffolds.

Poster Session

BAM-PW1141 - Optimization of the electroporation procedure for plasmid transformation of A. salmonicida subsp. salmonicida

Sabrina A Attere[1,2,3], Stéphanie Dallaire-Dufresne[1,2,3], Jean-Guillaume Émond-Rheaut[1,2,3], Kathrine H. Tanaka[1,2,3], Mélanie V. Trudel[1,2,3], Michel Frenette[2,4], Steve J. Charette[1,2,3]

[1]Institut de biologie intégrative et des systèmes, Université Laval, Quebec City, Canada, [2]Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, Quebec City, Canada, [3]Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Quebec City, Canada, [4]Groupe de Recherche en Écologie Buccale (GREB), Faculté de médecine dentaire, Université Laval, Quebec City, Canada

Aeromonas salmonicida subsp. salmonicida is an important fish pathogen. Molecular tools are required to study the virulence and the genomic stability of this bacterium. The establishment of an efficient electroporation-mediated transformation protocol in Aeromonas salmonicida subsp. salmonicida would allow easier and faster genetic studies. In the present study, we first designed the pSDD1 plasmid (4.1 kb) as a tool to set up an optimized electroporation protocol. Then electroporation conditions have been systematically tested to create a protocol that allows a maximum of transformants. Using optimal conditions (25 kV/cm, 200 Ω, 25 µF) the electroporation efficiency was up to 1 x 105 CFU/µg DNA. The electroporation procedure was also tested with another plasmid of 10.6 kb and with three different strains of A. salmonicida subsp. salmonicida. It appeared that the strains had important difference in their electro-transformation competency with the 01-B526 strain being the easiest to electroporate especially with the pSDD1 plasmid. This plasmid was stably maintained in 01-B526 transformants, just as the rest of the endogenous plasmids contained in this bacterium but can be easily cured by removing the selection. This is the first efficient electroporation protocol reported for Aeromonas salmonicida subsp. salmonicida. It offers new possibilities for the study of this bacterium.

Poster Session

BAM-PW1143 - Investigation into phage endolysin as antibacterial treatment for Gram-negative bacteria; focusing on Avian Pathogenic Escherichia coli

Ji-Yun Lee[1], Chrispian Theron[1], Charlotte Boucher[1], Robert Bragg[1]
[1]University of the Free State, Bloemfontein, South Africa

Antibiotic resistance is a worldwide challenge and in certain countries there have been bans applied against the use of antibiotics as a move towards solving this problem. As antibiotics are used for growth promoters and treatment in the food animal industry, bans against its use have a major negative impact in terms of production of healthy birds and poultry products. Thus, there is a need for alternative solutions and bacteriophage treatment is progressively a viable option. The lytic ability of phage to break open and kill bacterial cells, upon maturation of phage virions, serves as the basis of phage therapy research. There is also research being done on phage-related products including the expression of virolysins/endolysins which have yielded positive results for treatment on Gram-positive bacteria, but not with Gram-negative bacteria. Recent research has indicated that phage endolysins may likewise be used for treatment against Gram-negative bacteria. The aim of this study is to investigate the application of an expressed lytic gene from bacteriophage for treatment against Avian Pathogenic Escherichia coli field strains. Phage Lambda (λ) is chosen for its specificity for Escherichia coli and its well-documented DNA genome sequence which is available. The gene of interest is synthesized and further expressed using a yeast protein expression vector system. The expressed endolysin is used to treat a collection of Avian Pathogenic Escherichia coli, Gram-positive bacteria and non-pathogenic Escherichia coli in order to determine the efficacy of the lytic property of the endolysin. This is done both in the presence and absence of lipopolysaccharide penetrating agents to establish the optimal conditions under which the expressed endolysin remains functional. Part of the challenge is to work towards a useful product that can be used in the poultry market.

Poster Session

BAM-PW1145 - Molecular serotyping for Avibacterium paragallinarum isolates

Arina Jansen-Hitzeroth[1], Elke Coetsee[1], Charlotte Boucher[1], Robert Bragg[1]
[1]*University of the Free State, Bloemfontein, South Africa*

Avibacterium paragallinarum is the causative agent of infectious coryza (IC), an upper respiratory tract disease that occurs primarily in chickens. This disease has a huge economic impact in the poultry industry with a 10-40% decrease in egg production. Currenly A.paragallinarum is serotyped by making use of the modified Kume serotyping scheme, where the bacterium is classified into 3 serogroups (A, B and C) and 9 serovars (a-1-A4), B-1 and C1-C4). The main method by which this is achieved is the haemagglutination and haemagglutination inhibition (HA/HI) assays. This technique is however limiting as it is very subjective. This technique is suitable to serotype to the serogroup level, but HI test is not able to serotype accurately up to serovar level. Vaccine failures are a major problem that accurs due to no or poor cross-protection occurring especially between the C-serovars of A. paragallinatum. This problem will be overcome by having a more accurate serotyping technique available for the disgnosis of IC. A serotyping PCR was developed which distinguished beween the Modesto (C-2) and SA-3 (C-3) A. paragallinarum isolates which are the major cause of IC in South Africa. A serotyping multiplex PCR was performed and the same sized band was observed for all the reference isolates tested. Restriction digest results indicated a different banding pattern observed for the Modesto (C-2) and SA-3 (C-3) strains. There is no, to limited cross protection between the serovar C2 and C3 strains thus accurate diagnosis is very important for the control of the disease through the use of correct vaccines. This method was also used to distinguish between all the reference isolates of A. paragallinarum.

Poster Session

BAM-PW1147 - Screening of Lactobacilli and Bifidobacteria from Bangladeshi indigenous poultry for their potential use as probiotics

K. B. M. Islam[1], Syeeda Shiraj-Um-Mahmuda[2], Afroj Jahan[3], A. A. Bhuiyan[4]
[1]Department of Medicine & Public Health, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, [2]Dhaka Medical College, Dhaka, Bangladesh, [3]Gonosastho Community Based Medical College, Dhaka, Bangladesh, [4]Bangladesh Livestock Research Institution, Dhaka, Bangladesh

The use of imported probiotics in poultry is gradually being increased in Bangladesh. But surprisingly, no probiotic bacteria have been isolated yet in Bangladesh even though the existence of scavenging native poultry as potential source that are seemingly more resistant to GIT infection as well as other diseases. Therefore, the study was undertaken to isolate, identify and characterize the potential probiotic Lactobacillus and Bifidobacteria strains from Bangladeshi indigenous poultry, and to evaluate their suitability to use in poultry industry. Crop and cecal samples from 61 healthy indigenous birds of different age and sex with no history of clinical illness or antibiotic therapy for last six months were used to isolate potential probiotics strains following conventional cultural methods. A total 300 isolates were identified following physical, biochemical and molecular methods that belonged to the genus Lactobacillus and Bifidobacteria. An auto-aggregation test was performed for 192 and 108 isolated lactobacilli and bifidobacteria strains, respectively. Twelve lactobacilli isolates and 7 bifidobacteria isolates were selected because of their convenient aggregation. In vitro tests including antibacterial activity, resistance to low pH, resistance to bile extract, ability to produce H_2O_2, cell surface hydrophobicity and adhesion activities on crop epithelial cells were performed for evaluation of probiotic potential of each strain. Under the in vitro conditions and with respects to the probiotic traits, the lactobacilli; Lactobacillus salivarius, L. crispatus, L.johnsonii and the bifidobacteria, Bifidobacterium thermophilum and B. bifidum were found to be potential probiotic strains. Thus, they are proposed to be evaluated for their in vivo probiotic properties. If the proposed strains are found suitable as the probiotics to be used in commercial poultry industry, it is expected that the local probiotics would be more beneficial and would save the huge amount of money that Bangladesh spends every year for the importation of such materials from abroad.

Poster Session

BAM-PW1149 - Characterization of the antiviral activity of Actinobacillus pleuropneumoniae against the porcine reproductive and respiratory syndrome virus

Jérémy A Ferreira Barbosa[1], Josée Labrie[1], Francis Beaudry[2], Carl A. Gagnon[1], Mario Jacques[1]
[1]GREMIP, Faculté de Médecine Vétérinaire, Université de Montréal, Canada, [2]GREPAQ, Faculté de Médecine Vétérinaire, Université de Montréal, Canada

Porcine reproductive and respiratory syndrome virus (PRRSV) is the leading pathogen in swine industry. Our laboratory recently demonstrated that unidentified low molecular weight molecules in the culture supernatant of Actinobacillus pleuropneumoniae can block in vitro replication of PRRSV in SJPL infected cells. The characterization of this inhibitory activity can provide clues for the development of new prophylactic and therapeutic treatments against PRRSV. Therefore, the aim of this investigation was to study the antiviral activity of A. pleuropneumoniae. We hypothesised that A. pleuropneumoniae can induce cell cycle disruption of SJPL cells. To test our hypothesis, SJPL cells were treated with A. pleuropneumoniae culture supernatant. The cell cycle and cell proliferation were then analyzed. First, cell cycle analysis using flow cytometry, indicated that the A. pleuropneumoniae supernatant significantly shifted the cell population towards an arrest in the G2/M phase ($n=15$; $p\leq0.0001$). Second, we observed that the A. pleuropneumoniae supernatant significantly reduced cell proliferation ($n=8$; $p\leq0.05$). To further test our hypothesis, SJPL cells were infected with PRRSV and treated with 3,3'-methylenediindole (DIM), a molecule known to cause an arrest in the G2/M phase and a reduction in cell proliferation. Cells treated with this molecule did not support PRRSV replication, suggesting that disruption of the cell cycle and cell proliferation can inhibit the replication of PRRSV. The proteomes of SJPL cells that were either treated or not with A. pleuropneumoniae culture supernatant were then compared using westernblot analysis. A modulation of proteins implicated in the regulation of the cell cycle was indeed observed. In conclusion, the modulation of proteins associated with the regulation of the cell cycle of SJPL cells causes an inhibition of PRRSV replication.

Poster Session

BAM-PW1151 - Innate immune responses of Chinese native chicken lines against infection with Salmonella enterica serovar Enteritidis

Zhiming Pan[1], Qiang Fang[1], Shizhong Geng[1], Xilong Kang[1], Qiuxia Cong[1], Xinan Jiao[1]
[1]*Yangzhou University, Yangzhou, China*

The peripheral blood mononuclear cells (PBMCs), isolated from the chickens of four Chinese native lines (Qingjiaoma, Sanhuang, Wugu, and Xueshanma), were in vitro exposured to Salmonella enterica serovar Enteritidis (SE) strain 50041, and the mRNA expression levels of relative TLRs (TLR4, TLR5 and TLR15) and cytokines (IL-1β, IL-6, TGF-β4, CXCLi2 and CCLi2) were determined using qRT-PCR. It was showed that Wugu and Xueshanma lines expressed higher levels of TLR4 and TLR15 mRNA than the two others, while TLR5 was expressed less in the Sanhuang line than in others; Wugu and Xueshanma lines produced more IL-1β, IL-6, CXCLi2 and CCLi2 mRNA than Qingjiaoma and Sanhuang lines; the Qingjiaoma line showed significantly higher level of TGF-β4 mRNA than the Xueshanma line at 4 h post-infection ($p<0.05$). Furthermore, the mRNA expression levels of relative TLRs and cytokines in spleens and cecal tonsils of three chicken lines (Sanhuang, Wugu, and Xueshanma) after oral infection with SE were evaluated using qRT-PCR. After SE infection, three TLRs mRNAs were expressed much higher in spleens of the Xueshanma line and cecal tonsils of the Wugu line than others. Wugu and/or Xueshanma lines produced higher expression levels of IL-1β, IL-6 and CCLi2 mRNA in spleens and cecal tonsils, and the expression of these cytokines in cecal tonsils of the Sanhuang line were downregulated compared to the uninfected chickens. In addition, infection with SE also increased CXCLi2 mRNA expression in spleens of the Xueshanma line and cecal tonsils of the Wugu line, which suggested that Wugu and/or Xueshanma lines had certain advantages in the inflammatory cytokine response to SE infection. It was demonstrated by the experiments of in vitro and in vivo infection that Wugu and/or Xueshanma lines be more responsive to SE infection in the aspect of innate immunity.

Poster Session

BAM-PW1153 - Diversity in the Lac family Phosphotransferase system of Enterococcus hirae

Isha Katyal[1], Janet Hill[1]
[1]*Department of Veterinary Microbiogy, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada*

Diversity in phosphotransferase systems (PTS) plays a role in determining the carbohydrate utilization potential and the success of an isolate in an environment. Previous work in our lab has shown that Enterococcus hirae isolates obtained from feces of healthy pigs aged 15 weeks (E.hirae ecotype 2, E.hirae-2) could utilize a broader range of carbohydrates than isolates obtained from feces of healthy pigs aged three weeks (E.hirae-3) and nine weeks (E.hirae-1). Specifically, it was observed that 92% (36/39) of E.hirae-2 isolates could utilize lactose as a sole carbon source in comparison to 16% (10/64) of E.hirae-1 isolates and none (0/5) E.hirae-3 isolates. To explain this phenotypic difference, we investigated diversity in the Lac family phosphotransferase systems of six E. hirae isolates (two isolates from each ecotype). The Lac family PTS repertoire was identified from the annotated draft genome sequence of each isolate. A PTS was considered complete if the enzyme II components EIIA, EIIB and EIIC were present, while an incomplete PTS lacked one or more of these components. Four complete lactose/cellobiose specific PTS were observed in each of the isolates but none of them was annotated as lactose specific. Further, it was seen that EIIC components were more abundant (7-10 per genome) than EIIB (4-5 per genome) or EIIA (3-6 per genome) components. This is consistent with previous studies where "orphan" EIIC have been observed. A complete lactose specific PTS operon containing an EIICB fusion domain, an EIIA component, a 6-phospho-β-galactosidase and a lactose regulator was observed only in E.hirae-2 isolates. Although an orthologous operon was detected in E.hirae-1, the predicted EIICB fusion domain gene was disrupted. The operon was completely absent in E. hirae-3 isolates. Further experiments to confirm the involvement of this operon in lactose uptake are ongoing.

Poster Session

BAM-PW1157 - Defining a minimal cell: essentiality of small ORFs and ncRNAs in a genome-reduced bacterium

Maria Lluch Senar[1,2], Javier Delgado[1,2], Wei-Hua Chen[3], Verónica Lloréns-Rico[1,2], Francis J. O'Reilly[3], Judith A.H. Wodke[1,2,4], E. Besray Unal[1, 2], Eva Yus[1,2], Sira Martínez[1,2], Tony Ferrar[1,2]
[1]EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), Barcelona, Spain, [2]Universitat Pompeu Fabra (UPF), Barcelona, Spain, [3]European Molecular Biology Laboratory, Heidelberg, Germany, [4]Theoretical Biophysics, Humboldt-Universität zu Berlin, Berlin, Germany

Identifying all essential genomic components is critical for the assembly of minimal artificial life. In the genome-reduced bacterium Mycoplasma pneumoniae, we found, that small ORFs (smORFs; <100 residues), accounting for 10% of all ORFs, are the most frequently essential genomic components (57%), followed by conventional ORFs (53%). Essentiality of smORFs may be explained by their function as members of protein and/or DNA/RNA complexes. In larger proteins, essentiality applied to individual domains and not entire proteins, a notion we could confirm by expression of truncated domains. The fraction of essential non-coding RNAs is considerably higher (40%) than of non-transcribed regions (18%), pointing to the important functions of the former. The data provide an accurate view of a minimal genome, revealing an unexpected hidden layer of ncRNAs and smORFs with essential functions.

Poster Session

BAM-PW1000 - Discovering new cell envelope development pathways by identifying functional linkages between genes of unknown function through mutational approaches

Kara Neudorf[1], Elizabeth Vanderlinde[2], Christopher Yost[1]
[1]University of Regina, Regina, Canada, [2]University of Saskatchewan, Saskatoon, Canada

Gram-negative bacteria have an outer membrane which serves as a protective barrier against environmental stresses and as an interface during host-microbe interactions. Mutations that result in structural defects in the outer membrane often increase a cell's sensitivity to various stressors and impair host-microbe interactions. We are studying an uncharacterized four gene operon and investigating its role in cell envelope function in Rhizobium leguminosarum. The operon is highly conserved among the alphaproteobacteria, and consists of a moxR-like AAA+ ATPase (RL3499), a hypothetical protein (RL3500), and two large transmembrane proteins (RL3501 and RL3502). Mutation of the operon results in sensitivity to membrane disruptors, an inability to sustain growth on glycine or peptide rich media, where the mutant cells become enlarged, circular and distorted. Gain of function suppressor mutants have been isolated for a RL3500 non-polar mutant at a frequency of 1.28 x10^-6 CFU/mL on TY and minimal media with 3mM glycine. The suppressors were confirmed through PCR to maintain the original in-frame deletion to RL3500, suggesting a secondary site mutation. Transposon mutagenesis was conducted in an attempt to identify the secondary site suppressor locus. This screen lead to the identification of a broadly conserved tetratricopeptide repeat (TPR) containing protein RL0936, predicted to be involved in protein-protein interactions. RL0936 was found to be highly conserved based on a phylogenetic analysis. RL0936 was also identified as being up-regulated in the suppressor mutant suggesting that when RL0936 is over-expressed, it enables the RL3500 mutant to grow on peptide and glycine rich media. In addition, when RL0936 is mutated, it results in a decreased ability to form biofilms and increased sensitivity to desiccation. Mutations also result in sensitivity to detergents such as SDS and an increased NPN uptake, suggesting a disrupted outer membrane and a role in proper cell envelope function.

Poster Session

BAM-PW1002 - Assessing host-specificity of Escherichia coli with a supervised learning logic-regression-based analysis of single nucleotide polymorphisms in intergenic regions

Shuai Zhi[1], Qiaozhi Li[1], Edward Topp[2], Thomas Edge[3], Yutaka Yasui[1], Norman Neumann[1]
[1]University of Alberta, Edmonton, Canada, [2]Agriculture and Agri-Food Canada, Ottawa, Canada, [3]National Water Research Institute, Environment Canada, Ottawa, Canada

Background: Currently, no common perspective has emerged on whether Escherichia coli (E. coli) displays clear host specificity/adaptation. Despite this, the statistical methods currently used for assessing host-specificity tend to focus on unsupervised learning methods (i.e., cluster-based analysis), which may not be appropriate methodologically. This work proposes a supervised learning logic-regression-based analysis for statistically assessing the concept of host-specificity of E. coli and identifying genetic biomarkers of E. coli that are associated with colonization of specific hosts using intergenic sequences. Methods: we utilized a logic regression that uses DNA sequence of three intergenic regions in 780 E. coli isolates from 15 animal hosts as input data to build logic regression models to discriminate E. coli by host sources. Results: The intergenic regions uspC-flhDC, csgBAC-csgDEFG, and asnS-ompF can be PCR amplified in 97.6%, 98.7%, and 98.2% of studied isolates respectively. Logic regression successfully discriminate E. coli from several animals with high sensitivity and specificity. The fivefold cross validation showed the highest sensitivity of 82% was observed in deer with a specificity of 98%. The second and third highest sensitivity of 77% and 67% were observed in muskrat and moose respectively with their specificity all equal to 99%.The lowest sensitivity of 4% was found in cat, while the second lowest sensitivity of 5% was found in goose and gull. For the other host groups, the sensitivity ranges from 21% in dog to 61% in coyotes Conclusions: The recombination of logic regression and DNA sequences information from intergenic regions provide a novel and robust approach to explore host specific genetic patterns in E. coli population. The results indicated that E. coli might not be host-general as previously proposed by some studies but rather presents a certain level of host-specificity in general.

Poster Session

BAM-PW1004 - Characterization of a glycosyl hydrolase family 73 (GH73) from Clostridium difficile

Ryan Miller[1], Joshua Jones[1], Garrett Holmes[1], Zachary Suter[1], Christopher Reid[1]
[1]*Department of Science and Technology, Bryant University, Smithfield, USA*

The major structural component of the bacterial cell wall, peptidoglycan (PG), is composed of alternating beta-1,4 linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), cross-linked by peptide tetramer extending off of MurNAc. This project characterizes the protein encoded by cd1034 from Clostridium difficile 630.The gene has been tentatively annotated to encode a mannosyl-glycoprotein endo-β-N-acetylglucosaminidase (GlcNAcase), which cleaves the beta-1,4 glycosidic linkage between GlcNAc and MurNAc. Based on sequence alignment, CD1034 from C. difficile is classified in the Carbohydrate Acting Enzyme database (CAZy) as a member of glycosyl hydrolase family 73 (GH73). This family is usually involved in daughter cell separation during vegetative growth, and they often hydrolyze the septum after cell division. Currently, there is no kinetic data for this family and conflicting information regarding the mechanism of action for GH73. The goal of this project is to biochemically characterize GH73 enzymes using CD1034 from C. difficile as a model. An engineered form of CD1034 lacking the N-terminal transmembrane domain was generated for use in this study. Activity of purified CD1034 was confirmed using turbidometric and reducing sugar assays with Micrococcus luteus PG as substrate. Muropeptide analysis of CD1034 reactions by HPLC and mass spectrometry revealed endo-acting activity and confirmed glucosaminidase activity. Additionally, enzymatic assays with N-deacetylated PG demonstrated a requirement of the 2-acetamido group for activity.

Poster Session

BAM-PW1006 - Modification of quantitative composition of Campylobacter jejuni outer membrane vesicles (OMVs).

Renata Godlewska[1], Joanna Klim[1], Agnieszka Wyszyńska[1], Paweł Wierzchowski[1], Elżbieta Katarzyna Jagusztyn-Krynicka[1]

[1]*University of Warsaw, Faculty of Biology, Department of Bacterial Genetics, Warsaw, Poland*

Campylobacter jejuni is the most prevalent cause of food-borne gastroenteritis in the developed world. Like other Gram-negative bacteria it constitutively releases outer membrane vesicles (OMVs) during cell growth that probably plays roles in bacterial survival, virulence and pathogenesis. CjaA is an extracytoplasmic, glycosylated and highly immunogenic protein present in the proteomes of both clinical and environmental isolates. The protein which belongs to family 3 solute-binding proteins, component of the ABC transport system is localized mainly in the inner membrane (IM). The CjaA protein has been identified as component of C. jejuni OMVs. Because CjaA antigen and OMVs (generally) are promising candidates for the development of an anty-Campylobacter vaccine we decided to obtain OMVs with a higher content of CjaA antigen than those produce by wt cells. To achieve this goal we constructed C. jejuni strains with cjaA gene knockout harboring shuttle vectors with additional copy of cjaA gene: 1) wt cjaA gene with its own promoter; 2) cjaA gene with point mutation (C20A) which alters the location of protein from IM to periplasmic; 3) cjaA gene with point mutation (N139A) in N-linked glycosylation site (nonglycosylated form of the CjaA). The quantification of CjaA amount in OMVs produced by above mentioned strains were carried out using ELISA assay. Surprisingly content of CjaA in OMVs produced by strains carrying genes with point mutations (C20A and N139A) was lower in comparison to the wild-type strain. However OMVs of the strain carrying wt cjaA gene copy on plasmid content approximately four times more CjaA protein than OMVs of wild-type strain. Probably point mutations altered CjaA protein stability, resulting in the reduced amount of protein in cell and consequently in OMVs, despite a higher number of copies of the gene (compared to wt 81176 strain).

Poster Session

BAM-PW1008 - Cryoelectron microscopic evaluation of two life cycles of the bacterial predator Bdellovibrio

Ryan Chanyi[1], Cezar Khursigara[2], Susan Koval[1]
[1]University of Western Ontario, London, Canada, [2]University of Guelph, Guelph, Canada

Two species of the bacterial predator Bdellovibrio are reliant on a Gram-negative prey cell for survival. B. bacteriovorus invades the periplasmic space of its prey, degrading the cytoplasmic contents for growth, termed periplasmic growth. Recently our lab isolated a new species, B. exovorus, which does not invade but remains on the exterior of the prey cell throughout predation, termed epibiotic growth. For decades, visualization of predation characteristics has relied on conventional transmission electron microscopy. However there are many artefacts due to chemical fixation and heavy metal staining which do not allow imaging of predator-prey interactions in a near unaltered state. Cryoelectron microscopy has allowed us to clearly demonstrate and compare the two contrasting life cycles used by Bdellovibrio species. Upon initial attachment, B. bacteriovorus begins to modify the prey cell, such that it rounds up prior to entry and become a flexible bdelloplast. The predator is constricted at the site of entry during invasion, and leaves a plug-like structure there after invasion has occurred. Our observations suggest the loss of the flagellum by B. bacteriovorus during entry is required to reseal the pore in the outer membrane of the prey cell created during invasion. During predation, and prior to division, B. bacteriovorus was pressed along the length of the cell to the cytoplasmic membrane of the prey cell. This is thought to increase the surface area to allow efficient transport of nutrients into the predator. B. exovorus was observed to penetrate slightly into the outer membrane of the prey cell and disrupt the peptidoglycan at this point of attachment. Empty stalked or swarmer cells were clearly seen in cocultures. Using cryoelectron tomography, detailed three-dimensional representations of the attachment sites of the periplasmic and epibiotic predators were obtained.

Poster Session

BAM-PW1010 - Structural investigations of bacterial magnetotactic organelle formation and positioning

Nancy Hom[1], Ertan Ozyamak[2], Arash Komeili[2], Justin Kollman[1]
[1]Department of Anatomy and Cell Biology, McGill University, Montreal, Canada, [2]Department of Plant and Microbial Biology, University of California, Berkeley, USA

A unique group of magnetotactic bacteria are able to create multiple internal compartments, each of which serves as an enclosed space for controlled formation of a single magnetic nanocrystal. These organelles, called magnetosomes, are positioned in a line running the length of the bacterium, where they serve essentially as a compass needle to align the cell along the earth's magnetic field. The magnetic response appears to allow these bacteria to swim in a directed search toward regions with optimal growth conditions. Though the protein components, genetics and biochemistry of magnetosomes have been extensively studied, the three-dimensional structure of the magnetosome is not well-characterized. Using cryo-electron tomography, we are interested in obtaining a high-resolution map of the intact organelle in order to gain insight into the global magnetosome architecture. Additionally, we are investigating the structures of individual, purified, protein components necessary for magnetosome assembly and organization. The actin homolog MamK is required for the linear positioning of magnetosomes. We have determined the structure of MamK filaments by cryo-EM and homology modeling, revealing a unique filament structure with two twisted strands that are in register, rather than staggered as in other actin homologs. We are now interested in elucidating the specific molecular contacts for MamK assembly. Using MamK mutants, we performed negative-stain EM and biochemical characterizations to determine sites of important filament interactions. We have found that mutations at the cross-strand interface lead to increased filament dynamics, though the filament structure is the same as wildtype. In contrast, mutations at the intersubunit interface along each strand completely abolish filament assembly. We are also studying the structures of other magnetosome protein components, such as those necessary for maintaining structure and for controlling crystal growth, with the goal of gaining a complete view of the mechanisms of magnetosome development.

Poster Session

BAM-PW1012 - Structural and biochemical analysis of a unique phosphatase from Bdellovibrio bacteriovorus reveals its structural and functional relationship with the protein tyrosine phosphatase class of phytase

Robert Gruninger[1], John Thibault[2], Michael Capeness[3], Robert Till[3], Brent Selinger[2], Elizabeth Sockett[3], Steven Mosimann[4], Andrew Lovering[5]

[1]Lethbridge Research Centre, Agriculture & Agri-Foods Canada, Lethbridge, Canada, [2]Department of Biological Sciences, University of Lethbridge, Lethbridge, Canada, [3]Centre for Genetics and Genomics, School of Life Sciences, University of Nottingham, Medical School, Nottingham, UK, [4]Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Canada, [5]Institute of Microbiology and Infection & School of Biosciences, University of Birmingham, Birmingham, UK

Bdellovibrio bacteriovorus is an unusual delta-proteobacterium that invades and preys on other Gram-negative bacteria and is of potential interest as a whole cell therapeutic against pathogens of humans, animals and crops. Protein tyrosine phosphatases (PTP) are an important class of enzyme involved in desphosphorylating a variety of substrates, often with implications in cell signaling. The B. bacteriovorus open reading frame Bd1204 is predicted to encode a PTP of unknown function. Bd1204 is both structurally and mechanistically related to the PTP-like phytase (PTPLP) class of enzymes and possesses a number of unique properties not observed in any other PTPLPs characterized to date. Bd1204 does not display catalytic activity against some common protein tyrosine phosphatase substrates but is highly specific for hydrolysis of phosphomonoester bonds of inositol hexakisphosphate. The structure reveals that Bd1204 has the smallest and least electropositive active site of all characterized PTPLPs to date yet possesses a unique substrate specificity characterized by a strict preference for inositol hexakisphosphate. These two active site features are believed to be the most significant contributors to the specificity of phytate degrading enzymes. We speculate that Bd1204 may be involved in phosphate acquisition outside of prey.

Poster Session

BAM-PW1014 - The bacterial cell envelope and its origin

Elitza Tocheva[1], Eric Matson[2], Kit Pogliano[3], Yves Brun[4], Jared Leadbetter[1], Grant Jensen[1]
[1]Caltech, Pasadena, USA, [2]University of Wisconsin, Oshkosh, USA, [3]UCSD, La Jolla, USA, [4]Indiana University, Bloomington, USA

The cell envelopes of most bacteria fall into two major classes. Gram-positive bacteria are surrounded by a single membrane and a thick layer of peptidoglycan (PG), while Gram-negative bacteria are surrounded by two membranes and a thin PG layer. The cell envelope maintains the shape of bacteria and provides the first line of defense from the environment. In addition, it can elicit strong immunogenic responses in animals and is therefore a major target for antimicrobials. Understanding the composition, structure and function of the bacterial cell envelope is of major importance for understanding human health and disease. To characterize the transitions in membrane remodeling and PG synthesis in Gram-positive and Gram-negative bacteria we tracked the complex morphological changes occurring during endospore formation. For our studies, we focused on the canonical Gram-positive sporulating bacterium Bacillus subtilis and on our recently-discovered Gram-negative sporulating bacterium, Acetonema longum. Using electron cryotomography, phylogeny and biochemical approaches, we showed that sporulation is an ancient event that may have given rise to the outer membrane in bacteria and that A. longum represents a missing link by being both able to sporulate and retain an outer membrane. Further comparative analysis of A. longum and B. subtilis showed that during sporulation, both Gram-negative and Gram-positive cells can transform a thin cell walls into a thick cortex and back into a thin cell wall upon germination. Since peptidoglycan can gradually be interconverted between these two states and since the synthetic machinery is conserved among Gram-positive and Gram-negative bacteria, our data show that the cell walls of these cell envelope classes must share the same basic architecture with glycan strands running circumferentially around the cell. Furthermore, our studies provide the first experimental observation in support of Gram-positive bacteria giving rise to the Gram-negative bacteria and reveal the extensive structural and functional similarities between the two main envelope classes.

Poster Session

BAM-PW1016 - Isolation of carotenoid-producing yeasts and potential improvement by UV mutagenesis

Amal Ahmed[1], Hesham Gibriel[1]
[1]*Ain-Shams University, Cairo, Egypt*

The study was carried out to evaluate the Improvement of carotenoid productivity of the Yeast wild type strain by mutagenesis treatment using UV radiation. Agricultural wastes have been used as a carbon source for yeasts to produce carotenoid (1,2). Pigmented yeasts were isolated from different plants grown in Egypt such as tomato, maize, cotton, soy bean and sunflower leaves. Yeast isolates were identified by morphological and reproduction characteristics, along with physiological and biochemical tests. Data of morphological characteristics revealed that all the isolated yeasts produced red, soft, mucoid and oval colonies. All yeast isolates were identified as Rhodotorula glutinis species. Two R. glutinis mutants (M1 and M2) were resulted after 5 min subjection to UV radiation of the wild type. In R. glutinis M1, the highest production of dry cell weight, volumetric carotenoid production and cellular carotenoid accumulation were 15.28 g/l, 7.237 mg/l and 474µg/g dry yeast respectively. While in R. glutinis M2, these values were 16.52 g/l, 7.516 mg/l and 454 µg/g dry yeast respectively.The cost of carotenoid production by fermentation can be minimized by optimizing its process, using highly pigmented-producing microorganisms cultured in cheap industrial by products.

Poster Session

BAM-PW1018 - Technological and probiotic properties of Lactobacillus fermentum isolated from fura, a West-African fermented millet

James Owusu-Kwarteng[1], Fortune Akabanda[2], Kwaku Tano-Debrah[1], Lene Jespersen[3]
[1]University of Ghana, Legon-Accra, Ghana, [2]University for Development Studies, Navrongo, Ghana, [3]University of Copenhagen, Faculty of Life Sciences, Copenhagen, Denmark

Fura is a spontaneously fermented millet-based food consumed in West-Africa. Lactobacillus fermentum strains isolated from fura were identified by (GTG)5-based rep-PCR fingerprinting and sequencing of their 16S rRNA genes were assessed for various technological and probiotic properties in-vitro, with the aim of selecting potential starter cultures with probiotic effects. A total of 176 L. fermentum strains were assessed for various properties including rate of acidification, exopolysaccharides production, amylase activities and antimicrobial activities against selected pathogens. Furthermore, 48 of the 176 L. fermentum strains were assessed for resistance to low pH and bile salts, bile salt hydrolysis, haemolytic activities and resistance to 9 antibiotics. L. fermentum strains were clustered into 3 groups represented by 36%, 47% and 17% being fast, medium and slow acidifiers respectively. About 8% of the L. fermentum strains showed strong exopolysaccharides production potentials, 78% showed weak to moderate exopolysaccharides production whereas 14% did not produce exopolysaccharides. Amylase activity was generally weak or not detected for all strains. Strains of L. fermentum produced bacteriocin-like substances that only weakly inhibited the growth of Staphylococcus aureus, Escherichia coli, Enterococcus and Listeria monocytogenes, but none of the strains exhibited antimicrobial activity against Bacillus cereus. After exposure of 49 strains to pH 2.5 for 3 h, 12.2%, 26.5%, 30.6%, 12.2% and 18.4% showed counts of >8 logcfu/ml, 6 to <8 logcfu/ml, 3 to <6 logcfu/ml, 1 to <3 logcfu/ml and <1 logcfu/ml respectively. Majority (82%) of the tested strains were resistant to bile salt (0.3% (w/v) oxgall), whiles a few (8% strains) of L. fermentum exhibited partial bile salt hydrolase activity. The L. fermentum strains showed variable resistance to the different antibiotics whereas no strain exhibited α, β or ɣ-haemolytic activity. Strains of L. fermentum showing desirable technological and probiotic properties are recommended for starter culture development.

Poster Session

BAM-PW1020 - Prevalence and quantitative risk assessment of microbial pathogens from fresh produce within the Philippines

Pierangeli Vital[1,2], Kris Genelyn Dimasuay[1,2], Kenneth Widmer[3], Windell Rivera[1,2]
[1]Institute of Biology, University of the Philippines, Philippines, [2]Natural Sciences Research Institute, University of the Philippines, Philippines, [3]International Environmental Analysis and Education Center, Gwangju Institute of Science and Technology, Korea

This study is the first attempt in the Philippines to conduct a comprehensive assessment of the microbiological quality of retailed local fresh produce, namely, bell pepper, cabbage, carrot, lettuce, and tomato. Out of 300 samples from both open air markets and supermarkets, 16.7% of the samples tested positive for thermotolerant Escherichia coli, 24.7% for Salmonella spp. and 47% for somatic phages. Results show that counts range from 0.30 to 4.03 log10 CFU · g-1 for E. coli; 0.66 to ≥ 2.34 log10 MPN · g-1 for Salmonella spp.; and 1.30 to ≥ 3.00 log10 PFU · g-1 for somatic phages. Statistical analysis show that there was no significant difference in E. coli, Salmonella spp., and phage microbial counts between open air and supermarkets (α = 0.05) signifying the similarities in microbial content of the produce. TaqMan® and AccuPower® Plus DualStar™ real-time polymerase chain reaction (RT-PCR) confirmed the presence of these organisms. The relatively high prevalence of microorganisms observed among fresh produce surveyed signify a potential hazard to food safety. The pathogen data were used together with the survey of residents consuming fresh produce for microbial risk assessment. Estimated annual risks of infection range from 2.6 x 10-4 to 2.6 x 10-2, assuming 1% and 100% of an individual's produce servings are contaminated, respectively. This information can benefit farmers, consumers, merchants, and policy makers towards disease detection and prevention. Keywords: fresh produce, Escherichia coli, Salmonella spp., somatic phage, real-time PCR, risk assessment

Poster Session

BAM-PW1022 - Control of Listeria monocytogenes in fresh cheese using lactic acid bacteria as protective culture

Márcia Coelho[1], Susana Ribeiro[1], Henrique Rosa[1], Lurdes Dapkevicius[1], Célia Silva[1]
[1]CITA-A, Department of Agricultural Sciences, University of the Azores, Angra do Heroísmo, Portugal

In recent years, there has been a particular focus on the application of antimicrobial compounds produced by lactic acid bacteria (LAB) as natural preservatives to control the growth of spoilage and pathogenic bacteria in foods. The purpose of this study was to evaluate the anti-listeria activity of LAB isolates in fresh cheese. A total of 116 LAB isolates from an artisanal cheese (Pico cheese) were screened for antimicrobial properties. Eight isolates were found to produce bacteriocins against Listeria monocytogenes. The bacteriocin-producing isolates were identified by 16S rRNA sequencing analysis as Lactococcus lactis (1 isolate) and Enterococcus faecalis (7 isolates). Fresh cheese was made from pasteurised cow's milk inoculated with bacteriocin-producing LAB and artificially contaminated with approximately 6.0 log cfu/ml of L. monocytogenes. Numbers of L. monocytogenes were monitored during storage of fresh cheese at refrigeration temperature (4 °C) for up to 15 days. All isolates controlled the growth of L. monocytogenes, although some Enterococcus were more effective in reducing the pathogen counts. The reduction was of approximately 4 log units compared to the positive control after 7 days of incubation. In comparison, an increase of 4 log cfu/g in pathogen numbers was detected at the same time point in the absence of bacteriocin-producing LAB. The combination of bacteriocin producing Enterococcus sp. L3A21M3 and L3A21M8, or L3B1K3 and L3A21M3, optimized the reduction of L. monocytogenes counts in fresh cheese, reducing in approximately 5 log units after 7 days. In conclusion, the present work demonstrates that using bacteriocin-producing isolates in the manufacture of fresh cheese might contribute to prevent the growth of undesirable pathogenic bacteria such as L. monocytogenes. Some isolates presented excellent potential to be used as adjunct/protective cultures for cheese making.

Poster Session

BAM-PW1024 - Effect of pomegranate (Punica granatum L.) juice on the inhibition of wound bacterial infection

Jehan Al Abrahim[1]

[1]Princess Nora Bint Abdul Rahman University, Riyadh, Kingdom of Saudi Arabia

Inhibition of Pomegranate (Punica granatum L.) Juice on Wound of bacterial infection was studied by using Egyptian and Qassimi grantum types, which is Considered Saudi cultivars. Several granatum juice concentrations of 75, 50, 25, 10 and 5% which were diluted with distilled water or with honey bee and then filtered through bacterial filters, were used in this study. Results indicated that complete inhibition zone of Proteus spp. and Pseudomonas aerugenosa was obtained with 10% juice dilution of Qassimi granatum type, while partial inhibition zone with Serratia marcescenes bacteria occurred in case of 25% juice dilution of the same granatum juice. Complete inhibition of Proteus spp. and Salmonella typhimurium was obtained with the juice of granatum 25% dilution. However, partial inhibition of Proteus spp. and Pseudomonas aerugenosa bacteria occurred with the aforesaid granatum juice dilution. It was noticed that the inhibition effect of granatum juice increased when honey bee was used to dilute the juice of granatum.

Poster Session

BAM-PW1026 - Rapid detection of pathogenic E. coli using microfluidic chip-based real-time PCR

Soon Han Kim[1], Seok Hwan Kim[1], Young HO Koh[1], Jin Heo[1], Hee Jeong Shin[1], Yong Hoon Kim[1], In Sun Joo[1], Sung Woo Kim[2], Sang Jae Lee[1], Kun Sang Park[1]

[1]Ministry of Food and Drug Safety, Cheongwon-gun, Korea, [2]NANOBIOSYS INC., Seoul, Korea

Rapid and accurate detection of food borne pathogens is an important food safety tool. While traditional culture-based methods are considered the gold standard, their time-consuming and labor-intensive features make these assays particularly cumbersome. To develop a rapid and sensitive detection method, a microfluidic chip-based real-time PCR approach has been developed. Twenty one target genes of pathogenic E. coli such as EPEC(bfpU, eae), ETEC(eltA, eltB, sta), EIEC(icsA, gtrA, gtrB, gtrII), EHEC(stx1A, stx1B, stx2A, stx2B, hlyA, hlyB, hlyC, hlyD), EAEC(aggR, astA) and DAEC(afaE-1, afaE-3) were selected to design target specific primers. By using these target genes, we were able to detect pathogenic E. coli within 30 minutes. This method validated according to the international guidance, has an ability to detect virulence factors rapidly and widely for samples related to each type of E. coli infection. Therefore, the method developed here may be used for the upcoming foodborne illness investigation.

Poster Session

BAM-PW1028 - Poly-gamma glutamic acid as cryoprotectant for probiotics

Iza Radecka[1], Aditya Bhat[1], Olajumoke Adebayo[1], Terry Bartlett[1]
[1]*University of Wolverhampton, Wolverhampton, UK*

Probiotics are live microorganism which when administered in adequate amount confer various health benefits on the host. However, due to their sensitive nature to different environmental conditions during freeze drying, storage and ingestion, the amount of probiotics reaching the stomach and the small intestine are often insufficient and thus have little or no health benefit for the host. This research was targeted at improving the survival of 2 probiotic organisms – Bifidobacteria longum and Bifidobacteria breve – during storage in orange juice and in simulated gastric juice using poly-gamma glutamic acid- a non-toxic biodegradable and water soluble biopolymer produced by Bacillus subtilis natto – as a protective material. Results obtained show that poly-gamma glutamic acid was effective on both probiotic organisms in orange juice as there was little or no loss in viability for probiotic organisms coated with poly-gamma glutamic acid (from 9log to 5log), over a period of 28 days while a reduction in colony count for probiotic organisms that were not coated with poly-gamma glutamic acid was observed. Results obtained from the simulated gastric juice studies show that coating the probiotic organisms with poly-gamma glutamic acid enhanced the viability of both probiotic organisms; there was little or no reduction in viability (from 9log to 8log) in the coated probiotic organisms after 4hours while a reduction in colony count was observed in probiotic organisms that were not coated with poly-gamma glutamic acid.

Poster Session

BAM-PW1030 - Clarification of the mechanism of inhibiting the biofilm formation of pathogenic bacteria on fresh produce by food additives

Md Tariqul Islam[1], Aya Ogura[1], Chikako Machida[1], Ken-ichi Honjoh[1], Takahisa Miyamoto[1]
[1]Laboratory of Food Hygienic Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

We have shown that ε-polylysine (PL), Milk Serum Protein (MSP) and Sucrose Fatty Acid Ester (SE18) effectively reduced biofilm formation of several foodborne pathogens including Salmonella Typhimurium, Listeria monocytogenes and Staphylococcus aureus. Moreover, the combination of 0.001% PL and 0.25% MSP effectively reduced biofilm formation for both Gram-positive and -negative pathogenic bacteria. This study was conducted to clarify the mechanism of inhibiting the biofilm formation of pathogenic bacteria by these safe food additives on plastic petri dish. Salmonella Typhimurium was incubated in 0.1% Bacto-Soytone with or without 0.001%PL, 0.25%MSP and 0.05% SE18 for 2 h. Total RNA was prepared from the cells after incubation for 0 and 2 h. The cDNA reverse-transcribed from the RNA was applied to DNA microarray analyses using Agilent Microarray Analysis System. At the initial stage of biofilm formation, after incubation for 2 h without additives, the cells upregulated transcription of 252 genes and downregulated 357 genes more than 2-fold of that of the 0-h cells. Compared to the expression in untreated cells, in the presence of 0.001%PL, 320 and 90 genes were upregulated and downregulated, respectively, more than 2-fold after 2 h of incubation. In case of 0.25%MSP the upregulated and downregulated genes were 67 and 38, respectively; whereas 80 and 13, respectively for 0.05% SE18 treated cells. The curli and fimbriae genes such as csg, yde and yhc and cellulose production genes like yhj and adr were upregulated after 2 h of incubation in untreated cells. Those important genes for biofilm formation were downregulated in PL treated cells. However, those genes were not downregulated in the MSP and SE18 treated cells. In these cases, there might be other genes involved in adhesion inhibition process and the possible genes are flagella and motility genes such as fli and flg, which were upregulated in those samples.

Poster Session

BAM-PW1032 - StepOne real time identification of Listeria monocytogenes and Salmonella spp. in food of animal origin

Simona Kunová[1], Alica Bobková[1], Ľubomír Lopašovský[1], Peter Haščík[1]
[1]Slovak University of Agriculture, Nitra, Slovak Republic

The main aim of the present study was to evaluate the presence of Salmonella spp. and Listeria monocytogenes in animals products. We used the PrepSEQ Rapid Spin Sample Preparation Kit for isolation of DNA and SensiFAST SYBR Hi-ROX Kit for the real-time PCR performance. We could detect strain of Salmonella spp. and Listeria monocytogenes in samples (swabs) in the investigated samples without incubation. We detected contamination of meat and dairy products pathogenic bacterium Listeria monocytogenes in 50 samples using StepOne real time PCR. We found 19 positive samples. The real-time PCR system appears to be extremely useful in the rapid screening of food samples, allowing for the rapid identification of Salmonella spp. and L. monocytogenes. The positive samples were evaluated on the basis of Ct values. The minimum Ct value of Listeria monocytogenes found in meat and milk products was 14.53 and the maximum value was 28.40. There were fifty samples of meat and dairy products investigated using a StepOne Real-Time PCR and 13 samples evaluated as negative for the presence of Salmonella. We focused on the presence stn (Salmonella enterica ser. Typhimurium DT096), sef and PEF (Salmonella enterica ser. Enteritidis SE7) genes for the molecular diagnosis of Salmonella. It is a fast, reproducible, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future. Our results indicated that the Step One real-time PCR assay developed in this study could sensitively detect Salmonella spp. and Listeria monocytogenes in food of animal origin. The Poster was supported by the project: Development of International Cooperation for the Purpose of the Transfer and Implementation of Research and Development in Educational Programs conducted by the Operational Program: Education, ITMS code: 26110230085.

Poster Session

BAM-PW1034 - The cluster lmo0036-lmo0043 of Listeria monocytogenes contains genes coding for arginine deiminase and agmatine deiminase that are involved in acid tolerance

Changyong Cheng[1], Jianshun Chen[1], Baozhu Qi[1], Ye Xia[1], Chun Fang[1], Ying Shan[1], Weihuan Fang[1]
[1]*Zhejiang University Provincial Key Laboratory of Preventive Veterinary Medicine, Hangzhou, China*

Listeria monocytogenes is resistant to acidic conditions where it encounters in the environments and in vivo. We attempted to decipher the functional aspects of the gene cluster lmo0036-lmo0041 of L. monocytogenes that contains arginine deiminase (ADI) and agmatine deiminase (AgDI) systems as well as the regulatory mechanisms upon acidic stress. Gene knock-out and complementation were approached to decipher the functions of the enzymes. Transcription of arcA (lmo0043 encoding arginine deiminase) as well as aguA1 and aguA2 (lmo0038 and lmo0040 coding for putative agmatine deiminases) was significantly increased in L. monocytogenes cultures subjected to acidic stress at pH 4.8. Deletion of arcA and aguA1, but not aguA2, impaired growth of L. monocytogenes under acidic condition at pH 5.5, and reduced its survival in synthetic human gastric fluid at pH 2.5 and in murine stomach. Bacterial load in the spleen of mice inoculated with arcA deletion mutant was significantly lower than the wild-type strain. AguA1 has optimal activity 25℃ and over a wide range of pH from 3.5 to 10.5. However, AguA2 showed no deiminase activity. Site-directed mutagenesis generated a number of catalytic mutants for AguA1 and AguA2. All mutants of AguA1 completely lost their enzymatic activity. Interestingly, AguA2 acquired agmatine deiminase activity only when Cys157 was mutated to Gly. The reverse was seen in AguA1 with G157C mutation that lost the enzyme activity. Thus, we have discovered G157 as a novel residue other than the known catalytic triad (Cys-His-Glu/Asp) that is also critical for the enzyme activity in L. monocytogenes. Furthermore, we found that argR, the arginine repressor, acts on arginine metabolic pathways and on sigB in response to acidic stress. We conclude that L. monocytogenes contains functional ADI and AgDI systems that mediate acid tolerance. Of the two putative agmatine deiminases, only AguA1 is functional.

Poster Session

BAM-PW1036 - Viability of new potential probiotic strains with anti-inflammatory effects during production and ripening of low fat Cheddar cheese

Véronique Demers-Mathieu[1,4], Sabrine Naïmi[1], Julie Audy[2], Émilie Laurin[3], Ismail Fliss[1], Daniel St-Gelais[4]
[1]Dairy Science and technology (STELA) Research Center and Institute of Nutraceuticals and Functional (INAF), Université Laval, Québec, Canada, [2]Agropur coopérative, St-Hubert, Canada, [3]Aliments Ultima Inc., Longueuil, Québec, Canada, [4]Centre de Recherche et de Développement sur les aliments, St-Hyacinthe, Canada

Recent studies have shown that, the ingestion of specific probiotics (Lactobacillus, Bifidobacterium) can reduce the metabolic syndrome in humans due to their anti-inflammatory effects. No cheeses containing anti-inflammatory probiotics are currently available in Canada. In order to show beneficial effects, probiotics must maintain good viability during the production and ripening of cheese. The objective of this study was to successfully incorporate new probiotic isolates strains with anti-inflammatory effects in low fat Cheddar cheese. Three Lactobacillus and two Bifidobacterium strains isolated from infants or adults feces were selected. These strains were identified by 16S genetic identification, the lactis subspecis of Bifidobacterium animalis were confirmed by specific Q-PCR primers and the subspecis of Lactobacillus strains were confirmed by RpoB primers. The anti-inflammatory effects of these new isolate strains were evaluated in vitro using murine macrophage cell line J774.1. The production of each of the pro-inflammatory nitric oxide (NO) and the anti-inflammatory cytokine (IL-10) were determined in presence and absence of lipopolysaccharide (LPS). To evaluate the impact of process parameters on the viability of the probiotics, the five probiotic strains were used in the production of cheese. Potential probiotic strains induced significant decrease in NO production in both basal and LPS-stimulated macrophages and were able to increase IL-10 production by macrophages. Cheese composition was similar, whatever the type of probiotic isolate strains used. During cheese production, the viability of some isolated strains was more affected during the cooking and/or salting steps. During the ripening, the viability of bifidobacteria was more affected than lactobacilli. The viability of lactococci was dependant of the type of probiotic strain used during the production. In the next study, some of these cheeses will be tested in vivo on mice to determine survival of probiotics into gastro-intestinal transit and their anti-inflammatory effect.

Poster Session

BAM-PW1038 - Effect of Lactobacillus plantarum BS25 on cholesterol levels in vitro and in BALB/C mice (Mus musculus L.)

Cherry Joy Tumampo[1], Rio May Llanes[1], Arvie Joy Buncaras[1], Ernesto Balolong[1], Marilen Balolong[1], Leslie Michelle Dalmacio[1]

[1]*University of the Philippines, Manila, Philippines*

The risk of developing cardiovascular disease due to high cholesterol level has driven the development of functional foods that can maintain normal or lower cholesterol levels. In this study, the ability of Lactobacillus plantarum BS25, a candidate probiotic isolated from a fermented rice-shrimp mixture, to reduce cholesterol levels in vitro and in vivo was determined. A mango-soymilk drink and a chocolate drink containing Lactobacillus plantarum BS25 (LpBS25) were prepared. The effect of LpBS25 on cholesterol levels was first determined in vitro through a cholesterol assimilation assay. Results showed that LpBS25 isolates alone were able to assimilate an average cholesterol concentration of 32.36 µg/mL and that LpBS25 in the fermented drink can assimilate an average of 96.11 µg/mL cholesterol in vitro. Cholesterol assimilation in the fermented drink was observed to be significant ($p<0.05$) compared to the uninoculated drink, with only 3.89 µg/mL cholesterol reduction in the latter. Chocolate drink containing LpBS25 was orally administered to hypercholesterolemic BALB/c mice to determine its effect on the lipid profile. Two set ups were made: A) hypercholesterolemic mice not given LpBS25 chocolate drink and B) hypercholesterolemic mice given LpBS25 chocolate drink. Hypercholesterolemia was induced by feeding the mice 3% cholesterol-enriched diet for 9 weeks then the mice in group B were given chocolate drink containing 109 to 1011 CFU/ml LpBS25 every other day for 5 weeks. The mean serum cholesterol levels measured at baseline and after induction of hypercholesterolemia (week 9) were significantly different for both groups ($p=0.031$). Descriptive statistics shows that at post treatment, the mean serum cholesterol reduction in the mice fed with LpBS25 is higher (48.05 mg/dL) than that of the control group (28.00 mg/dL). These results suggest that LpBS25 can be an effective inoculum in developing probiotic drinks and can provide potential health benefits, such as cholesterol reduction, to consumers.

Poster Session

BAM-PW1040 - Evaluation of Salmonella growth in peanut confectionery products

Dennis Leandro[1], Izabel Silva[1], Eduardo Duarte[1], Maristela Nascimento[1]
[1]*Institute of Food Technology, Campinas, Brazil*

Due to recent large outbreaks, peanuts have been considered a product of potential risk for Salmonella. Usually, peanut products show a low water activity (aw) and high fat content, which contribute to increasing the thermal resistance and survival of Salmonella. The aim of this study was to evaluate the behavior of Salmonella Typhimurium during storage of three different peanut confectionery products (A, B and C). Samples of 200 g were inoculated with two different Salmonella inoculum levels, 3 log cfu/g (Test 1) and 6 log cfu/g (Test 2), and stored in a B.O.D. at 27-28°C with 60-70% of relative humidity. The initial aw of the products A, B and C were 0.27, 0.41 and 0.70, respectively. In Test 1, after 15 days of storage, the aw of all samples remained practically stable. At the same time, Salmonella reductions of 2.91 log cfu/g for product A, 1.87 log cfu/g for product B, and >3.74 log cfu/g for product C were observed. After 20 days, only product A showed a slight increase in aw and a low number of Salmonella cells was recovered from product B, while in products A and C it decreased to below the detection limit. Regarding Test 2, after 15 days, the aw of products A and B increased to 0.32 and 0.45, respectively. A high reduction (>5.27 log cfu/g) in Salmonella count was obtained in product C, whereas product A showed the lowest death rate. After 20 days, no significant change was observed in aw and Salmonella reduction was 1.40 log cfu/g for both products A and B, while C showed counts below the detection limit. The results of this study indicate that Salmonella survives longer in peanut confectionery products with aw lower than 0.45.

Poster Session

BAM-PW1042 - Microbial investigation of industrial liquid egg white

Clarisse Techer[1], Amina Daoud[1], Sophie Jan[1], Michel Gautier[1], Florence Baron[1]
[1]*Agrocampus Ouest, Rennes, France*

Although egg white is known as expressing efficient antimicrobial properties under physiological conditions, little is known on the microbial quality of industrial egg white. This issue is of great importance when egg white enters into the composition of highly perishable products, such as chilled egg-based desserts. Due to the high thermo-sensitivity of its constitutive proteins, liquid egg white is stabilized at low heat-treating times and temperatures (2 to 6 min at 55°C to 57°C in France). The remaining of heat-resisting bacteria may shorten the shelf-life of the desserts, with damaging health and economical consequences. The objective of this study was to investigate the microbial quality of raw and pasteurized liquid egg white products collected in a French company at two consecutive warm and cold seasons. A total of 63 samples were analyzed, including 30 raw and 33 pasteurized (at 57°C for 6 min) liquid egg white products. The level and type of bacterial contamination was evaluated by pour-plating in PCA (incubation for 24h at 30°C under aerobic conditions) and 16SrDNA sequencing of the colonies. The average counts were fairly low and a broad range of population was highlighted from one sample to another, namely 1.7 ± 1.6 log CFU/mL (<1 CFU/mL to 8.3 log CFU/mL) and 0.8 ± 0.9 log CFU/mL (<1 CFU/mL to 3.6 log CFU/mL) for raw and pasteurized samples, respectively. Raw egg white mainly contained the Gram negative Enterobacter and Escherichia genera while the Gram positive Enterococcus genus prevailed in the pasteurized samples. This first report on the microbial quality of industrial egg white confirms the efficiency of the pasteurization step to eradicate Gram negative bacteria. However, it also highlights the need to control egg white contamination with heat-resisting Enterococcus for a better control of spoiling issues, and particularly in egg-based desserts supporting further bacterial growth.

Poster Session

BAM-PW1044 - Oral administration of Lactobacillus plantarum RGU-Lp1 represses inflammatory responses in mice

Yuko Kato-Mori[1], Shigefumi Kishida[1], Katsuro Hagiwara[1]
[1]Rakuno Gakuen University, Ebetsu, Japan

Some of lactic acid bacteria (LAB) have potential role for relief of inflammation and those effects vary in the LAB strain. Administrations of the certain LAB strains repress the production of inflammatory cytokine in vivo. Recently, we identified Lactobacillus plantarum RGU-Lp1 (Lp1) that induce IFN-γ expression in lymphocytes, but never be colonized in mice. To investigate immune modulatory effect of LAB strains, we examined immunological properties of LAB in BALB/c mice and investigated an anti-inflammatory effect of Lp1 using LAB-deleted mice. Lp1 was administered to BALB/c mice 4 weeks, the gene expression of TNF-α and IL-1β in LPS induced splenocyte were examined by real-time RT-PCR. Interestingly, the expression of IL-1β was suppressed by Lp1 administration. To investigate whether IL-1β repression is attributed to a specific LAB species, BALB/c mice were treated with the combined antibiotics (penicillin, streptomycin, and kanamycin) to eliminate LAB from gastrointestinal (GI) tract. After the antibiotics treatment, the mice were divided into 3 groups; 1st group had no Lactobacilli in GI tract, 2nd group was administered with Lp1, and the last group was administered with Lactobacillus plantarum ATCC8014. Then, those mice were induced inflammation in lung by OVA sensitization. After the sensitization, the pathological change in lung was scored and compared with other groups. The pathological score was lowest in Lp1-treated mice, but the mice-lacking LAB marked the highest score. The gene expression of IL-1β in the cells from bronchial lavage fluid (BALF) was suppressed significantly in those of Lp1-treated mice. The results indicate that the Lactobacillus plantarum RGU-Lp1 in mice GI tract is efficacious in repressing IL-1β expression by the OVA-induced inflammation, and may have a role of anti-inflammatory effect.

Poster Session

BAM-PW1046 - The application of CRISPR subtyping used for surveillance of Salmonella contamination

Qiuchun Li[1], Fei Zhao[1], Jinlin Huang[1], Yachen Hu[1], Zhiming Pan[1], Xinan Jiao[1]
[1]*Jiangsu Key Laboratory of Zoonosis, Yangzhou University,Yangzhou, China*

As CRISPR has been developed in molecular subtyping of different pathogens, we tried to evaluate the CRISPR on typing of Salmonella. First, we used a quantitative method to acquire data on Salmonella contamination of whole raw chickens (fresh and frozen) available to consumers between April 2011 and March 2012. In total, 240 samples were taken and overall contamination rate of 33.75% for Salmonella were detected. Among the isolated 81 Salmonella contaminated chickens, 78 strains were identified in 28 different serotypes. The major prevalent serotype was Salmonella Typhimurium (34.6%). The CRISPR genotyping method and PFGE was then used to distinguish them. The result showed that both evolutionary tree constructed by the two methods were similar, even though the CRISPR subtyping method did not show higher discriminatory power than PFGE analysis. Another interesting detection was that the number and arrangement of CRISPR spacers among different isolates were quite different. We detected 148 different spacers in CRISPR1 and 119 in CRISPR2 among these 29 strains. The CRISPR spacers and arrangement of these spacers have been confirmed to be related with the strain development in different environments. So we constructed the spacer library of Salmonella Typhimurium and compared it with the reported spacers in database. The result showed that the library is very useful and important for strain tracking and surveillance of Salmonella contamination based on this powerful subtyping tool.

Poster Session

BAM-PW1048 - Sprouts and stems show different pigmentatin behavior from the flesh of garlic (Allium sativum L.)

Lee Sri[1], Kyu Kyung[1], Joo-Hyoung Cho[2], Chang-Ho Chung[2]
[1]Dept. of Food Science, Sejong University, Sejong, S. Korea, [2]Dept. of Culinary Science and FoodService Management, Sejong University, Sejong, S. Korea

Sprouts and stems did not turn green while flesh of the same garlic formed intense green pigmentation upon crushing. The levels of thiosulfinates and amino acids of both flesh and sprouts/stems of the same garlic were not significantly different, implying that neither of the two greening elements are limiting in sprout/stems. When total garlic thiosulfinates extracted from the greening garlic were added additionally to homogenized sprouts/stems, green pigmentation appeared, suggesting that sprouts/stems contain necessary amounts of amino compounds. When thiosulfinates extracted from fresh sprouts/stems were added to heated greening garlic flesh (therefore it does not turn green), the heated flesh turned green, suggesting that sprouts/stems contain color developing thiosulfinates necessary for greening reactions. When sprouts/stems were added to homogenized greening flesh, greening intensity of the flesh was reduced progressively as the level of sprouts/stems increased. When sprouts/stems were heated at 80C for longer than 30 seconds but shorter than 5 min, the homogenized sprouts/stems turned green suggesting that the greening inhibitory principle of sprouts/stems were destroyed by heating. Alliinase which is essential for green reaction survives such heating. Sprouts/stems seem to contain factor(s) interfering the greening reaction by somehow consuming color developing thiosulfinates. Since the inhibiting activity of sprouts/stems was inactivated by mild heating, the inhibitory principle was postulated to be extremely heat-labile protein(s).

Poster Session

BAM-PW1050 - Aflatoxin producing capacity of Aspergillus flavus in maize cultivated in selected local governments in Ibadan, Nigeria

Abosede Fawole[1], Gbemisola Onipede[1], Samuel Omotosho[1]
[1]*The Polytechnic, Ibadan, Ibadan, Nigeria*

The aflatoxin producing capacity of Aspergillus flavus isolated from locally stored maize was investigated in this work. Three samples each from four selected locations were screened for the presence of aflatoxins, using Extraction method and Thin Layer Chromatography (TLC). All four aflatoxins B1, B2, G1, and G2 were detected in the samples with highest ppb of 24.10, 7.67, 4.89 and 10.78 respectively. Twenty five isolates of Aspergillus flavus (all L-type) were obtained from the samples and were used to colonize aflatoxin-free maize sample (ACR.97TZL COMPI-W) obtained from International Institute of Tropical Agriculture (IITA), Ibadan. Only aflatoxins B1, B2 and G1 were detected in the kernels colonized by isolates. Fifteen isolates (60%) secreted B1 aflatoxin at 20 ppb and above while thirteen isolates (52%) secreted aflatoxin B2 above 20ppb. Six isolates (24%) secreted aflatoxin G1 above 20ppb and none (0%) of the isolates secreted G2-aflatoxin up to 20 ppb. Most of the Aspergillus species produced aflatoxins at a level higher than the U. S. Food and Drug Administration (FDA) regulations (20 ppb) and can cause exposure per person above the Nigerian recommended maximum of 7.6 µg day-1 for food stuff. These results suggest that variable management practices should be put in place to reduce contamination across the agro-ecological zones in Ibadan to safeguard the health and welfare of maize consumers. Key words: aflatoxins, fungi, maize, contamination. Correspondence to: A.O. Fawole, Biology Department, The Polytechnic, Ibadan, Nigeria. E-mail: fawiiwife@yahoo.com

Poster Session

BAM-PW1052 - A study on mononuclear cell VDR expression and HPLC assay for plasma levels of cholecalciferol and 13-cis retinoic acid in tuberculous patients

Amitava Chakrabarti[1], Anand Srinivasan[1], Debasish Hota[1], Dheeraj Gupta[1], Dibyajyoti Banerjee[1]
[1]PGIMER, Chandigarh, India

Objective: To estimate the concentration of cholecalciferol and 13-cis retinoic acid (RA) in the plasma and pleural fluid of patients with tuberculosis (TB) against controls and to measure the mononuclear cell VDR expression in TB patients and healthy controls. Methods: Plasma levels of cholecalciferol and 13-cis-RA were measured in 22 patients with TB and healthy controls and their pleural fluids levels were measured in 6 TB patients and diseased controls by established high-performance liquid chromatography based procedure. VDR expression was determined by measuring the mRNA expression of VDR gene by Polymerase Chain Reaction (PCR) method. Results: Cholecalciferol levels in plasma and pleural fluid of patients with TB and healthy controls were 67.45 (10.71) nmol/L and 21.40 (8.58) nmol/L compared with 117.43 (18.40) nmol/L ($P < 0.001$) and 94.73 (33.34) nmol/L ($P = 0.0049$), respectively. 13-cis-RA level in the plasma of TB patients and healthy controls were 1.51 (0.72) nmol/L and 6.67 (0.81) nmol/L ($P < 0.001$), respectively. 13-cis-RA was not detectable in pleural fluid. VDR expression was less in TB patients against the healthy controls. Conclusion: There is a combined deficiency of cholecalciferol and 13-cis-RA in TB patients. Because cholecalciferol and 13-cis-RA are in equilibrium with active ingredients of vitamins A and D, we feel that there is a combined deficiency of these vitamins in patients with TB. There is also a decreased expression of VDR in TB patients. It has been proved that concomitant vitamin A and D supplementation can kill intracellular Mycobacterium tuberculosis in vitro. Therefore, the observations made can pave the path for a trial of combined supplementation of available formulations of vitamin A and D for novel anti-tubercular drug therapy. Because such an approach is host-based it has potential to treat multidrug- resistant and extensively drug-resistant forms of TB too."

Poster Session

BAM-PW1054 - Collection, management, development, and practical use of pathogen resources in Korea

Kwang-Jun Lee[1], Soonyoung Han[2]

[1]Pathogen Resource TF, Center for Infectious Diseases, National Institute of Health, Korea Centers for Disease Control & Prevention, Chungbuk, Korea, [2]Center for Infectious Diseases, National Institute of Health, Korea Centers for Disease Control & Prevention, Korea

Pathogen resources are closely connected with healthcare in that they are one of the most important raw materials in order to develop vaccine, treatment, and diagnostic reagents against infectious diseases. Recently, they are debated in the process of adopting Nagoya protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization to the convention on biological diversity. Based on their importance, Korean government leads and supports the collection, management, development, and practical use of pathogen resources through National Culture Collection for Pathogens (NCCP) as the centralized pathogen resource bank. The most distinctive feature of NCCP is that its collecting resources are focused on "human-originated pathogens": these are isolated from patient's specimen asked for laboratory testing in KNIH or collected through three regional banks in university hospitals. From the primary isolates, resources valuable at a country level were sorted and deposited in NCCP as a central bank with the information including clinical data, epidemic background, resource characteristics, and the isolation information. NCCP currently comprise about 4,500 items, including bacteria, fungi, viruses, and the derivative materials. The whole resources are preserved in dual security system of identity card/fingerprint and vein recognition systems based on the infectious characteristics of pathogens. Among them, about 1,300 strains are opened to the public through NCCP on-line Catalogue database (http://nccp.cdc.go.kr) for distribution. Additionally, NCCP is trying to improve the usability of the standardized resources through resource development projects. They include developments of reference strains for all sorts of guidelines using Korean isolates, educational strain sets for medical students or diagnostic agents, and standard materials for researches on infectious diseases. Thus, NCCP establishes a public infrastructure for culture collection specific to pathogens and contributes to scientific communities by maintaining and serving high-quality microbial resources in medical science and laboratory healthcare.

Poster Session

BAM-PW1056 - Effect of the catalase on radiation stress in the budding yeast Saccharomyces cerevisiae

Takuto Nishimoto[1], Masakazu Furuta[2], Mitihiko Kataoka[1], Masao Kishida[1]
[1]Grad.Sch.Tecnol, Univ.of Osaka Pref, Sakai, Japan, [2]Grad.Sch.Environ.Sci., Univ.of Osaka Pref., Sakai, Japan

Background and Purpose: Two actions of ioninzing radiations ara known on living organisms. One is the direct action which causes breaks on a single or a double strand of cellular DNAs by ionization of DNA molecules. The other is the indirect action in which reactive oxygen species (ROS), mainly hydroxy radical (OH·), generated by radiolysis of water molecules within cells and culture media outside of the cells attacks the DNA and the other cellular components. In order to investigate the detail defense mechanism of the actions of ionizing radiations, we focused the effect of catalase, an antioxidative enzyme, on the ROS generated within Saccharomyces cerevisiae cells by the indirect action of the ionizing radiations. Methods and resaults: Wild type cells and isogenic strains cta1 and ctt1 disruptants deleted for (catalase A and T, respectively) were exposed to ^{60}Co gamma-rays at various doses (0-4kGy). The cta1 disruptant and the wild type strain showed similar radiation sensitivity in cell killing by ^{60}Co gamma-rays. In contrast, ctt1 disruptant showed significantly more cell killing by ^{60}Co gamma-rays. They showed the similar trend in cell killing by hydrogen peroxide, one of the ROS. Furthermore the Catalase-T overexpressed strain showed dramatically increased catalase activity, survival against the irradiation and hydrogen higher than ctt1. These results suggest that catalase-T plays an important role of a sufficient increase in tolerance to the exposure of ^{60}Co gamma-rays.

Poster Session

BAM-PW1058 - Supporting the discoverability of research objects by connecting research and researchers with ORCID

Subba Rao Chaganti[1], Rebecca Bryant[2]
[1]University of Windsor, Windsor, Canada, [2]ORCID, Urbana, USA

A long-standing challenge within the research community has been the inability to reliably connect individuals with their contributions, including articles, datasets, and other research objects. Researchers also struggle to make connections with potential collaborators. ORCID , an open, non-profit, and community-driven organization, provides a unique and persistent identifier to researchers, connecting them with their activities through integration in research workflows. Since its launch in October 2012, the ORCID registry has grown steadily and organizations within the interconnected publishing, funding, and academic research communities have integrated the ORCID identifier into their workflows. Support from the Alfred P. Sloan Foundation has enabled ORCID to support the integration with variety of research platforms, including VIVO, DSpace and Hydra/Fedora repository tools, research data life cycle management tools like HubZero, and the Reactome biological pathways knowledge base data center. This poster will provide an overview of ORCID and how ORCID iDs can be obtained and profiles curated. The poster will also provide examples of how platforms like VIVO , Reactome , and HubZero are integrating ORCID iDs into their systems, better enabling the open collection, dissemination, archiving, and discovery of a broader range of research objects as well discovery and visibility of the researchers who create them. 1 http://orcid.org/ 2 http://orcid.org/blog/2013/09/27/announcing-orcid-adoption-integration-program-awardees 3 http://vivoweb.org/ 4 http://www.reactome.org/static_wordpress/about/ 5 http://hubzero.org/

Poster Session

BAM-PW1060 - The glucose/acetate transition of Escherichia coli

Brice Enjalbert[1], Pierre Millard[1], Muriel Cocaign-Bousquet[1], Jean-Charles Portais[1], Fabien Létisse[1]
[1]LISBP/INSA, Toulouse, France

It is well described that the enterobacterium Escherichia coli produces acetate when growing on glucose. When glucose is exhausted, this acetate is then consumed. The resulting glucose/acetate transition (and hence, the switch from glycolysis to gluconeogenesis) is a natural model to study dynamic adaptation. Exponential growths on glucose and on acetate as the only substrate have been well described in the literature. However, experimental data and knowledge are scarce about the acetate metabolism during and after the growth on glucose. Here, several unexpected features were unraveled by investigating the physiological and molecular properties of the acetate metabolism during the different stages of the glucose/acetate transition. First, during the glucose consumption phase, the acetate accumulation was shown to result from both a constant flux of acetate production and an adjustable flux of acetate consumption. Second, the transition was proved to be a fast process, with the metabolite and transcriptional patterns being set up in minutes after glucose exhaustion. Third, acetate consumption is rapidly observed after glucose exhaustion but this metabolism is catabolic without biomass formation. Artificial stabilization of the acetate concentration allows observing an eventual induction of the glyoxylate shunt genes and anabolism on acetate but only 5 hours after glucose exhaustion. This delay is dramatically reduced proportionally to acetate concentration from a threshold of 16mM acetate. During the catabolic use of acetate, the cells are able to swiftly resume growth on glucose. We therefore hypothesize the finality of such a metabolic organization is to allow for a rapid switch on a better substrate. This work highlights the complex relationship of E. coli to acetate. Acetate metabolism and the many mechanisms of the so-called stationary phase are only beginning to yield their secrets.

Poster Session

BAM-PW1062 - DNA Encapsulation: a new method for DNA long-term preservation at room temperature

Dominique Clermont[1], Sylvain Santoni[2], Safa Saker[3], Maïté Gomard[1], Eliane Gardais[3], Chantal Bizet[1]
[1]Institut Pasteur, Collection de l'Institut Pasteur, Paris, France, [2]UMR AGAP, INRA, Montpellier, France, [3]Banque d'ADN et de cellules, Généthon, Evry, France

Background: Current methodologies rely on maintaining DNA samples in cold environments during storage procedures and transport processing. In view of the exponential increase of the number of DNA samples to be stored on one hand, and the cost and the risks of freezers' breakdowns on the other hand, room temperature storage of dehydrated DNA appears as an alternative solution having significant advantages, particularly in long term DNA storage and in the area of sample shipment. Through the French call IBiSA (Infrastructures en Biologie, Santé et Agronomie), a new procedure for room temperature storage of DNA was evaluated whereby DNA samples from human tissues, bacteria and plants were stored under an anoxic and anhydrous atmosphere in small glass vials fitted in stainless-steel, laser-sealed capsules. Methods: Samples were stored dry at room temperature for various time periods to assess any degradation as compared with frozen control samples. The study included accelerated ageing by using a high temperature (76°C, 50% relative humidity). Results: No detectable degradation of the DNA occurred for 18 months at room temperature. At 76°C, after 30 hours of storage, it was necessary to include a small adjustment to the process in order to avoid losses through aggregation or adsorption. In these conditions, the recovered DNA exhibited no detectable degradation. Its quality was confirmed using spectrophotometry, fluorometry, gel electrophoresis and pulsed field gel. Furthermore, no interference or inhibition in PCR applications was evidenced. Conclusion: This study including accelerated ageing results has demonstrated the interest of this DNA storage procedure for room temperature storage.

Poster Session

BAM-PW1064 - Apoptosis induction and cytotoxicity of Vibrio cholerae non-O1/non-O139 isolated from clinical and environmental sources in Thailand

Boonnapa Kanoktippornchai[1], Chariya Chomvarin[1], Chariya Hahnvajanawong[1], Thitima Nutrawong[1]
[1]Khon Kaen University, Khon Kaen, Thailand

Vibrio cholerae non-O1/non-O139 is capable of producing sporadic outbreaks of cholera-like diarrhea; however, the pathogenic mechanisms of this bacterium remain unclear. The objectives of this study were to: 1) compare the apoptosis induction and cytotoxicity between V. cholerae non-O1/non-O139 with hlyA positive and hlyA negative strains; 2) determine the molecular mechanisms of apoptosis induction between V. cholerae non-O1/non-O139 with hlyA positive and hlyA negative strains; and 3) compare those activities between clinical and environmental V. cholerae non-O1/non-O139 isolates. Using cytotoxicity assay and apoptosis assays (ethidium bromide/acridine orange (EB/AO) staining, caspase-3 activity and DNA fragmentation), V. cholerae non-O1/non-O139 that carried hlyA, produced significantly higher cytotoxic activity (70.6%) and apoptosis induction (59.6%) than hlyA negative strains (37.0% and 37.5%, respectively). Western blot analyses revealed that V. cholerae non-O1/non-O139 hlyA positive strains had significantly increased expression of Bax and active caspase-3 and significantly decreased expression of NF-κB and Bcl-2 than hlyA negative strains. Expression of BID in V. cholerae non-O1/non-O139 hlyA positive and negative strains was not significantly different, whereas the truncated BID was not found, indicating that V. cholerae non-O1/non-O139 induced apoptosis through mitochondria-dependent apoptosis pathway and not extrinsic pathway. V. cholerae non-O1/non-O139 isolated from clinical sources had significantly higher cytotoxic activity (79%) and apoptosis induction (65.2%) than bacteria isolated from environmental sources (63% and 54.6%, respectively). Our results indicate that hlyA production plays a role in cytotoxicity and apoptosis induction. The pathway of apoptosis induction should be at least activated through a mitochondria- dependent apoptosis pathway.

Poster Session

BAM-PW1066 - Phylogenetic analysis of the H-NS superfamily of proteins in Enterobacteriaceae.

Stephen Fitzgerald[1], Stefani Kary[1], John Stavrinides[1], Daniel Stoebel[2], Andrew Cameron[1]
[1]University of Regina, Regina, Canada, [2]Harvey Mudd College, Claremont,USA

H-NS and H-NS-like proteins are widespread in Enterobacteriaceae where they play essential roles in chromosome organization and the regulation of global gene expression. Structural and functional information about this family of proteins is primarily derived from studies investigating the chromosomally encoded H-NS and StpA paralogues in the model organisms E.coli and S. Typhimurium. Such studies enabled the identification of numerous H-NS-like proteins throughout members of the enterobacterial family based on sequence and functional similarities. However, very little is known about the diversity and evolutionary relatedness of H-NS-like proteins within the Enterobacteriaceae. This is further complicated by the presence of plasmid-borne H-NS-like proteins many of which move freely amongst enterobacterial species on self-transmissible plasmids. Studies investigating the inter-relatedness of H-NS-like proteins frequently demonstrate that while significant overlap in structure and function exists, each protein also has unique biological roles suggesting a certain amount of functional divergence has taken place between members. Using a phylogenetic approach we have generated an H-NS-like protein family tree from representative Enterobacteriaceae. This led to the identification of four evolutionary and likely functionally distinct sub-groups of chromosomal H-NS-like proteins: H-NS, StpA, Hfp and a group confined to the genera Pantoea and Erwinia. In conjunction with H-NS and StpA, a third chromosomally encoded H-NS-like protein, Hfp, was recently described in certain E. coli isolates. We have identified this protein in most representative species within the Enterobacteriaceae and vastly increased the number of species known to encode three chromosomal H-NS-like proteins. Extending our analysis to plasmid-borne H-NS-like proteins we also identified four distinct sub-groups, each corresponding with our identified chromosomal H-NS-like protein sub-groups, indicating multiple independent origins of plasmid-borne H-NS-like proteins. Work is currently underway to validate each functional group experimentally and to assess the impact of the co-existence of members from each functional group within a single species.

Poster Session

BAM-PW1068 - Characterization of extracytoplasmic function sigma factor (MSEEG_0405) in Mycobacterium smegmatis

Shiau-Ting Hu[1], Pei-Yu Chen[1], Yin-Shiu Lo[1], Sheng-Hui Tsai[1]
[1]*Institute of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan*

Extracytoplasmic sigma factor (Ecf) belonging to the group of σ70 family, could sense environmental stress and cause specific genes to be transcribed. The gene MSMEG_0405 of Mycobacterium smagmatis, which is located on glycopeptidolipid (GPL) locus, has been predicted to be a regulatory protein Ecf and be associated with Sap protein (MSMEG_0404). In this study, we firstly demonstrated Ecf indeed interacted with Sap by bacterial two-hybrid assay. Several genes on GPL locus were inhibited by Lsr2, and the gene expression of GPL locus was associated with colony morphology, sliding mobility and biofilm formation. To investigate the relationship of Lsr2 and Ecf, the function and stress of Ecf, the Δlsr2, Δecf, and ΔecfΔlsr2 mutants were constructed. When using antibiotics as environmental stresses, the ΔecfΔlsr2 double mutant revealed more sensitive to amikacin, tetracycline, minocycline and tigecycline than wild type. It was found that the growth rate of Δlsr2 or Δecf single mutant was slightly slower than wild type, and the ΔecfΔlsr2 double mutant was the slowest strain. The slower growth of ΔecfΔlsr2 may be the reason causing bacteria more sensitive to antibiotics. As to the mechanism that deletion of Ecf and Lsr2 causes this slower growth remains investigation. In order to screen the stresses of Ecf more efficiently, we constructed the Gaussia luciferase reporter system to test the promoter activity of ecf and lsr2 genes under oxidative stress (5 mM H2O2), acidic pH (pH 6), and high temperature shock (48oC). As a result, no significant effect on the expression of ecf and lsr2 was observed by the above stresses.

Poster Session

BAM-PW1070 - Developing a high throughput chemogenomic approach for profiling bioactives against Burkholderia cenocepacia

April Gislason[1], Ruhi Bloodworth[1], Wubin Qu[2], Xuan Li[3], Chenggang Zhang[2], Silvia Cardona[1]
[1]University of Manitoba, Department of Microbiology, Winnipeg, Canada, [2]Beijing Institute of Radiation Medicine, State Key Laboratory of Proteomics, Cognitive and Mental Health Research Center, Beijing, China, [3]University of Minnesota Duluth, Department of Mathematics and Statistics, Duluth, United States

Development of novel antibiotics against multi-drug resistant Gram-negative bacteria like Burkholderia cenocepacia, is crucial in order to contend with the pervasive evolution of antibiotic resistance. Previously, we developed a chemogenomic approach to match antibiotics to their respective targets. In this enhanced sensitivity assay (ESA), a library of B. cenocepacia K56-2 conditional growth (CG) mutants of essential genes was exposed to antibiotics. Downregulating the copy number of essential genes and exposure to sublethal concentrations of an antibiotic caused defective growth of the CG mutant expressing low levels of the antibiotic target. We are developing the ESA into a high throughput screen by pooling and coculturing strains, followed by detection through multiplexed sequencing. To achieve this, primers were designed to amplify the transposon-genome interface of 56 CG mutants in one multiplex reaction. The number of cycles and primer concentrations for the multiplex PCR were optimized to achieve efficient and balanced amplification of the CG mutants. Indices were then added to the amplicons so each pool could be analyzed en masse in one sequencing run. To measure depletion, 27 CG mutants were grown clonally then pooled together in equal amounts or ten-fold depleted by cell density (OD600nm). The genomic DNA of each CG mutant pool was extracted and used as the template for a multiplex PCR to amplify the transposon-genome interface. The resulting amplicons were indexed and sequenced using the Illumina MiSeq platform, and the relative abundances of each ten-fold depleted CG mutant compared to the evenly pooled CG mutants. The ten-fold depletion of 25 CG was detected, demonstrating that the multiplexed sequencing is able to detect and quantify the relative abundances of these CG mutant strains. The high throughput ESA is being further developed for the identification of gene target-bioactive matches in B. cenocepacia K56-2.

Poster Session

BAM-PW1072 - Characterizing the distribution of epistatic effects using a sequence-based assay in Escherichia coli

Melissa Kohlman[1], Alex Wong[1]
[1]Carleton University, Ottawa, Canada

Epistasis, the modification of the fitness effects of a mutation by other mutations, has important consequences for the trajectory of adaptive evolution and for the shape of the "adaptive landscape". Nonetheless, the extent of epistasis, and the shape of the distribution of epistatic effects (DoEE) is poorly characterized. We are developing a high-throughput method for describing the DoEE. The basis of this experiment will be the expression of a set of 4123 E. coli ORFs (the ASKA collection) on a common antibiotic resistance mutation background, which not only provides antibiotic resistance, but also has negative effects on growth in the absence of antibiotics. Fitness of each ORF will be determined using a sequence-based assay on the resistant and wild-type backgrounds, providing a rapid measure of background-dependent fitness effects for over 4000 constructs. In addition to indicating epistasis, a lethal interaction may indicate a potential target for antibiotic drug design based on the gene being overexpressed. It may also indicate overexpression mutations that, in combination with the resistance mutation, rescue the decrease in growth effects. This information is useful, as it might indicate possible secondary mutations that occur after the initial resistance mutation and increase antibiotic resistance.

Poster Session

BAM-PW1074 - Spatial heterogeneity of soil bacterial diversity along Riparian Zone of Eutrophic Lake Wetland in Mongolian Plateau

Ji Zhao[1], Linhui Wu[1], Jingyu Li[1], Jifei Xu[1]
[1]*College of Environment and Resources of Inner Mongolia University, Hohhot, China*

Soil bacteria are important drivers for nearly all biogeochemical cycles in wetland ecosystems. Soils and sediments from Wuliangsuhai wetland, a previously uncharacterized natural wetland located in western Inner Mongolia, China, were surveyed to characterize the structure and diversity of its microbial communities using barcoded pyrosequencing based on the 16S rRNA gene. Samples were collected along a transect that spanned vegetated uplands, exposed lakebed sediments, and water-logged locations. The overall microbial communities were unexpectedly diverse. Six phyla of Proteobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Firmicutes and Deinococcus-Thermus exist in all samples. Proteobacteria was the most abundant phylum. The relative abundance of Proteobacteria in lake sediments was higher than other soil samples, showing that Proteobacteria maybe have important roles in lake sediment at the phylum level. At the genus level, the dominant genera were Thiobacillus, Sulfurimonas, Sinobacteraceae, Anaerolineaceae and Caldilineaceae. Sulfurimonas was the dominant genus in lake sediments, and higher than that in other samples. Further, we used multivariate statistics to identify the relationships shared between sequence diversity and heterogeneity in the soil environment. The relative abundances of bacterial groups at different taxonomic levels correlated with total phosphorus (TP), Total nitrogen (TN) and ammonium nitrogen centrations, but little or no relationships to organic carbon (OC). Eutrophic relative factors had a strong impact on the bacterial community structure, ammonium nitrogen was the most important factor explaining the differences in bacterial communities across samples. Hence, soil factors affect the bacterial community structure, which has profound consequences on ecosystem function.

Poster Session

BAM-PW1076 - Microbial communities and functioning in boreal forest soil and coarse woody debris under intensified biomass harvests

Emily Smenderovac[1,3], Nathan Basiliko[1,3], Kara Webster[2,3], John Caspersen[3]
[1]Laurentian University, Sudbury, Canada, [2]Canadian Forest Service, Sault Ste Marie, Canada, [3]University of Toronto Faculty of Forestry, Toronto, Canada

Use of forest biomass for the purpose of bioenergy production is increasing in Canada as concerns over fossil fuel use grow. Although best practices of conventional forest harvesting and silviculture are generally sustainable, intensified biomass removal has greater potential to impact ecosystem functioning and sustainability, including through large reductions in organic matter input to soils through reduced slash and subsequent woody debris. Microbial communities in soil and plant litter are key players in nearly all aspects of forest biogeochemical cycles and ecology. Changes in microbial communities in forest soils and coarse woody debris (CWD) could be valuable indicators of future ecosystem functioning. In order to determine how intensified biomass harvesting impacts boreal forest microbial communities, research plots of jack pine near Chapleau, Ontario, which were differentially harvested with, tree length (“business as usual”), full tree, stumped, and bladed clear cut treatments, were compared to unharvested control plots and nearby old-growth forest. Sequencing (on the Roche 454 platform) and T-RFLP of fungal, bacterial, and archaeal rRNA genes in soils and CWD was performed to determine whether the treatments or wood decay stage lead to differences in microbial community structure. Microbial enzyme activities and community-level physiological profiles (through substrate induced respiration) were characterized to determine whether functional differences exist across soils regarding the ability of the soils to degrade lignose, cellulose and access specific nutrients. The most apparent differences between harvest treatments and controls were seen in the functional capabilities of the communities present in the organic soil layer, though all treatments had similar responses, regardless of biomass removal rate. In CWD, sequencing results revealed species that are indicative of decay stage despite the broad ecological differences related to source material. CWD communities may allow for modeling of future diversity impacts of intensified harvesting when post-harvest soil diversity is not immediately detectable.

Poster Session

BAM-PW1078 - Purification and characterization of autoinducer-2 inactivating enzyme in periodontopathogenic bacterium Eikenella corrodens

Fariha Mansur[1], Mohammad Karim[2], Yuichiro Noiri[3], Hiroyki Azakami[1]
[1]Yamaguchi University, Yamaguchi, Japan, [2]Islamic University, Kushita, Bangladesh, [3]Osaka University, Suita, Japan

Previously, we reported that periodotopathogenic bacterium Eikenella corrodens secretes autoinducer-2 (AI-2) as a communication signal. Although its maximum expression was observed during the mid-exponential growth phase, AI-2 activity was remarkably decreased when they entered the stationary phase. We suggested that the E. corrodens has a novel AI-2 inactivation system which produces an autoinducer-2-inactivating enzyme during its stationary phase. To purify AI-2 inactivating enzyme of E. corrodens, cell-free culture supernatant of E. corrodens grown until stationary phase was fractionated by ammonium sulfate precipitation and separated by anion exchange chromatography. As a result, AI-2 inactivation activity was detected from some fractions. SDS-PAGE analysis of these fractions suggested that AI-2 inactivating enzyme could be purified completely. The results of N-terminal amino acid sequence analysis suggested that purified protein might be outer-membrane porin. Thus, I constructed porin gene-defecient (ΔporA) strain. AI-2 production of ΔporA strain was not decreased even after stationary phase, whereas that of wild-type strain was decreased. Furthermore, AI-2 activity of MHF was decreased in a dose-dependent manner. These results suggested that porin protein might be involved in AI-2 inactivation of E. corrodens. AI-2 precursor, 4,5-dihydroxy-2,3- pentanedione (DPD), was inactivated by the addition of porin. This result suggested porin protein might be involved in the inactivation of DPD, MHF, and AI-2 by similar mechanism. I investigated the effect of AI-2 inactivation on biofilm formation ability of E. corrodens. Biofilm efficiency for ∆porA E.corrodens was slightly greater than wild type. This suggested that AI-2 inactivation by porin might affect the biofilm formation, maturation and detachment in E. corrodens. Prevention of quorum sensing is one of the key steps to limit the progression of disease. AI-2 inactivation enzyme may become a potential therapeutic agent for treating many diseases including periodontal disease.

Poster Session

BAM-PW1080 - Influence of seasonal temperature variation on methanogen communities and methanogenesis in manure storage tanks

Maialen Barret[1,2], Marc Auffret[1], Nathalie Gagnon[1], Edward Topp[3], Lucie Masse[1], Daniel Massé[1], Guylaine Talbot[1]
[1]Dairy and Swine Research and Development Centre AAFC, Sherbrooke, Canada, [2]Ecole Nationale Supérieure d'Agronomie Toulouse, Tolosan, France, [3]Southern Crop Protection and Food Research Centre AAFC, London, Canada

In 2008, 12% of the greenhouse gas (GHG) emissions from the Canadian agricultural sector originated from manure management. In liquid manure storages, methane is the primary GHG. Methane emissions result from the combination of successive microbial anaerobic processes including conversion of organic matter into soluble organics, volatile fatty acids (VFAs) like acetate, H2, formate and CO2. Generally in manure storages, the syntrophic interaction between hydrogenotrophic methanogens and acetate-oxidizing bacteria is the dominant pathway in methanogenesis. The objective of this study was to quantify the impact of temperature on methanogen community dynamics and activity in manure. Shallow and bottom manure samples were collected and incubated in the laboratory with varying temperatures (4; 12; 20 and 25 °C) over a storage period of 150-200 days. VFAs and CH4 production were measured at least once a week. In parallel, methanogen community structure and composition were characterized by LH-PCR targeting the mcrA gene, and amplicons were sequenced. Data were analysed by nonmetric multidimensional scaling and other statistical tools (PC-ORD package). Methane production increased with temperature, and the relationship was well described with an Arrhenius equation. Activation energy values calculated in dairy manure were 1.2 fold higher than those in swine manure, indicating CH4 emission rates (mL/kg-manure/day) were more responsive to temperature in the dairy tanks. VFAs concentrations and CH4 emission rates were inversely related. Initially, methanogen community structures were significantly different in all tanks. Then, a site-specific variability was observed based on temperature, incubation time and depth. A more significant variability in community composition was observed for shallow samples in both swine manure tanks explained by Methanoculleus enrichment. Both Methanosarcina and Methanocorpusculum were enriched in dairy manure. A better understanding of the methanogenic community dynamics and energetics would help to assess the potential methane emission from manure storages.

Poster Session

BAM-PW1082 - Stress-induced aggregate formation by Gram-negative rodent pneumonic bacteria, CAR bacillus

Fumio Ike[1], Ayako Kajita[1], Hiromi Sakata[1], Mitsuo Sakamoto[2], Toshiaki Kokubo[3]
[1]Experimental Animal Division, RIKEN BioResource Center, Tsukuba, Japan, [2]Japan Collection of Microorganisms, RIKEN BioResource Center, Tsukuba, Japan, [3]Fundamental Technology Center, National Institute of Radiological Sciences, Chiba, Japan

The cilia-associated respiratory bacillus (CARB), an unclassified, extracellular, Gram-negative filamentous bacterium, colonizes the ciliated respiratory epithelium of rodents and causes persistent respiratory diseases. Recently, we developed CARB single culture system (liquid culture) and determined its full genome sequences. BLAST (nr) search of about 1,200 coding sequences (CDS) indicated that 45% of CDS were function-unknown or predicted protein genes. In order to study gene expression regarding pathogenesis, we sought phenotypic change of CARB in various conditions. When cultured in low attachment flask, CARB divided and grew in planktonic and floating state. Then, some bacteria gathered to form aggregates, which were propidium-iodide stainable and constituted of bacteria, fibers and mucus substances. In this study, we point out the importance of aggregate formation in CARB. [Materials and Methods] SMR strain of CARB was grown in Vero E6 cell culture supernatants (Vero-sup). Lyophilization was performed at RIKEN JCM. Freeze-dried CARB was revived by adding Vero-sup. Morphology of CARB was monitored under phase contrast microscopy and no contamination was confirmed by PCR. [Results and Discussion] CARB formed 'floating aggregates' in low attachment cultureware. We transferred the aggregate-form CARB on a glass bottom dish, then, the bacteria emerged from the aggregates one after another immediately after the transfer and attached to the surface of a glass, suggesting that the 'floating aggregates' were induced by a stress (attaching surface unavailable, in this case). To clarify this assumption, we paid attention to a lyophilization stress. Rehydrated CARB were composed of two types of shape: broken-form (shredded, folded or shrunken) and aggregate-form. Broken-form CARB showed no growth, but the bacteria of fine structure came into sight from aggregate-form soon after the rehydration and started good proliferation few days later. Consequently aggregate formation may work as a shelter against some kind of stress in CARB

Poster Session

BAM-PW1084 - Fungal dominant species and diversity index at the Rhizosphere soil of red pepper for the growth stage

Kab-cheol Kim[1], Do-young Ko[1], Seong-soo Cheong[1]
[1]Jeollabuk-Do Agricultural Research and Extention Services, Ik-san City, Korea

To investigate fungal dominant species and the diversity index at the rhizosphere soil of red pepper during growth stage by pyrosequencing analysis from May to November 2012, the samples were investigated at Agricultural Research & Extension Services Iksan-City, Jeollabuk-Do, Korea. The total bacteria DNA from samples were directly extracted and then PCR amplification was performed by using primers targeting from D1 to D3 regions of the 16S rRNA gene with the extracted DNA. Diversity index of Fungi were estimated by Shannon methods, and the each index of fungi on rhizosphere was changed 6.12 on May, 5.54 on June, 5.45 on July, 5.20 on September, and 5.93 on November. Compared with Eztaxon database, Dominant genera of fungi on rhizosphere soil were identified as Eukarya on May and July, Pseudeurotium on June, Leotiomycetes on September, and Chaetomium on November.

Poster Session

BAM-PW1086 - Analysis on alkane-oxidizing methanogenic community from a production water of an oil seep in Japan

Kohei Nakamura[1], Naoya Shinzato[2], Seikoh Saitoh[2], Hiroaki Aoyama[2], Kazuhiro Takamizawa[1]
[1]Gifu University, Gifu, Japan, [2]University of the Ryukyus, Okinawa, Japan

Methanogenic alkane degradation has been firstly reported by Zengler et al (Nature, 1999). Since then, many reports have documented the structures of methanogenic communities degrading alkanes or crude oil. However, mechanism of the reaction still remains to be clarified. In particular, the initiator microbe(s) of alkane degradation is regarded as a key player of the reaction and seems to be phylogenetically diverse, hence their diversity and functions should be further studied. In this study, we constructed and analyzed alkane-oxidizing methanogenic community from a production water of an oil indication. Among examined alkanes (C_8, C_{12}, and C_{16}), the community preferably degraded octane. About 85% of electron from degraded octane was consumed for methanogenesis after 441 day of incubation at 30°C. Pyrosequencing analysis based on bacterial 16S rRNA gene showed that the most dominant taxa were changed from the phylum *Proteobacteria* in the production water into *Firmicutes* in the culture with alkanes. At the family-level classification, uncultured *Peptococcaceae* bacteria (composed of 48% of total bacterial reads) were dominated and followed by *Syntrophaceae* (16%) in the culture with alkanes. The function of the former was uncertain yet, but related sequences were also found in oil sands tailings and oil reservoirs. The relatives of the latter were often found in similar environments and their function were supposed to be the initiator microbes of alkane degradation. Pyrosequencing and MPN-PCR analyses showed that the number of alkylsuccinate synthase alpha subunit genes, which is a key enzyme for anaerobic alkane degradation by some cultured microorganisms, did not correlate with increase of reads classified as *Syntrophaceae* after incubation with alkanes, indicating that this enzyme seems not to involve in the degradation of alkanes in our alkane degrading culture which is similar in a report by Embree et al (ISME J, 2013).

Poster Session

BAM-PW1088 - The Impact of Lactobacillus rhamnosus GG to intense care unit patients on respiratory and gastrointestinal tract microbiomes

Daphnée Lamarche[1], Michelle Shah[1], Nicole Zytaruk[1], Deborah Cook[1], Michael Surette[1]
[1]McMaster University, Hamilton, Canada

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in the Intense Care Unit (ICU) and has the highest mortality rate among nosocomial infections. Despite all the prevention strategies already in place, the ongoing morbidity, mortality and the cost associated with VAP are still a significant socioeconomic burden on the health care system. Moreover, late-onset VAP is more likely caused by multidrug-resistant bacteria such as Pseudomonas aeruginosa or Acinetobacter species, resulting in infections that are more difficult to treat. Therefore, it is essential to develop novel simple and inexpensive techniques avoiding the use of antibiotics to prevent VAP. A meta-analysis of several studies has suggested that probiotics reduce the incidence of VAP in the ICU. To investigate this more rigorously, a randomized control trial, PROSPECT, is assessing the impact of the administration of Lactobacillus rhamnosus GG on VAP prevalence. As part of this trial, we are examining the microbiome of patients using both culturing and molecular approaches for samples from the lower respiratory tract (endotracheal aspirate and bronchoalveolar lavage) and from the gastrointestinal tract (nasogastric tube aspirate, stool). In order to determine if the differences correlate with the disease and the probiotic administration, we are monitoring changes in the patient's microbiome by sequencing the V3 region of the 16S rRNA gene. We observed polymicrobial colonization of the lower airways even in the absence of VAP. Furthermore, we are also trying to recover L. rhamnosus GG from the samples. We have found the probiotic in few endotracheal aspirates and in bronchoalveolar lavages. The recovery from the respiratory tract is unexpected and it needs to be determined if this may be beneficial or detrimental.

Poster Session

BAM-PW1090 - Microbial and pathogen community profiling within land-based aquaculture systems using culture-based and molecular-based techniques

Nicole Kteily[1], Robin Slawson[1], Brent Wootton[2]
[1]Wilfrid Laurier University, Waterloo, Canada, [2]Centre For Alternative Wastewater Treatment, Fleming College, Lindsay, Canada

The mitigation of potential pollutants from land-based aquaculture practices (e.g. solids, nutrients, chemicals, and pathogenic microorganisms) is an important issue. Various treatment strategies used in aquaculture wastewater treatment include membrane filtration, phosphorus removal, and UV-irradiation. However, more research is required regarding the impact of these strategies on microbial communities present in land-based aquaculture systems. Of particular interest is gaining a better understanding of microbial community balance on the retention of organisms with pathogenic potential, including the fecal indicator bacteria *E.coli* and *Enterococcus* spp., as well as the fish pathogens *Yersinia* spp. and *Aeromonas* spp. The diversity of microorganisms within these communities can be described in terms of measureable characteristics (such as genetic structure or carbon metabolism), which can be applied to environmental samples to generate a traceable profile of the community. The objective of this research is to assess microbial communities, including specific pathogens, within various land-based aquaculture treatment systems. Community and pathogen analysis will take place both before and during implementation of selected treatment technologies, using complementary phenotypic (culture-based) and genotypic (molecular-based) methods such as community level physiological profiling (CLPP) and denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA, respectively. Quantitative PCR (qPCR) will be used to enumerate selected pathogen removals from the wastewater. Preliminary data suggests that microbial community stability within aquaculture systems is influenced by factors such as system design, treatment technology, and fish population. Presented findings will include an assessment of the overall microbial communities along with pathogen-specific profiles. In summary, the identification of these profiles will provide more in-depth information regarding the impact of treatment strategies on the development and stability of the microbial population exposed to various treatment strategies, as well as on those organisms detrimental to human or fish health.

Poster Session

BAM-PW1092 - Quorum sensing controls 4-hydroxy-3-methyl-2-alkylquinolines biosynthesis in Burkholderia ambifaria

Servane Le Guillouzer[1], Annelise Chapalain[1], Marie-Christine Groleau[1], Mariane Seguin[1], Jean-Philippe Dumais[1], Eric Déziel[1]

[1]*INRS - Institut Armand-Frappier, Laval, Canada*

Quorum sensing (QS) is a global regulatory mechanism of gene expression depending on bacterial density. Bacteria synthesize and secrete signaling molecules whose concentration in the environment provides an indication on bacterial density. These molecular signals, perceived by bacteria of the same population, trigger regulatory cascades synchronously in the whole population leading to bacterial activities coordination. Pseudomonas aeruginosa is an opportunistic pathogen responsible for many nosocomial infections. Its pathogenicity is attributed to multiple virulence factors who are mainly under QS control through signaling molecules belonging, on the one hand, to the N-acyl-L-homoserine lactones (AHLs) family and, on the other hand, to the 4-hydroxy-2-alkylquinolines (HAQs) family. Genes from the pqsABCDE operon are mainly involved in the biosynthesis of HAQs. We have shown that putative signaling molecules analogous to HAQs, called 4-hydroxy-3-methyl-2-alkylquinolines (HMAQs) are also produced by several bacterial species belonging to the Burkholderia genus. These are produced through an operon homologous to the pqsABCDE operon called hmqABCDEFG. While the main actors involved in regulating the expression of the pqsABCDE operon are known, the regulatory mechanism of the hmqABCDEFG operon expression hasn't been elucidated yet. Our preliminary data indicate that, in the opportunistic pathogenic bacterium Burkholderia ambifaria, a member of the Burkholderia cepacia complex responsible for important morbidity and mortality among individuals with cystic fibrosis, the hmqABCDEFG operon expression, like the pqsABCDE operon, is under QS control through molecular signals of the AHLs family. However, while the pqsABCDE operon expression is modulated by HAQs themselves, the expression of hmqABCDEFG isn't autoregulated. Since HAQs play a predominant role in regulating the expression of Pseudomonas aeruginosa virulence genes, HMAQs could be involved in bacterial pathogenicity as well. It is therefore crucial to investigate the regulatory mechanism of the hmqABCDEFG operon expression which could ultimately provide new potential therapeutic targets.

Poster Session

BAM-PW1094 - An assessment of variation in feed-dependent phylogenetic and functional classification of microbial communities in rumen of Mehsani buffalo

Nidhi Gohil[1], Nirmal Kumar J.I.[1], Jitendra Solanki[2], Chaitanya Joshi[2]
[1]Institute of Science and Technology for Advanced Studies and Research, V. V. Nagar, India, [2]Departments of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, India

The complex microbiome of rumen acts as a potential fermentative bioreactor which is vastly involved in plant cell wall degradation and biomass utilization thereby providing unique genetic resource for cellulose and hemicelluloses degrading microbial enzymes that could be used in the production of bio-fuels. To gain a greater understanding of the ecology and metabolic potential of these microbiome, we examined the changes in microbial taxonomic and functional gene profile using shotgun semiconductor sequencing with the change in diet composed of varying proportion of green roughage (4 animals) and dry roughages (4 animals) with grains . The sequencing of 48 metagenomes revealed that the Bacteroidetes were dominated at phyla level and Prevotella at genus level. The ratio of Firmicutes to Bacteroidetes was found to be higher in solid fraction as compared to liquid fraction. In solid fraction of dry roughage group, the significant increment ($p<0.05$) in Bacteroidetes abundance was observed with increment of roughage concentration. At genus level, Clostridia were significantly increasing with the increment in roughage concentration. SEED database based annotation at subsystem level revealed higher carbohydrate metabolism function. A comparison of glycoside hydrolase and cellulosome functional genes revealed more glycoside hydrolase (GH) encoding genes with higher fiber diet and significant difference in Carbohydrate Active Enzymes (CAZy) family composition between Green and Dry roughage groups of liquid fraction. The present study concludes that the change in feed has pronounced effect on the rumen microbial diversity and their metabolic profile and it also provides a base to understand modulating behavior of microbiota which can be manipulated to improve livestock nutrient utilization efficiency as well as for targeting the efficient degradation of complex carbohydrate molecules.

Poster Session

BAM-PW1096 - Multilocus sequence analysis (MLSA) of the genus Bacteroides and related taxa by using the dnaJ, gyrB, hsp60, recA, rpoB, and 16S rRNA genes

Mitsuo Sakamoto[1], Moriya Ohkuma[1]
[1]Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Tsukuba, Japan

Multilocus sequence analysis (MLSA) was performed on representative species of the genus Bacteroides and related taxa, including the genera Barnesiella, Butyricimonas, Dysgonomonas, Odoribacter, Parabacteroides, Paraprevotella, Porphyromonas, Prevotella and Tannerella. The genes selected, dnaJ, gyrB, hsp60, recA, rpoB and 16S rRNA were amplified by direct PCR and then sequenced from 107 species (some data were retrieved from the public database). Neighbor-joining (NJ) and maximum-likelihood (ML) phylogenies of the individual genes were compared. The data confirm that the potential for discrimination of the genus Bacteroides and related taxa is greater using MLSA of housekeeping genes than 16S rRNA genes. Among the housekeeping genes analyzed, the dnaJ gene was the most informative gene, followed by the gyrB gene. Analyses of the concatenated sequences (4589 bp) of all six genes revealed robust phylogenetic relationships among the species used when compared with the single-gene trees. This study demonstrates that MLSA of housekeeping genes is a valuable alternative technique for the identification and classification of species of the genus Bacteroides and related taxa. This work was supported by a research grant (2009-2011) of IFO (Institute for Fermentation, Osaka, Japan), and also by a Grant-in-Aid for Scientific Research (No. 23580126) from the Japan Society for the Promotion of Science to M. S.

Poster Session

BAM-PW1098 - Assessing authenticity of microbial genomic DNA as reference materials using NGS

Seil Kim[1], Hyo-Jin Yang[1], Sook-Kyung Kim[1]
[1]*Korea Research Institute of Standards and Science (KRISS), Daejeon, Korea*

The microbial genomic DNA reference materials (RM) can be used for the quantification and the identification of microorganisms and the methods based on the genomic DNA materials. As the RM is used as a standard, the assurance of the genomic DNA RM is very crucial. However, assessing the authenticity of microbial genomic DNA material is difficult. Although 16S rRNA gene is de facto standard phylogenetic molecular marker, it cannot be used determine the authenticity of the genomic DNA. The sequences of some strains are very similar although they were different species. DNA-DNA hybridization method (DDH) is a gold standard to determine taxonomical position of a specific strain. However it is very laborious and time-consuming. It also does not produce precise and accumulative results. Average nucleotide index (ANI) is a very robust and sensitive method based on genomic sequences to determine genetic distance of two strains. ANI is a method can replace DDH by in silico genomic comparison. Although ANI requires whole genomic sequence information, the power of next generation sequencing (NGS) enables to obtain genomic sequence information very cheaply. To determine the criteria for ANI, two Gram-negative (Salmonella typhimurium and Escherichia coli) and three Gram-positive bacterial strains (Bacillus subtilis, Staphylococcus aureus, and Listeria monocytogenes) were used for the comparison. The sequencing of the various microbial genomic DNA was carried out using Illumina MiSeq. Obtained raw sequences were assembled and the assembled sequences were used as subject of ANI calculation. In this study, we determined the criteria for the assessment of the authenticity using ANI. The minimum coverage of the draft genome and ANI similarity for identification was obtained can be used for quality control of production of the microbial genomic DNA RM.

Poster Session

BAM-PW1100 - Whole genome multi-locus sequence type analysis of six Bordetella species

Sofia Hauck[1]
[1]*University of Oxford, Oxford, UK*

Bordetella pertussis, an obligate human pathogen with low genetic diversity, remains endemic globally despite widespread vaccination. Recent pertussis outbreaks have included other Bordetella species, such as the emerging B. holmesii, as co-infections or as co-occurring outbreaks. Misdiagnosis and underdiagnosis are both common, due to varied symptoms and tests with poor sensitivity or specificity. A whole genome analysis of six species in the Bordetella genus was conducted to determine genetic similarities and phylogeny, to clarify the evolutionary background of emerging species and to aid in the search for better genetic markers for diagnosis and surveillance. Finished Bordetella genomes added to the Bacterial Isolate Genome Sequence Database (BIGSdb) were compared to determine the core genome. They and 96 newly sequenced draft genomes were analysed with multi-locus sequence typing (MLST) techniques, using the Bordetella core genome scheme, a ribosomal gene scheme applicable to all bacteria (rMLST) and a scheme of 32 virulence-associated genes. The first two schemes' results generated phylogenetic trees with similar topology, supporting rMLST as representative of the whole genome and as a scalable, backwards compatible, phylogenetically consistent identification system. The virulence scheme showed that species in the genus have different antigenic profiles, consistent with the observed lack of cross-protection. B. holmesii has none of the major toxins or adhesins found in B. pertussis. B. pertussis was confirmed to be clonal and appears to have undergone a selective sweep when vaccination was introduced. B. bronchiseptica was found to be polyphyletic and include B. pertussis and B. parapertussis, supporting the classification of the latter two as subspecies of B. bronchiseptica. B. holmesii was found to be at least the third independent case of Bordetella evolving to infect human hosts. New Bordetella species may be emerging to fill the niche vacated by B. pertussis due to extensive and specific vaccination.

Poster Session

BAM-PW1102 - Novel bacteria isolated from the ultrapure water of a Hungarian power plant

Erika Tóth[1], Zsuzsa Kéki[1], Anita Vengring[1], Zalán Homonnay[1], Peter Schumann[2], Károly Márialigeti[1]
[1]Eötvös Loránd University, Budapest, Hungary, [2]DSMZ, Braunschweig, Germany

A Gram-positive actinobacterium and a group of Alphaproteobacteria were isolated from the ultrapure water of the water purification system of a Hungarian power plant from R2A and from highly oligotrophic media. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain IV-75T belonged to the suborder Micrococcineae in the family Intrasporangiaceae. Its closest phylogenetic relative is Arsenicicoccus bolidensis CCUG 47306T (94.3%). The closest relatives of strains PI_31, PI_25 and PI_21T are Bosea minatitlanensis AMX51T (93.43%) and Bosea thiooxidans DSM 9653T (93.36%), similarity to all other taxa is less than 93.23%. Description of these novel bacteria was based on polyphasic approach. The strain IV-75T exhibited a rod-coccus cell cycle, it was strictly aerobic, non-motile, catalase-positive and oxidase-negative. The peptidoglycan contained meso-diaminopimelic acid and MK-10H(4) was the major menaquinone. The polar lipid pattern contained phosphatidylglycerol, two unidentified phospholipids, one glycolipid and several other lipid components. The major fatty acids were anteiso- C15:0, C18:1 w9c and C16:0. The members of the Alphaproteobacteria group were regular Gram negative rods, strictly aerobic and rather inactive in degradation of various carbon sources. The major isoprenoid quinone of these strains was Q-10, the major cellular fatty acids of strains PI_31, PI_25, PI_21T were C18:1w7c and 11Me18:1w7c. Based on the moderate levels of 16S rRNA gene sequence similarity and the unique combination of chemotaxonomic characteristics, strain IV-75T was considered to represent a novel species of a new genus, for which the name Aquipuribacter hungaricus gen. nov., sp. nov. was proposed. The proposed name of the Alphaproteobacteria group is Phreatobacter oligotrophus - the type strain is PI_21T(=DSM 25521T =NCAIM B 02510T).

Poster Session

BAM-PW1104 - Paenibacillus acervicinus KUDC4121 sp. nov., Isolated from Rhizosphere of a Acer okamotoanum, Ulleungdo Island in the Republic of Korea

Ye-Ji Hwang[1,2], Sa-Youl Ghim[1]
[1]School of Life Sciences and Institute for Microorganisms, Kyungpook National University, Daegu, Korea, [2]KNU Creative BioReasearch Group (BK21 plus project), Kyungpook National University, Daegu, Korea

Strain KUDC4121 (=DSMZ 24950, KCTC 13870) was isolated from the rhizosphere of Acer okamotoanum, a native plant in Ulleungdo island in the East Sea of the Republic of Korea in September 2006. Strain KUDC 4121 comprised Gram positive, motile, rods and is capable of growth from 18-37 °C and from pH 6.0-7.5 with optimal growth conditions of 30 °C and pH 7.0. It grows on tryptic soy agar media containing up to 0.5% NaCl (w/v). Cell length ranges from 2.0 to 2.5 μm. This strain is oxidase and catalase positive, and does not hydrolyze starch and casein. The G + C content of genomic DNA was 48.28 mol%. The major fatty acids were anteiso-C15:0 (62.5%) and iso-C16:0 (11.5%). The DNA-DNA relatedness between strain KUDC4121 and the reference strain was all relatedness ratio were lower than 70%. Phylogenetic analysis based on 16s rRNA gene sequence showed KUDC4121 belonging to genus Paenibacillus. The closest type strain was P. chondroitinus DSM 5051T with 97.8% similarity followed by P. alginolyticus DSM5050T (97.6%), P. pocheonesis Gsoil 1138T (97.5%), P. frigoriresistens YIM 016T (97.5%) and P. pectinilyticus RCB-08T (97.2%). Based on combined phenotypic properties and phylogenetic and genetic data, strain KUDC4121 can be considered as a novel species of the genus Paenibacillus.

Poster Session

BAM-PW1106 - Utilization of polymerase chain reaction(PCR) and QuantiFERON-TB Gold for identification of Mycobacterium tuberculosis among Iraqi patients with reference to their blood groups

Mohemid Al-Jebouri[1], Nuha Wahid[2]

[1]College of Medicine,University of Tikrit,Tikrit,Iraq, [2]Kerkuk Teaching Hospital,Kerkuk,Iraq

Tuberculosis is an infectious disease caused by the bacillus Mycobacterium tuberculosis. It typically affects the lungs (pulmonary TB) but can affect other sites i.e extra-pulmonary TB. The current study aims to find out a possible association between the different blood group ABO and Rh system among the HIV negative tuberculosis patients, and attempt to explore some immunological diagnostic facts about the disease. The patient were examined for the presence of TB utilizing QuantiFERON-TB Gold In-Tube assay and polymerase chain reaction (PCR) in comparison with routine tests like acid fast bacilli(AFB) smear, OnSite TB rapid test, erythrocyte sedimentation rate,vaccination,tuberculin and chest X-ray examinations. . Out of 300 (patients and controls) 50 selected patients and controls were diagnosed with QuantiFERON-TB-Gold in tube in relation to blood group the highest positive result in patients was found in O+ blood group (45%). While in relation to PCR real-time out of 150 patients, 19 selected patients were diagnosed with PCR and the highest positive result was found in B+ blood group (50%). It was found that QFT-GIT was more sensitive than tuberculin skin test (TST). In close contact that was BCG-vaccinated, the QFT assay appeared to be a more specific indicator of latent TB infection than TST. According to PCR it was concluded that the positivity result of PCR real-time found in sputum than serum samples. Seroprevalence of M. tuberculosis antibodies was relatively high in IgG antibody.The results of other tests like OnSite TB rapid test, erythrocyte sedimentation rate,tuberculin and chest X-ray in relation to QuantiFERON-TB Gold In-Tube assay, polymerase chain reaction (PCR) and blood groups were variable and some times highly significant differences were concluded.It was concluded that QuantiFERON TB Gold In Tube showed high sensitivity and specificity . Real-time polymerase chain reaction (PCR) is a specific tool for early diagnosis of TB.

Poster Session

BAM-PW1108 - Non-specific DNA detected by PCR with mycoplasma-specific primers from the 16S-23S rRNA intergenic spacer region

Celeste Perez[1], Daniela Feliciotti[1], Celeste Colucci[1], Mónica Tous[1]
[1]*INEI ANLIS "Carlos G Malbran", Buenos Aires, Argentina*

Mycoplasma detection must be routinely performed to ensure the absence of these adventitious agents in cell cultures. To this end, we use home-brew nested PCR to amplify the 16S-23S rRNA intergenic spacer region of mycoplasmas that detects all species of mycoplasma contaminants in cell culture. PCRs were carried out under good laboratory practices. Controls included negative and positive specimens previously tested by Höescht stain and ELISA and water as no template control. Amplicon lengths vary within 370-490 bp in the first round and 219-365 bp in the second round. Non-specific amplicons sized approximately 800 bp appeared sporadically in the second round. Two fragments derived from water and from a positive control were sequenced. BLAST analysis revealed 92% and 93% identity with strains of uncultured bacterium clones 18-1 to 18-4 and W4-1. After breakdown the procedure, we searched for potential powder sources. Twelve samples of low-powder gloves of different brands used to clean labware package, paper towels, Sontara wipes and glove box cardboard were tested by our home-brew PCR. An 800bp band was observed in one of the glove samples, but not in successive replicates. Bands of approximately 330 bp were amplified and sequenced from two cardboard samples, BLAST analysis of the amplicons showed 89% identity with Rubrobacter xylanophilus which has some strains that degrade hemicellulose. The 800pb band from low powder gloves had the same random and sporadic occurrence as previous PCRs, thus there should be another DNA source we could not identify. Interestingly, we amplified bacterial DNA from the cardboard that is usually air exposed when the tear-off strip of the box is broken. The length of the 330 bp fragment detected was within the expected size range of mycoplasma amplicons. This finding aware us about an unexpected source of contamination that could lead to false positive results.

Poster Session

BAM-PW1110 - Development of a PCR-based molecular epidemiology method for Pseudomonas aeruginosa

Masahiro Suzuki[1], Kazuhiro Yamada[1], Miyako Aoki[1], Masakado Matsumoto[1], Yoshitsugu Iinuma[2]
[1]Aichi Prefectural Institute of Public Health, Nagoya, Japan, [2]Kanazawa Medical University, Kahoku, Japan

Pseudomonas aeruginosa is one of the major causes of nosocomial infections. Molecular epidemiological analyses, such as pulsed field gel electrophoresis (PFGE), facilitate understanding of the transmission routes of nosocomial infections. PFGE is considered the golden standard of molecular epidemiology. However, it is a time-consuming and technically demanding method, and therefore cannot be used widely in ordinary clinical laboratories. In this study, we aimed to develop a molecular epidemiology method by detecting distribution patterns of open reading frames (ORFs), which we call PCR-based ORF typing (POT). ORF candidates for molecular typing were chosen by comparing whole genome sequences of *P. aeruginosa* PAO1, PA7, UCBPP-PA14, and LESB58. ORFs used for typing were selected based on distribution patterns of ORF candidates among clinical isolates. Primers for multiplex PCR detection of selected ORFs were designed. The PCR products were electrophoresed on 4% agarose gels. Genotypes of POT were compared to those obtained using PFGE and multilocus sequence typing (MLST). Ten ORFs from genomic islets, 5 ORFs from genomic islands, bla_{IMP}, and bla_{VIM} were adopted for POT. These 17 ORFs and a *P. aeruginosa* marker were detected using 9-plex and 10-plex PCR systems. Distribution patterns of 10 ORFs from the genomic islets were correlated with those obtained using MLST. On the other hand, the 5 ORFs from the genomic islands and beta-lactamases showed diversity among strains classified into the same clonal complexes. Strains collected from the same outbreaks showed the same POT type. These strains were classified into the same cluster determined by PFGE with >80% homology. Molecular epidemiology of *P. aeruginosa* can be carried out within 4 hours using the POT method. Since this method is very easy to perform even in ordinary clinical laboratories, it is a valuable tool for daily infection control measures.

Poster Session

BAM-PW1112 - Biosecurity and development of an impedimetric immunosensor to detect Staphylococcus aureus in hospital critical areas

Ana Cristina Vasconcelos Fialho[1], Antonio Aparecido Pupim Ferreira[2], Cristina Paiva de Sousa[3], Manuel Fernando Araújo-Moreira[3]

[1]Universidade Federal do Piaui, Teresina, Brazil, [2]Universidade Estadual Paulista, Araraquara, Brazil, [3]Universidade Federal de Sao Carlos, Brazil

Fundamentals in biosecurity are essential to protect man, animals and environment from risks that can be found in work activities. These activities involve teaching, research, extension and technological production that can put people in constant dangerous regarding within this context. At health level, biosecurity is a relevant instrument to the hospital infection control, establishing from methods and techniques, the way to be adopted to prevent and control the risks of an infection. In Brazil, among other diseases, hospital infection can reach 15% and it is placed on the third category in the list of problems caused in a hospital environment. This work describes the development of an impedimetric immunosensor constructed to detect pathogenic bacteria, and specifically Staphylococcus aureus in critic hospital areas aiming to contribute to the control of hospital infection. Different parameters were studied: direct immobilization of S. aureus protein A in transductor surface; immobilization of protein A in cystamine (CYS) and glutaraldehyde (GA); monolayers self-organized (MSO); different incubation times and dilution to modifiers, protein A and antibody anti-S. aureus; blockage with bovine serum albumine (BSA) and immunoassay. Electrochemical techniques (spectroscopy by electrochemical impedance (SEI) and cyclic voltammetric (CV)) and non-electrochemical (microscopy) were utilized to the characterization and immunosensor construction. SEI and CV data showed better results with MSO 2 x 10-2 mol L-1 and GA 2.5% with incubation times of 2 and 1 h, respectively. Protein A was immobilized 1:20 overnight and from monoclonal antibody anti-S. aureus (tincub = 3 h) and blockage with BSA 0,5% (tincub = 1 h). In the interaction antibody-antigen, we utilized S. aureus ATCC 6535 with 30 min incubation. The immunosensor developed indicated to be an effective method in monitoring the bacteria in hospital and could be applied as an indicative and effective instrument to the S. aureus detection in nosocomial critical areas.

Poster Session

BAM-PW1114 - Microbial genome assembly and finishing: Automated and manual tools

Marta Matvienko[1], Martin Simonsen[2], Poul Liboriussen[2], Peder Roed Lindholm Nielsen[2], Jesper Jakobsen[2], Steffen Mikkelsen[2], Henrik Sandmann[2], Søren Mønsted[2], Jannick Dyrløv Bendtsen[2]

[1]CLC bio, a Qiagen Company, Davis, USA, [2]CLC bio, a Qiagen Company, Aarhus, Denmark

De novo genome assembly and genome finishing is becoming increasingly important in the sequencing projects. The hybrid data comprised of short and long reads can complement each other to generate high quality assemblies. Here we demonstrate how an assembly of a microbial genome can be optimized using the CLC Microbial Genome Finishing Module. The module is a collection of tools for identifying, visualizing and solving problems in genome assemblies from short NGS reads, long NGS reads, and Sanger reads. The publicly available MiSeq and PacBio data for E. coli were used to optimize the parameters for genome assembly and finishing. The contigs were assembled from MiSeq data in the Genomics Workbench. To evaluate and improve the assembly, we used the Microbial Genome Finishing Module. The contigs were analyzed using the Analyze Contigs tool. This identified and annotated the problematic regions that needed further attention. Those were the regions with low, high, single-stranded, unstable coverage, and regions with unaligned read ends. After manual inspecting and editing mappings in these areas, we used the automated Join Contigs tool with the option to join contigs with uncorrected PacBio reads. This step dramatically reduced the number of contigs , and increased the N50 length. Besides using long reads, the Join Contigs tool provides an automated way of joining contigs based on other types of analyses: paired reads that are mapped to different contigs are used to identify the neighboring contigs, the distance between them, and orientation; BLAST alignment of contigs against each other; when a closely related reference is available, the contigs are joined using the BLAST alignments to the reference. To close the remaining gaps in the genome, the primers for the ends of all contigs, and for the low coverage regions, could be designed using the automated primer design tool.

Poster Session

BAM-PW1116 - Multilocus genotypic analysis of Escherichia coli O157:H7 strains isolated from different food products in Egypt

Mohamed Elhadidy[1], Mahmoud Mohammed[2], Walid Elkhatib[3]
[1]Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt, [2]Department of Food Control, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt, [3]Department of Microbiology & Immunology, Faculty of Pharmacy, Ain Shams University, African Union Organization St. Abbassia, Cairo, Egypt

In this study, E. coli O157:H7 strains(n = 33) recovered from different food sources in Egypt were characterized using molecular assays that can discriminate E. coli strains into genotypes exhibiting various levels of pathogenic potential. Genotypic characterization included lineage-specific polymorphism assay (LSPA6), Shiga-toxin-encoding bacteriophage insertion site assay (SBI), Clade 8 typing, Tir (A255T) polymorphism, variant analysis of Shiga toxin 2 gene (Stx2a, and Stx2c), and antiterminator Q genes (Q933 and Q21). LI/II (76.0%), SBI 1 (60.6%), Clade 8 (69.7%), Tir T (72.7%) and Stx2c (45.5%) were found to be significantly ($P<0.05$) overrepresented as compared to other genetic markers among E. coli O157:H7 strains. Bivariate analysis of genetic markers revealed statistically significant ($P<0.01$) pairwise correlations including LSPA-Clade, LSPA-Tir (A255T), Q21/Q933 variants-SBI genotypes, and Tir (A255T)-Clade types. Furthermore, multivariable analysis confirmed the presence of significant association between LSPA from one side and Clade types as well as Tir (A255T) from the other side. To the best of our knowledge, this is the first study that reported the characterization of these genetic markers among E. coli O157: H7 population in Middle East and Africa.

Poster Session

BAM-PW1118 - The mass spectrometry biotyper system identification of chicken's gastrointestinal tract bacteria after bee's products application to the broiler diet

Jana Petrová[1], Lukáš Hleba[1], Peter Haščík[1], Miroslava Kačániová[1]
[1]*Slovak University of Agriculture, Nitra, Slovak Republic*

The main topic of this study was to examine the effect of bee products on the Enterococci colonization of chickens gastrointestinal tract and Enterococci identification with MALDI TOF MS Biotyper. Bee pollen and propolis were administered into both feed mixtures. The first experimental group was treated with the bee pollen in feed mixture with the addition of 800 mg pollen per 1 kg of compound and the second group was treated with the propolis with the addition of 800 mg propolis per 1 kg of compound. There were investigated qualitative counts of Enterococci in ceca of 42-day-old chicken (COOB 500) using MALDI TOF MS Biotyper method in this experiment. The counts of Enterococci on Slanetz-Bartley agar were isolated. Enterococcus cells, isolated from gastrointestinal tract, were detected using MALDI TOF MS Biotyper. The counts of CFU of Enterococci were compared between the experimental and the control treatment. The lowest count was detected in the control experimental group. Bacterial species were directly identified from one bacterial colony; each colony was covered with 2 ml of matrix solution without other supplements. We used MALDI Biotyper 3.0 software to compare the first 100 peaks of each spectrum to our MALDI TOF mass spectrum database previously updated. We identified the species range of the genera Enterococcus in the intestinal tract of chickens by using MALDI TOF MS Biotyper. There were detected E. avium, E. casseliflavus, E cecorum, E. faecalis, E. faecium, E. gallinarum, E. hirae and E. malodoratus. There were the most frequent species of E. avium, E. faecium, E. faecalis and E. gallinarum in the experimental groups. The Poster was supported by the project: Development of International Cooperation for the Purpose of the Transfer and Implementation of Research and Development in Educational Programs conducted by the Operational Program: Education, ITMS code: 26110230085.

Poster Session

BAM-PW1120 - The application of pyrosequencing in periopathogen determination in aortic valves and in sulcular fluid

Tatjana Janatova[1], Stepan Podzimek[1], Jaroslav Mysak[1], Lenka Sabova[2], Tomas Prskavec[3], Zdenek Broukal[1], Jana Duskova[1]

[1]The Institute of Clinical and Experimental Dental Medicine, First Faculty of Medicine and GUH, Prague, Czech Republic, [2]Institute of Microbiology, Academy of Sciences, Prague, Czech Republic, [3]Second Surgical Clinic of Cardiovascular Surgery, GUH and First Faculty of Medicine, Prague, Czech Republic

Periodontitis may be a risk factor for inflammatory-degenerative diseases of heart and big vessels. The influence of periodontal pathogens in this respect needs further investigation. Aims: The aim of this clinical-laboratory study was to test the presence of periodontal bacteria DNA in the samples of aortic valves extracted during their surgical replacement and to assess the concomitant presence of the same periodontal bacteria DNA in periodontal environment. Material and methods: Fifteen patients, scheduled for the aortic valve replacement surgery were involved in the study. In the pre-operation stage they were periodontally examined and the samples of periodontal pockets contents were taken. Samples of impaired valves were taken during the surgical valve-replacement intervention. It was very difficult to isolate microbial DNA from aortic valves even if the standard isolation method of microbial DNA from sulcular fluid samples yielded enough material.Therefore five different methods were used for the isolation of bacterial DNA from valves samples. Both samples were processed in molecular microbiologic techniques using the 454 pyrosequencing for the qualitative detection of selected periodontal bacteria. The 454 pyrosequencing technique was used to detect the putative periodontal pathogens Tannerella forshytia, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Fusobacterium nucleatum, Campylobacter rectus, Eikenella corrodens, Treponema denticola and many others. Results: The 454 pyrosequencing technology showed a very infrequent presence of periopathogenic microbial DNA in aortic valves samples, on the other hand, samples from periodontal pockets contents of the same patients revealed the standard pattern of periopathogenic microflora. Conclusions: The role of periopathogenic microflora in chronic inflammation process in aortic valves was not proved in this study. Acknowledgement: The study was supported by research project PRVOUK-P28/LF1/6 of the Ministry of Education, Youth and Sports, Czech Republic and by projects NT 13087-3 and NT 14164-3 of the Internal Grant Agency, Ministry of Health, Czech Republic.

Poster Session

BAM-PW1122 - Whole genome sequence identification of a public culture collection strain

Philip Shwed[1], Nicholas Petronella[2], Jennifer Crosthwait[1], Deborah Ashby[3]
[1]Environmental Health Science and Research Bureau, Health Canada, Ottawa, Canada, [2]Bureau of Food Surveillance and Science Integration, Health Canada, Ottawa, Canada, [3]New Substances Assessment Division, Health Canada, Ottawa, Canada

Microorganisms that are obtained from public culture collections can serve as reference, research or biotechnology materials. However, cultures submitted to these collections may have been deposited before taxonomic identities were firmly established and require authentication. In this study, one culture deposited as Bacillus subtilis, displayed 16S rDNA nucleotide sequence characteristic of the Bacillus cereus (Bc) group. The deposit's genome was sequenced by the Illumina MiSeq, assembled into de novo contigs via the Velvet algorithm package and annotated by the Prokka annotation software. Comparison of marker genes via the Bc Multi Locus Sequence Typing website (http://pubmlst.org/ bcereus/) showed a close, but non-exact match to an existing strain. In order to determine relatedness to other sequenced Bc genomes, a whole genome phylogenetic tree was constructed, based on all identified coding sequences from all Bc group genomes (140 strains). The dendogram allowed identification of several close relatives, including the Bc type strain (Bc ATCC 14579T). Since Bc group chromosomal sequences display limited differences in gene content, the genome was also searched and PCR tested for vegetative insecticidal protein (vip3), or crystal protein (cry) gene presence. Neither were detected that would indicate B. thuringiensis identity. The study suggests that whole genome sequencing and phylogenetics may be useful for the authentication of strains and laboratories with access to next generation sequencers could help in the quality control of public culture collection strains.

Poster Session

BAM-PW1124 - Diversity within a Pseudomonas aeruginosa population occurs in response to selective pressures in the cystic fibrosis lung

Shawn Clark[1,2], Julio Diaz Caballero[3], Heather Maughan[3], Pauline Wang[3,4], Sylva Donaldson[4], Yvonne Yau[1,5], Valerie Waters[6], Elizabeth Tullis[7], David Guttman[3,4], David Hwang[1,2]

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada, [2]Latner Thoracic Surgery Research Laboratories, University Health Network, Toronto, Canada, [3]Department of Cell & Systems Biology, University of Toronto, Toronto, Canada, [4]Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, Canada, [5]Department of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Canada, [6]Department of Pediatrics, Infectious Diseases, Hospital for Sick Children, Toronto, Canada, [7]Department of Medicine, Division of Respirology, University of Toronto, Toronto, Canada

Chronic airway infections caused by Pseudomonas aeruginosa are a major contributor to pulmonary deterioration and mortality in patients with cystic fibrosis (CF). Treating Pseudomonas-dominated infections presents challenges in those with CF largely due to the organisms' intrinsic resistance to many antibiotics. Current theories suggest that over time, a clonal P. aeruginosa strain evolves into multiple related lineages carrying airway-specific adaptive phenotypes needed for persistence. Our understanding of this phenomenon relies heavily on cross-sectional analyses of single isolates, while little is known about how periodic selection influences the overall dynamics of this pathogen population. The objective of our study was to assess patterns of change in P. aeruginosa population structure within a CF patient over time, by sampling and characterizing multiple longitudinally collected isolates at both the phenotypic and genetic levels. Sequential P. aeruginosa isolates were recovered from sputum produced by the same patient over a 12-month period. Isolates were categorized by colony morphology and screened for a panel of 38 phenotypic traits including biofilm formation and antibiotic susceptibility. The genome of each isolate was also sequenced using an Illumina platform. Genomic analysis of 235 sequenced P. aeruginosa isolates found two clonally related but genetically distinct sub-populations. A high degree of phenotypic heterogeneity was found across isolates; however, both populations could be distinguished by their differential in vitro susceptibilities to aztreonam and ceftazidime. Non-synonymous mutations were found at 3 independent sites within a gene potentially involved in β-lactam resistance. The frequency of isolates containing these specific phenotypes fluctuated rapidly over time and in parallel to prolonged intravenous and inhaled aztreonam therapies administered to the patient. This study enhances our understanding of the adaptive strategies used by P. aeruginosa in the CF lung and provides some initial insight into why P. aeruginosa infections, once established are so difficult to treat.

Poster Session

BAM-PW1126 - Investigating the translocation of high temperature protein B, the multifunctional chaperonin of Legionella pneumophila

Peter Robertson[1], David Allan[1], Elizabeth Garduno[1], Rafael Garduno[1]
[1]*Dalhousie University, Halifax, Canada*

Legionella pneumophila is an intracellular pathogen which survives and replicates in a Legionella-Containing Vacuole (LCV) within freshwater protozoa and the alveolar macrophages of immunocompromised humans. L. pneumophila High Temperature Protein B (HtpB) is a member of the chaperonin family of proteins whose main function is to assist in the folding of cytoplasmic proteins. HtpB is known to be multifunctional, as it is capable of mediating invasion of L. pneumophila into HeLa cells, attracting mitochondria, and delaying phagosome-lysosome fusion. In order to perform these functions, HtpB must be secreted from the bacterial cytoplasm to the bacterial surface, and must then further enter host compartments. While prior research indicates that HtpB does manage to translocate across the bacterial membrane and reach both the LCV and host cytoplasm, currently the mechanism by which HtpB is secreted is unknown. We report here that translocation of HtpB to the bacterial cell surface may be dependent upon the Dot/Icm secretion system, the major virulence factor in L. pneumophila. That is, dot/icm mutants accumulate HtpB in the periplasm, further suggesting that the translocation of HtpB from the bacterial cytoplasm to the periplasm (across the inner bacterial membrane) occurs in a manner independent of Dot/Icm. Using a fusion protein in which HtpB is fused to the adenylate cyclase of Bordetella pertussis, we demonstrate that the translocation of HtpB into the cytoplasm of U937-derived macrophages is dependent on a functional Dot/Icm system. This research will improve the understanding of the multifunctional virulence factor HtpB in L. pneumophila, a potentially fatal pathogen of immunocompromised populations.

Poster Session

BAM-PW1128 - NOD2 is not of importance for the course of mycobacterial infections in humans

Mohammad Salem[1], Jakob Benedict Seidelin[1], Steffen Robert Eickhardt-Sørensen[2], Thomas Bjarnsholt[2], Gerhard Rogler[3], Søren Riis Paludan[4], Ole Haagen Nielsen[1]

[1]Department of Gastroenterology, Medical Section, Herlev University Hospital, University of Copenhagen, [2]Institut of International Health, Faculty Of Health Sciences, University of Copenhagen, Denmark, [3]Division of Gastroenterology and Hepatology, Department of Internal Medicine, University Hospital Zürich, Zürich, Switzerland, [4]Department of Biomedicine, Aarhus University, Denmark

Recognition of peptidoglycan-derived muramyl dipeptide (MDP) by the cytosolic sensor nucleotide oligomerization domain 2 (NOD2) induces various crucial innate immune responses. MDP constitutes a key component in bacterial cell envelopes. Most bacteria carry the N-acetylated form of MDP (A-MDP), whereas N-glycolyl MDP (G-MDP) is frequently found on mycobacteria and related Actinomycetes. Clinical studies have linked Crohn's disease (CD) to mycobacterial infections, and several animal studies have additionally reported that G-MDP has greater NOD2-stimulating capacity than A-MDP. Disease associated gene variants of NOD2 produce a loss of function phenotype, and it has been hypothesized that this could explain the suggested involvement of mycobacteria in CD. In this study, the response pattern of two intracellular bacteria, Mycobactorium avium paratuberculosis (a G-MDP producing bacteria) and Listeria monocytogenes (an A-MDP producing bacteria) was investigated as well as G-MDP and A-MDP in peripheral blood mononuclear cells among patients with CD carrying the different genotypes of NOD2. It was revealed that the NOD2 system in humans is primarily sensing A-MDP. In addition, the recognition of Mycobactorium avium paratuberculosis was NOD2-independent and involved signalling through Toll-like receptor. Moreover, Mycobactorium avium paratuberculosis was characterized by greater cytokine production such as interleukin (IL)-1β and IL-10 than Listeria monocytogenes. These results demonstrate that A-MDP activates NOD2 with significantly greater potency than G-MDP in humans as opposed to rodents, and that that NOD2 is of minor importance for the reponse of innate immunity to Mycobactorium avium paratuberculosis.

Poster Session

BAM-PW1130 - PilG controls Pseudomonas aeruginosa twitching motility through cAMP-dependent and independent pathways

Ryan N.C. Buensuceso[1], Hosam Khalil[1], Sarah Farr[1], Nanette B. Fulcher[2], Martin Daniel-Ivad[1], Matthew C. Wolfgang[2], P. Lynne Howell[3], Lori L. Burrows[1]

[1]Department of Biochemistry and Biomedical Sciences and the Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Canada, [2]Department of Microbiology and Immunology, University of North Carolina School of Medecine, Chapel Hill, USA, [3]Hospital for Sick Children, Toronto, Ontario, Canada and Department of Biochemistry, University of Toronto, Toronto, Canada

Type IV pili (T4P), one of the major virulence factors of Pseudomonas aeruginosa, are responsible for surface adhesion and movement by twitching motility. Twitching involves cycles of T4P extension, adhesion to a surface, and retraction, and is regulated by physical and chemical inputs. The Pil-Chp system (Chp) is a putative Che-like phosphorelay controlling twitching and intracellular levels of the secondary messenger, cyclic AMP (cAMP). How Chp regulates pilus extension and retraction is unclear. Here we show that the CheY-like response regulator, PilG, and the retraction ATPase, PilU, localize mainly to the leading end of twitching cells, implying that they mark the active pole. In contrast, the CheY-like response regulator PilH is diffuse, consistent with its proposed role as a phosphate sink. Levels of the PilB, PilT, and PilU ATPases, and T4P extension were confirmed to be dependent on PilG's control of intracellular cAMP levels. However, our data also suggest a potential role for PilG in modulating T4P extension/retraction dynamics. We identify a PilG point mutant, D58N, which complements cAMP production and twitching motility in Chp signaling mutants, but is not phosphorylated by its upstream histidine kinase, ChpA. We also show that sequenced Pseudomonads fall into distinct complex and simple populations based on complexity of the encoded Chp cluster. These data highlight PilG's complex role in controlling T4P extension and retraction, and show that it is the terminal response regulator of the Chp system.

Poster Session

BAM-PW1132 - Regulation of quorum sensing in gram-negative bacteria due to uptake of signaling molecules onto the artificial receptors

Norihiro Kato[1,2], Chigusa Okano[1], Yuriko Takayama[1,2], Rei Kawakami[1,2], Ken-ichi Iimura[1,2], Eri Nasuno[1,2]
[1]Utsunomiya University, Utsunomiya, Japan, [2]CREST, JST, Japan

A quorum sensing (QS) system in Serratia marcescens could be successfully blocked by trapping transmitter molecules onto artificial receptor, cyclodextrin (CD), embedded in polymer gel membranes. S. marcescens possesses N-acylhomoserine lactone (AHL)-mediated LuxIR-type circuit which regulates biofilm formation, virulence factor production, etc. The LuxR-type protein SpnR can form the stable complex with N-hexanoylhomoserine lactone after increasing its concentration above a threshold due to cell multiplication of S. marcescens. Instead of the SpnR, other host molecules that specifically interacted with the AHLs were screened to artificially rcduce the local AHL concentration around cells. The cyclic oligosaccharide CD possesses hydrophobic cavity which can form inclusion complex with hydrophobic acyl-chain of the AHL in the culture broth, while the interaction in aqueous media was demonstrated by 1H-NMR and quartz crystal microbalance sensor. In this study, the CD hosts were embedded inside hydrogel matrices for the uptake of diffusible AHLs. The CD-modified hydrogel membranes and microparticles were easily prepared with polysaccharides including alginate and hydroxypropyl cellulose. The desired amounts of the immobilized CDs were immersed into a Luria-Bertani (LB) medium when a bacterial culture starts at 25ºC. The degree of QS inhibition was evaluated by determining amounts of antibacterial prodigiosin after the cell lysis because of the QS dependence of the prodigiosin production. The blocking effects of CDs on the AHL-mediated QS could be clearly visualized; production of the prodigiosin turns the color of the culture broth into red during the cell growth when the sequential QS process is normally activated. In the presence of CDs, the culture broth could keep the ordinal color even after the turbidity of the culture broth fully increased. The bacterial cell-cell communication (QS) could be blocked by keeping the AHL concentration low level as below the threshold due to forming CD-AHL complex in the culture broth.

Poster Session

BAM-PW1134 - Inhibitory control of quorum sensing due to enzymatic degradation of autoinducers by encapsulated bacteria

Rei Kawakami[1,2], Eri Nasuno[1,2], Naoyuki Takahashi[1,2], Ken-ichi Iimura[1,2], Tomohiro Morohoshi[1,2], Tsukasa Ikeda[1,2], Norihiro Kato[1,2]

[1]Utsunomiya University, Utsunomiya, Japan, [2]CREST, JST, Japan

Hydrogel encapsulated microorganisms could effectively inhibit a quorum sensing (QS) as one of the cell-to-cell communication systems depending on concentration of the transmitting signal, autoinducer. Quorum quenching bacteria which were isolated from environmental samples, could be encapsulated in droplets of pre-gel solution using jet dispensing and then the polymer solution jet was vigorously dropped into a cross-linking solution to gelate. Our target is to suppress N-acylhomoserine lactone (AHL)-mediated QS using AHL-degrading bacteria encapsulated inside monodisperse calcium alginate hydrogel beads. The non-contact jet dispensing system could be applied as time-saving way to mass production of embedded microorganisms; approximately 70,000 gel beads (300 micrometers across) were given by the hour. Inhibitory effects of the AHL-degrading bacteria were investigated by red pigment prodigiosin, of which production was regulated by concentration of N-hexanoylhomoserine lactone (C6HSL) and N-(3-oxohexanoyl) homoserine lactone as autoinducers in Serratia marcescens AS-1. Expression of the pig clusters was activated by autoinducer accumulation around cells during multiplication and then the prodigiosin synthase was begun to produce. The desired amounts of hydrogel beads embedded with some kinds of AHL-degrading bacteria including Acinetobacter sp. and Pseudomonas sp. were dispersed in a Luria–Bertani (LB) liquid medium when a bacterial culture starts with shaking. Prodigiosin amounts were determined by measuring absorbance at 534 nm (A534) of the extracts from cell pellets and then relative prodigiosin production was determined by A534 of control without any hydrogel beads. After 16 h culture, relative prodigiosin production normalized by control continuously kept below approximately 10-20% in the presence of hydrogel encapsulated AHL-degrading bacteria. Furthermore, degrading activity of the hydrogel beads could be demonstrated by decreasing concentration of C6HSL using HPLC system. These results suggest that AHL hydrolase can be produced continuously inside hydrogel beads and enzymatic inactivation of autoinducers are responsible for the QS inhibition.

Poster Session

BAM-PW1136 - Control of gene expression in Methylobacterium extorquens using small regulatory RNAs

Roqaya Imane[1], Martin Lamarche[2], Carlos Miguez[2], Jonathan Perreault[1]
[1]Institut National de la Recherche Scientifique Institut Armand-Frappier, Montreal, Canada, [2]Conseil national de recherches Canada CNRC, Montreal, Canada

Small regulatory RNAs (sRNAs) are short non coding RNAs that control gene expression in bacteria and have the ability to bind the messenger RNA (mRNA) and inhibit translation. They can be used in any application that requires the modification of gene expression, such as metabolic engineering: an approach that aims to produce high yields of chosen metabolites. This consists in identifying potential genetic targets whose inhibition would allow increased production of the desired metabolites. Several different types of sRNA(s) can be synthesized to target distinct genes within the metabolic pathways necessary for the production of the metabolite desired. The key element of the synthesized sRNA is a sequence complementary to the target. sRNA transcription also requires a promoter which will needs to be carefully cloned to avoid unnecessary sequences in the sRNA. The longer the sRNA sequence, the higher the possibility for non-specific interactions or a reduced binding efficiency with the desired target sequence can occur. While the engineered sRNA-system as been proven useful in E. coli, it has not been used in other bacteria yet. We have built such a system in Methylobacterium extorquens. A shortened-promoter has been cloned and shown to function to express the Green Fluorescent Protein (GFP) reporter gene. The next step is to target a GFP reporter with appropriate sRNAs in order to prove the feasibility of targeting any genes. M. extorquens is a methylotrophic gram negative bacterium, able to grow on methanol. It is thus a good candidate to avoid using agricultural products as a carbon source for microorganisms used in large scale biotechnology production.

Poster Session

BAM-PW1138 - Antisense RNA control of quorum-sensing and antibiotic production in Streptomyces

Matthew Moody[1], Marie Elliot[1]
[1]*Department of Biology and Institute for Infectious Disease Research, McMaster University, Hamilton, Canada.*

Streptomyces are sporulating bacteria with a complex, multicellular life cycle. After spore germination, *Streptomyces* grows vegetatively by hyphal tip extension and branching, forming a dense mycelial network. As nutrients become scarce, aerial hyphae emerge from the vegetative cells, growing into the air. These aerial structures are then subdivided, and mature to form chains of reproductive spores. In addition to their complex life cycle, *Streptomyces* are renowned for their ability to produce an abundance of secondary metabolites having medical importance (e.g. antibiotics, anti-cancer agents). *Streptomyces* co-ordinate antibiotic production and morphological differentiation in part by producing and responding to gamma-butyrolactone quorum-sensing molecules. We recently conducted RNA sequencing (RNA-seq) studies, with our goal being to explore the non-coding RNA (ncRNA) potential of three diverse *Streptomyces* species (*Streptomyces coelicolor*, *Streptomyces venezuelae*, and *Streptomyces avermitilis*). Some of the most highly-expressed and conserved non-coding RNAs we identified were located within antibiotic biosynthetic clusters, and included ncRNAs associated with the genes encoding gamma-butyrolactone synthases and receptors, which make up the quorum-sensing systems of *Streptomyces*. In the model species *S. coelicolor*, the genes encoding the gamma-butyrolactone receptor (*scbR*) and synthase (*scbA*) are divergently transcribed with overlapping 5′ untranslated regions (5′ UTRs). In addition to the antisense RNA that results from this divergent transcription, a second antisense RNA (ScbN) is expressed opposite the coding region of *scbA*, adding an additional layer of regulatory control within this region. Here we highlight the effects of ScbN expression on the production of gamma-butyrolactones and antibiotics in this model species, and present a model that encompasses this new level of regulation governing antibiotic production.

Poster Session

BAM-PW1140 - Aflatoxin producers as index for PurityY of poultry feed and meat. A preliminary study

Maimuna Habib[1], Kwanashie Clara[1], Kwaga Jacob[1], Abdu Paul[1], Junaid Kabir[1]
[1]A.B.U, Zaria, Nigeria

Majority of grain farmers reside in the northern part of Nigeria where storage facilities are traditional or locked up stores that lack proper ventilation. Families bring out their grains to clean up and if the grains change color or smell due to poor storage, they are sold to poultry feed toll millers as whole grains or ground fine particles, at very cheap price. Lack of awareness regarding the toxicity of fungi in four local government areas in Kaduna led to survey of mycotoxins producing fungi in poultry and poultry feed. A total of 180 feed types (20 Broiler starter, 20 Broiler super starter, 10 Broiler finisher, 50 Grower's (chick) mash, 80 Layer finisher) were grown on Rose Bengal Chloramphenicol agar. Microscopic examination on slides after staining with lactophenol blue. Of the 180 feed samples, 178 (98.9 %) yielded fungi of the mold type. Mold of the Aspergillus species was isolated from all the feed types without exception. From 180 feed samples cultured, 178 (98.9%) yielded a variety of molds such as A. fumigatus 134 (75.1 %), A. flavus 36 (20%), A. niger 5 (3%), A. teneus 3 (1.7%). Of the 500 tracheal swabs of apparently healthy chicken sampled, 450 yielded fungal growth comprising A. fumigatus 126 (25.1%), A. flavus 56 (10.5%), A. niger 28 (5.5%), A. teneus 21 (4.1%), A. ochraceaus 7 (1.4%), and other molds such as Penicillium curvularia, mucor, Rhizopus and candida spp. This study showed that fungi are present in all types of poultry feeds in the four local governments. From the results, fungi are present in all feed types and in the trachea of apparently healthy chickens. Given the high rate and spread of fungi in feeds and trachea of chicken, it is recommended that poultry feed producers/ millers add anti mold agents in feeds.

Poster Session

BAM-PW1142 - Evaluation of Brucella spp. in lymph nodes of pigs and wild boars with lymphadenitis by different media

Acacia F. Vicente[1], João M. A. P. Antunes[1], Gustavo H. B. Lara[1], Mateus S. R. Mioni[1], Susan D. Allendorf[1], Marina G. Peres[1], Camila M. Appolinario[1], Bruna L. D. Ribeiro[1], Marcio G. Ribeiro[1], Jane Megid[1]
[1]UNESP, Botucatu, Brazil

Brucella spp. is a Gram-negative facultative intracellular bacterium. This study aimed to evaluate three selective culture media (Brucella Agar, Farrell and CITA) and investigate the presence of Brucella spp. by microbiological culture in lymph nodes of pigs (n=50) and wild boar (n=50) with lymphadenitis, from slaughterhouses of the São Paulo state, Brazil. The results of the pigs and wild boar lymph nodes analyzed (100 lymph nodes corresponding to 200 plates), demonstrated 70 plates with fungal growth in Brucella Agar. In the same medium, there were bacterial growth in 59 plates. In CITA medium no fungal growth was observed, just bacteria in 15 plates. In Farrell medium there was any bacterial or fungal. All bacterial growth resulted as Gram-positive. In Brucella Agar medium the growth of these microorganism is justified by the absence of antimicrobials inhibitors, such as cycloheximide, which is present in Farrell supplement and inhibits the translation of mRNA by ribosomes, preventing the fungi protein synthesis. Fungal growth may also inhibit bacterial growth, leading to reduction in the sensitivity of bacteriological diagnosis. In CITA medium the addition of amphotericin B interacts with a steroid present in the membrane of the fungus resulting in loss of selective permeability of the membrane. The Farrell medium prevents the fungal and commensal bacterial grow common during the contamination of environmental collect due the antimicrobials concentrations presented. However, some antimicrobials present in its formulation are also high growing inhibitors of B. ovis and make difficult the growing of B. melitensis, B. suis and some strains of B. abortus. The combined use of CITA and Farrell medium showed better results inhibiting the contaminants and enable the isolation of Brucella species. The presence of Brucella spp. in lymph nodes of pigs and wild boar with lymphadenitis, collected in slaughterhouses, was not detected in this work.

Poster Session

BAM-PW1144 - Screening for Mu-like and HP2-like prophage sequences within the genome of Avibacterium paragallinarum reference isolates

Elke Coetsee[1], Robert Bragg[1], Charlotte Boucher[1]
[1]*University of the Free State, Bloemfontein, South Africa*

Avibacterium paragallinarum is the causative agent of infectious coryza, an upper respiratory tract disease that occurs primarily in chickens. This disease has a huge economic impact in the poultry industry with a 10-40% decrease in egg production. Prophages, which are bacteriophages within the lysogenic stage, have been found within the genome of other Pastuerallacae spp which include Haemophilus influenza, Actinobacillus actinomycetemcomitans, Mannheima haemolytica and Histophilus somni. Recently prophages resembling a Mu-like and HP2-like phage have been found in the Modesto (C-2) strain of A. paragallinarum by means of whole genome sequencing. Prophages can transfer new functions to the host bacterium thereby altering its virulence, for example, resistance to antibiotics, detoxification of heavy metals, acquisition and utilization of certain nutrients, evasion of predators or colonization of specific environments. Molecular techniques like PCR were used for prophage screening on the different reference isolates of A. paragallinarum. Our studies have shown prophage sequences present within the genome of A. paragallinarum, however not all of these sequences were present in all of the serovars of A. paragallinarum. The presence of these prophages could contribute to the virulence and pathogenicity of the different serovars, its presence or absence could also contribute to the occurrence of different serovars as well as the emergence of new serovars

Poster Session

BAM-PW1146 - The use of recombinant Beak and Feather Disease Virus capsid protein (BFDV CP) in diagnostic tool and experimental vaccine development

Chrispian Theron[1], Charlotte Boucher[1], Arina Jansen[1], Robert Bragg[1]
[1]*University of the Free State, Bloemfontein, South Africa*

Beak and Feather Disease (BFD) affects exotic birds such as parrots, and is a major problem for breeders and owners of these expensive birds. The disease not only presents a problem for the trade of these birds, but is also a burden to the health of wild birds of this species. The disease is caused by a virus of the Circoviridae family, commonly referred to as Beak and Feather Disease Virus (BFDV). The virus has so far proven to be unculturable in tissue cultures and embryonated chicken eggs, which has limited research on this virus, diagnosis of viral infection and vaccine development against this virus. Methods of treatment of the virus without the prerequisite of propagating it are therefore required. The BFDV genome contains two major open reading frames which encode a replicase (Rep) and a capsid protein (CP). Generally, haemagglutination (HA) and haemagglutination inhibition (HI) tests have been used for diagnosis, but are limited in their specificities and consistencies. PCR and qPCR methods targeting the Rep gene have been successfully applied as alternative diagnostic tests for the disease. The use of the capsid protein for the development of alternative diagnostic tests as well as the production of an experimental vaccine was investigated. An efficient and versatile yeast expression system was used to produce recombinant BFDV CP for use in diagnostic test and vaccine production. The recombinant protein was successfully used for alternative diagnostic tests such as plate agglutination and ELISA. An immune response was induced in chickens vaccinated with recombinant BFDV CP, demonstrating the potential for vaccine production against this virus using this approach. This research provided promising tools for diagnosis of the virus as well as a vaccine prototype against the virus. This research is currently being used as a model system for other similar applications.

Poster Session

BAM-PW1148 - Investigation of Pasteurellaceae isolated from Syrian and European hamsters

Henrik Christensen[1], Werner Nicklas[2], Magne Bisgaard[3]
[1]Department of Veterinary Disease Biology, University of Copenhagen, Denmark, [2]Microbiological Diagnostics, German Cancer Research Centre, Heidelberg, Germarny, [3]Horsevænget, Viby Sj, Denmark

Six strains isolated from Syrian hamsters and five from European hamsters were investigated by a polyphasic approach including data previously published. Strains showed small, shiny, regular, and circular colonies with smooth and shiny appearance, typical of Pasteurellaceae. The strains formed two monophyletic groups based on 16S rRNA gene sequence comparison to other members of Pasteurellaceae. Partial rpoB sequencing as well as published data on DNA-DNA hybridization showed high genotypic relationships within both groups. Menaquinone 7 (MK7) was found in strains of both groups as well as an unknown ubiquinone with shorter chain length than previously reported for any other member of Pasteurellaceae. A new genus with one species, Mesocricetibacter intestinalis gen. nov., sp. nov. is proposed for group 1 of Krause (taxon 24 of Bisgaard), whereas group 2 of Krause (taxon 23 of Bisgaard) is proposed as Cricetibacter osteomyelitis gen. nov., sp. nov. Major fatty acids of type strains of type species of both genera are C 14:0, C 14:0 3OH/C 16:1 ISOI, C 16:1 ω7c and C 16:0. The two genera are clearly separated by phenotype from existing genera of Pasteurellaceae.

Poster Session

BAM-PW1150 - Role of the capsular polysaccharide as a virulence factor for Streptococcus suis serotype 14

David Roy[1], Jean-Philippe Auger[1], Mariela Segura[1], Nahuel Fittipaldi[2], Daisuke Takamatsu[3], Masatoshi Okura[3], Marcelo Gottschalk[1]
[1]Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Saint-Hyacinthe, Canada, [2]Public Health Ontario, Toronto, Canada, [3]National Institute of Animal Health (NIAH), Tsukuba, Japan

Streptococcus suis is an important swine pathogen and a zoonotic agent responsible for causing meningitis and septicemia. Although serotype 2 is the most virulent and frequently isolated from both swine and human cases, serotype 14 is in emergence, with human cases described in different countries. Contrasting with the extensive studies on the serotype 2, our understanding of the serotype 14 pathogenesis is limited. In order to study the role of the capsular polysaccharide (CPS) of serotype 14 as a virulence factor, we constructed an isogenic knockout mutant of a highly conserved regulatory gene, cps14B. In addition, to study the effect of the absence of sialic acid on the expression of the whole CPS for this specific serotype, a defective mutant of a gene that codes for UDP-N-acetylglucosamine 2-epimerase, a gene involved in sialic acid synthesis (neu14C) was also characterized. Coagglutination assays and electron microscopy results showed a total loss of CPS in both mutants. Due to very similar phenotypic characteristics, further studies were carried out with cps14B only. Phagocytosis assays with murine macrophages showed high susceptibility to phagocytosis for Δcps14B compared to the well encapsulated wild-type strain. An in vivo CD1 murine model of S. suis infection was used to demonstrate attenuated virulence of the non-encapsulated mutant. Whereas there is a difference in the CPS composition between serotypes 2 and 14, this study demonstrated that the CPS of serotype 14 is also an important anti-phagocytic factor and a critical virulence factor. As for serotype 2, it is difficult to evaluate the role of sialic acid in virulence of S. suis serotype 14, since no CPS is produced in the absence of this sugar.

Poster Session

BAM-PW1152 - *Clostridium perfringens* in poultry feed

Ruben Pablo Schocken-Iturrino[1], Mariana Froner Casagrande[1], Marita Vedovelli Cardozo[1], Livia Boarini[1], Mariana Casteleti Beraldo-Massoli[1], Silvina do Carmo Pelicano Berchielli[1]
[1]São Paulo State University, Jaboticabal, Brazil

The poultry industry is one of the agricultural activities most developed in the world, including Brazil, due to the need to use animal protein in human diet. However, a problem commonly found in animal feed ingredients is contamination by pathogens such as Salmonella spp. and Clostridium perfringens, making essential the microbiological control, since ingestion of contaminated raw material can be the cause of serious problems for animals that ingest them, causing necrotic enteritis. Thus, this study aimed to investigate the presence of C. perfringens in animal meal (blood and feather meal, poultry viscera meal and meat and bone meal) and a vegetable mix with 40% soybean meal and 60% of corn. We analyzed 80 raw samples. Twenty-five grams of each sample were transferred to 225 ml of 1% peptone water (previously sterilized) and serial dilutions up to 10-6 were done subsequently subjected to a thermal shock to allow the spores to germinate and to remove contaminants. An aliquot of 1 mL of each dilution was transferred to petri dishes and DRCM agar (Reinforced Clostridial Medium diferential Merck®) was added by the pour plate method. Plates were incubated in anaerobic jars using the GasPak system at 37°C for 48h. Suggestive colonies of C. perfringens were submitted to Gram smears, then transferred to tubes containing brain-heart infusion and subjected to biochemical tests; lactose, maltose and sucrose fermentation, salicin, indole, nitrate, gelatinase, motility, and H2S production. From the 80 samples of raw ingredients used for manufacture feed analyzed, 60% were positive for Clostridium perfringens (17.5% meat and bone meal, 20% blood and feather meal, 10% poultry viscera meal and 12, 5% in vegetable mix) demonstrating the importance of controlling contamination by this agent in ingredients in order to avoid economic losses caused by the disease caused by this pathogen.

Poster Session

BAM-PW1154 - A microboilogical focus on the presentation of the health situation in the less developed world case study Cameroon

Irai Marie Joelle[1], Njeuyong Arnaud Paulin[1], Apouayame Oumarou[1]
[1]*Hope Medical Foundation, Yaounde, Cameroon*

In Cameroon, healthcare is offered either through government-run hospitals or through privately run clinics. This paper looks at factual evidence to describe the main challenges facing health care in Cameroon, including absenteeism, corruption, shortage of doctors/nurses, inefficiency and mismanagement. Background: Cameroon is still lagging way behind in health care services for the poor as well as the affluent. In recent years, our neighbours, Nigeria and Gabon have forged ahead in respect of expertise and experience of doctors, advancement of healthcare technologies and high quality hospitals and health management organizations. Methodology: This working paper looks at factual evidence to describe the main challenges facing health care in developing countries, including absenteeism, corruption, shortage of doctors, and mismanagement. Results: people, the report concludes, revealing also that the country currently has two doctors for every nurse – a scenario inverse of the World Health Organization's recommendation of having three nurses per doctor in a well-functioning healthcare system. The report's conclusion that roughly 80 per cent of the country's population still seek their first line of care from informal healthcare providers, i. traditional healers, faith healers and community health workers. Absenteeism, inefficiency and corruption are also seen common in current health infrastructures Conclusion: Based on the failures in the state run health care system identified by the Cameroon Health Watch report there will no doubt be a significant thrust to allow more private sector or non-governmental involvement in healthcare services. As the report itself notes, the potential benefits of harnessing the ubiquity and the influence of the informal healthcare service providers could be massive, with training and monitoring. objective to improve the quality of healthcare services and to address emerging health problems of Cameroon.

Poster Session

BAM-PW1156 - High frequency genetic variation of purine biosynthesis genes is a mechanism of stress survival success in the enteric pathogen Campylobacter jejuni

Andrew Cameron[1], Emilisa Frirdich[1], Steven Huynh[2], Craig Parker[2], Erin Gaynor[1]
[1]University of British Columbia, Vancouver, Canada, [2]United States Department of Agriculture, Albany, USA

Population heterogeneity gives bacteria a remarkable ability to survive in rapidly changing environments, because production of cells with variable phenotypes ensures success in different situations. Although pure cultures are assumed to be clonal and homogeneous, underlying heterogeneity in bacterial populations can be detected by exposure to stresses or single-cell analyses. The enteric pathogen Campylobacter jejuni, a leading agent of diarrheal disease, produced progeny that differ markedly from each other in their sensitivity and resistance to osmotic stress. These isolated progeny then produced many cells that retain the altered phenotype, but also revertants with the initial sensitivity or resistance restored. Whole-genome sequencing revealed sequence changes occurring solely in two phosphoribosyltransferase genes of the purine biosynthesis pathway: purF and apt, which act on the same substrate, phosphoribosyl pyrophosphate. We sequenced the apt and purF genes from another 96 single colony isolates from a wildtype, non-stressed population, and found extensive variation in both genes, including the insertion of large repeats, deletions, and polymorphisms, but no nonsense mutations, indicating significant variability with only viable alleles. A purF deletion mutant could not be generated, and an apt deletion mutant was severely impaired for survival. Testing the 96 colonies for other phenotypes, such as oxidative stress survival, revealed that certain allele combinations were associated with stress resistance or sensitivity. This stress response heterogeneity also correlated with the ability of C. jejuni to survive intracellularly in an epithelial cell model of infection. To assess the effect of osmotic, oxidative, and intracellular stress on the heterogeneous allele pool, next-generation amplicon sequencing revealed that specific conditions encountered both in the wild and in infection selected for unique subsets of purF or apt alleles. In conclusion, these hypervariable genes affect the fitness of individual cells, are important for infectious transmission from hosts, and have clear implications for human disease.

Poster Session

BAM-PW1158 - Comparison of culture, conventional and real-time PCR methods for Listeria monocytogenes in foods

Dong-Hyeon Kim[1], Jung-Whan Chon[1], Hong-Seok Kim[1], Dasom Choi[1], Jin-Hyeok Yim[1], Dae-Geun Hwang[1], Jong-Soo Lim[1], Kwang-Young Song[1], Jin-San Moon[2], Kun-Ho Seo[1]

[1]KU Center for Food Safety, College of Veterinary Medicine, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul 143-701, Korea, [2]Livestock Product Standard and Veterinary Epidemiology Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Korea

We compared standard culture methods as well as conventional PCR and real-time PCR for the detection of Listeria monocytogenes (L. monocytogenes) in milk, cheese, fresh-cut vegetables, and raw beef meat that have different levels of background microflora. No statistical differences were observed in sensitivity between the two selective media in all foods. In total, real-time PCR assay exhibited statistically excellent detection sensitivity ($p < 0.05$) and was less time consuming and laborious as compared with standard culture methods. Conventional culture methods showed poor performance in detecting L. monocytogenes in food with high levels of background microflora, generating numerous false negative results. While the detection of L. monocytogenes in fresh cut vegetable by culture methods was hindered only by Listeria innocua, various background microflora, such as Listeria innocua, Listeria welshimeri, Listeria grayi, and Enterococcus faecalis appeared on the two selective media as presumptive positive colonies in raw beef meat indicating the necessity of improvement of current selective media. It appears that real-time PCR is an effective and sensitive presumptive screening tool for L. monocytogenes in various types of foods, especially foods samples with high levels of background microflora, thus complementing standard culture methodologies. In conclusion, we suggest that real-time PCR be used for early screening of L. monocytogenes in food samples.

Poster Session

MEM-PW3001 - The antimicrobial effect of the royal jelly against microscopic filamentous fungi

Miroslava Kačániová[1], Jana Petrová[1], Alexander Vatľák[1], Lukáš Hleba[1], Attila Kántor[1], Katarína Rovná[1]
[1]*Slovak University of Agriculture, Nitra, Slovak Republic*

The Royal Jelly (RJ) is a yellowish-white, acidic secretion, with a slightly pungent odor and the taste produced by the honeybees. The potential antifungal activities of royal jelly were investigated in this study. This product was obtained from the honey bee hybrid Apis mellifera. There was identified a number of antimicrobial peptides including royalisin, apisimin, jelleines I,II,III,IV,10-HDA, apalbuminaα in the chemical copmosition of the royal jelly. The main purpose of this study was to investigate some antimicrobial effects of royal jelly produced by honeybees (Apis mellifera) and collected from Slovakia against two different types of fungi (Aspergillus niger and Penicillium expansum) and two species of yeasts (Candida albicans and Geotrichum candidum). There were prepared nine concentrations of pure royal jelly in the water. Then they were added into the bacterial strains seed layer cultured individually. We loaded hundred µl of nine concentrations of water soluble royal jelly extracts (10,20,30,40,50,60,70,80,90mg/ml) into the wells of the agar plates. The plates were kept for incubation at 30°C for 3-4 days and then the plates were examined for the formation of inhibition zone. The test was performed three times for each fungus culture and the inhibition zones were measured by caliper to get an average value. The samples in different concentrations showed an antifungal activity against tested fungi and yeasts. The diameter of the clear zone formed in each concentration was measured and correlated to the ability of the extracts to inhibit the growth of microorganisms. Interestingly, the results showed the variation of inhibitory effects of royal jelly samples on different strains of the microscopic filamentous fungi in-vitro. The Poster was supported by the project: Development of International Cooperation for the Purpose of the Transfer and Implementation of Research and Development in Educational Programs conducted by the Operational Program: Education, ITMS code: 26110230085.

Poster Session

MEM-PW3003 - Antifungal susceptibility and biofilm characterization of Candida species isolated from a Tertiary Care Hospital, Rawalpindi, Pakistan

Ayesha Aslam[1], Kanwal Rafique[1], Fariha Hasan[1], Aamer Ikram[2], Irfan Ali Mirza[2], Mahmoud A. Ghannoum[3], Aamer Shah[1]

[1]Quaid-i-Azam University, Islamabad, Pakistan, [2]Armed Forces Institute of Pathology, Combined Military Hospital, Rawalpindi, Pakistan, [3]DepartCase Western Reserve University and University Hospitals, Cleveland, USA

The purpose of this study was to investigate the prevalence, antifungal susceptibility and biofilm formation in different Candida species collected from a tertiary care hospital in Rawalpindi, Pakistan. Candida sp. were isolated from clinical specimens received in the Microbiology Laboratory of AFIP during the period of December 2012 to November 2013. Candida sp. were identified through CHROMagar, API 20C Aux and molecular method. Antifungal susceptibility was checked through disc diffusion and E-strips method. Biofilm formation was determined, quantified and characterizeds by different methods such as Congo red agar, dry weight, XTT reduction, confocal laser microscopy. Antifungal susceptibility of biofilm was checked through 96 wells plate method. Total 153 Candida sp. were collected and more than 50% of them were isolated from vaginal swabs collected from Gynaecology ward. Majority of Candida infections were hospital acquired (60%) and infection rate was high in females (69%) as compared to males (31%). Among five different Candida species, C. albicans were found in highest number (58.8%), followed by C. tropicalis (18.3%), C. glabrata (13.1%), C. krusei (7.2%) and C. parapsilosis (2.6%). All isolates were susceptible to amphotericin B where as resistance rate was low against voriconazol and caspofungin. Resistance to fluconazole was high in C. krusei (81.8%) followed by C. glabrata (65.0%), C. tropicalis (32.1%) and C. albicans (6.6%). 70% of Candida isolates were susceptible to caspofungin (<2 µg/ml). High MICs of Fluconazole (48 to ≥128µg/ml) was found for C. krusei. Biofilm formation was high in non-albicans species (56%) than C. albicans (44%) and were highly resistant to fluconazole. Biofilms formed by the Candida glabrata are significantly different probably due to the inability to form hyphae. This study will be helpful to devise an empirical therapy against antifungal resistant Candida species.

Poster Session

MEM-PW3005 - Evaluation of Antifungal Potential of Dunaliella salina and Phormidium autumnale against Plant Pathogenic

Perveen Kahkashan[1]

[1]*King Saud University, Riyadh, Saudi Arabia*

The antifungal potential of Dunaliella salina and Phormidium autumnale were evaluated against plant pathogenic fungi; Alternaria alternata, Cladosporium sp., Fusarium solani, Fusarium sp., and Fusarium oxysporum. Water, acetone, methanol and Methanol: Acetone: Diethyl ether extracts of tested algae showed weak to strong activity against one or more than one tested plant pathogenic fungi. However, none was able to inhibit the growth of A. alternata. The extracts prepared with the mixture of methanol, acetone and diethyl ether were found highly effective in controlling the growth of most of the plant pathogenic fungi, followed by methanol, acetone and water. Extract with combination of the three solvents methanol, acetone and diethyl ether of D. salina caused maximum reduction in the growth of F. oxysporum (64.4%). Whereas, Methanol:Acetone:Diethyl ether extract of P. autumnale exhibits maximum growth reduction against F. solani (62.2%). Water extract of D. salina registered almost negligible reduction in the mycelial growth of Cladosporium sp (0.1%). The crude extracts of algal species were analyzed by gas chromatography-mass spectrometry (GC-MS) and the main component in the crude extract of P. autumnale was 1-Hexyl-2-Nitrocyclohexane (91.7%) whereas in the extract of D. salina, 3-Methyl-2-(2-Oxopropyl) Furan (90%) was detected. Some important compounds; Butanal and Octanal were also detected in these crude extracts. This study will be helpful in exploring the suitable environmental friendly fungicides to be used against plant pathogenic fungi.

Poster Session

MEM-PW3007 - Some rare sugars reinforce the action of metronidazole on trichomonad

Masakazu Harada[1], Emi Kondo[2], Hiromi Hayashi[2], Masato Teranaka[1], Meiji Arai[1]
[1]Kagawa University, Kagawa, Japan, [2]University of Tokushima, Tokushima, Japan

Trichomonad parasites utilize glucose for energy production via metabolic pathways. Main therapeutic drug of protozoal parasites is metronidazole, which is activated coupled with the reduction of ferredoxin. On the other hand, some monosaccharides are known in very small amount in nature and are called “rare sugars”. They have been proved to have biological activities. For instance, D-allose inhibits the production of reactive oxygen species from neutrophils, and D-allulose (D-psicose) affects the growth of L1 larvae of *Caenorhabditis elegans*. So the utilization of those sugars by trichomonad and the interactions with metronidazole were examined using D-allose, D-allulose and *Tritrichomonas foetus*. Trichomonads were cultured in medium including F-bouillon and glucose. But when glucose was substituted to those sugars from the medium, they had never increased. So trichomonads turned out to be unable to metabolize D-allose and D-allulose. Moreover, when each sugar was added to the medium containing ED50 of metronidazole, the parasite density decreased significantly less than that in the medium with metronidazole only. D-Allose remarkably reinforced the action of metronidazole. It is suggested that those sugars would affect to the metabolic pathway of glucose and that metronidazole might be activated coupling with some steps. By using metronidazole with those sugars for treatment of trichomonad parasites, the drug dosage could be lowered and the development of drug resistance of parasites might be inhibited.

Poster Session

MEM-PW3009 - Proteomic analysis of Trichoderma cellulose catabolism

Hong-Shin Lin[1], Shu-Ying Liu[2], I Peng[3], Ming-Yu Hsieh[2], Kou-Cheng Peng[1]
[1]Institute of Biotechnology, National Dong Hwa University, Hualien, Taiwan, [2]Department of Molecular Biotechnology, Da-Yeh University, Changhua, Taiwan, [3]Department of Biotechnology, National Formosa University, Yunlin, Taiwan

To investigate the induced cellulose catabolism of Trichoderma, two dimensional gel electrophoresis (2-DE) was used to examine protein regulation during different time spanning with 1% of cellulose as the main carbon source. A total of 65 differentially expressed proteins were identified using MALDI-TOF MS. The identified proteins were classified into four categories based on their biological functions, including genetic information processing, carbohydrate metabolism, structural proteins, and hypothetical or unknown function. It has been proposed that while conidia germination, constitutively secreted cellulase decomposes cellulose into small various lengths of oligosaccharides and glucose. Glucose was then in-fluxed into cell that initiated a cascade of glucose-, sepharose-induced regulation that generated much more cellulose metabolism related proteins. Parts of these proteins were ex-fluxed to the oligosaccharides-rich surroundings & worked intensively to generate life-sustaining glucose. In this study, the increased level of extracellular cellulases was detected by specific enzymatic activity and zymogram. However, the extracellular reducing sugar concentration remained low and steady throughout the experiment. This could be due to Trichoderma secreted cellulases to decompose cellulose into oligosaccharides and glucose that were consumed, in the mean time, to meet its growth need. Increased dry weight of Trichoderma mycelia during the time course, plus decreased extracellular reducing sugar concentration when using glucose as the sole carbon source further supported this result. Interestingly, four zinc finger proteins were highly up-regulated, which suggested cello-oligosaccharides may provoke cellulase gene expression via specific gene regulators. These findings contribute to our understanding of cellulose catabolism of Trichoderma.

Poster Session

MEM-PW3011 - Cytological effects of NP-40 on fungal morphology

P. John Vierula[1]
[1]*Carleton University, Ottawa, Canada*

The addition of low concentrations of non-ionic detergents to growth media can be used to limit fungal colony size in experimental manipulations such as genetic transformations. The current study was undertaken to characterize the cytological changes induced by the detergent NP-40 on germinating spores and maturing colonies of the filamentous fungus Neurospora crassa. In the presence of NP-40, germinating conidiospores of this fungus produce oblong, stunted germ tubes instead of the usual long thin primary hyphae. New cell compartments which emerged from these germ tubes were spheroidal and assumed a striking, bud-like morphology. Scanning electron microscopy revealed numerous round buds emerging from some mother cells. The cell surfaces exhibited a dimpled texture as well as some pronounced outgrowths presumed to be nascent daughter cells. The cell walls of the detergent-induced buds retained more Calcofluor white compared to regular hyphae, suggesting some substantial changes to chitin content had occurred. Immunostaining for actin and tubulin revealed randomly oriented cytoskeletal elements. A preliminary study of soluble proteins extracted from detergent treated cells revealed a few significant changes in the charge profiles which are proposed to be the result of post translational modifications such as phosphorylation.

Poster Session

MEM-PW3013 - Role of small G-proteins in the human fungal pathogen Candida albicans

Hayet Labbaoui[1], Stephanie Bogliolo[1], Vikram Ghugtyal[1], Robert Arkowitz[1], Martine Bassilana[1]
[1]*CNRS/INSERM/University of Nice, Nice, France*

The switch from budding to filamentous growth, critical for the virulence of the human fungal pathogen Candida albicans, requires sustained polarized growth. Using reporters, we quantified the distribution of active Cdc42 and Rho1, two critical Rho-G proteins, during filamentous growth in C. albicans (1). During such polarized growth, a dramatic reorganization of cellular compartments and exo/endocytosis occurs. In Saccharomyces cerevisiae, Arf G-proteins and their regulators have been shown to function at the interface of membrane traffic and cell polarity, raising the question of their role in external-signal mediated filamentous growth in C. albicans. C. albicans has 3 Arf proteins and 2 Arf-like proteins. We examined the importance of these small G-proteins in C. albicans filamentous growth and cell wall integrity. Our results indicate that Arf2, but not Arf1, is a key factor for C. albicans viability, budding and filamentous growth as well as cell wall integrity. This is in contrast to the situation in S. cerevisiae, where Arf1 and Arf2 appear to be redundant and neither protein individually is required for viability. C. albicans Arf1 and Arf2 are over 90% identical and whether the relative functional importance of these proteins is due to different regulation of expression or to specific protein sequences is under investigation. Our results also indicate that Arl1, and to a lesser extent Arf3, is necessary for invasive filamentous growth; neither protein is required for viability or cell wall integrity. We are currently analyzing the localization of active Cdc42 and Rho1 in the defective mutants, together with the importance of these Arf GTPases for actin cytoskeleton organization and membrane traffic.

Poster Session

MEM-PW3015 - Identifying target genes of the fission yeast transcription factor Prz1 by genetic activation

Kate Chatfield-Reed[1], Gordon Chua[1]
[1]*University of Clagary, Calgary, Canada*

Only ten of the 91 transcription factor haploid deletions in *Schizosaccharomyces pombe* display significant differences in their generation times when compared to wild type in rich medium. These observations suggest that most transcription factors are either not required or not active under these conditions. As a result, transcriptome profiling of these transcription factor deletions, in rich medium, would likely not reveal the target genes. One approach to overcome this obstacle is to screen for synthetic-lethal interactions, with the transcription factor deletion strain, to find genes that genetically activate the transcription factor of interest. The genes that have synthetic-lethal interactions with the transcription factor correspond to genetic backgrounds in which this transcription factor gene is required for life. This means that the transcription factor should be active in the single deletion mutants with which it displays a synthetic-lethal interaction: we applied this approach to Prz1, a $Ca2^{+}$-calcineurin-activated transcription factor. A genome-wide SGA screen was performed with the *prz1* deletion mutant identifying several synthetic-lethal interactions, including $pmr1^{+}$ which encodes a calcium membrane transporter. Transcriptome comparison between the *prz1* and *pmr1* deletion mutants in rich medium identified several putative novel target genes of Prz1. Some of the genes up-regulated in the *pmr1* deletion overlapped with the genes that were up-regulated in response to Prz1 activation by calcium and tunicamycin. These results show the potential application of synthetic lethality in identifying the target genes of *S. pombe* transcription factors.

Poster Session

MEM-PW3017 - Nicotinamide metabolism in the fungal pathogen Candida albicans

Rahul Ghugari[1], Sarah Tsao[1], Mohammad Alsady[1], Eun-hye Lee[1], Martine Raymond[1], Alain Verreault[1]
[1]*Institute for Research in Immunology and Cancer, Université de Montréal, Canada*

Candida albicans is a major opportunistic human fungal pathogen that is generally harmless but can causes lethal systemic infections in immunosuppressed individuals. Nicotinamide (NAM), a form of vitamin B3, is a precursor of NAD+ that inhibits the *C. albicans* histone deacetylase Hst3. This leads to hyper-acetylation of histone H3 lysine 56 (H3K56) that, in turn, causes catastrophic DNA damage and lethality. We hypothesized that cells lacking the Pnc1 nicotinamidase, which converts NAM into nicotinic acid (NA), would be more sensitive to NAM than wild-type cells because the pnc1 mutant should accumulate higher amounts of intracellular NAM. Unexpectedly, this was not the case. We therefore decided to monitor the metabolic fate of NAM in wild-type and pnc1 mutants. We developed a mass spectrometry assay to determine the concentrations of NAM and other NAD+ metabolites. Following addition of excess NAM, its intracellular concentration went up rapidly (roughly 200-fold in <3min) in wild-type and pnc1 mutants. Surprisingly, the NAM concentration was not higher in pnc1 mutants but, consistent with the nicotinamidase activity of Pnc1, we could not detect NA in pnc1 mutants treated with excess NAM. This demonstrates that Pnc1 is the only nicotinamidase in *C. albicans*. Remarkably, pnc1 mutants treated with tetra-deuterated nicotinamide (heavy NAM) were able to synthesize heavy NAD+. The fact that pnc1 mutants can convert excess NAM into NAD+ may, at least in part explain why pnc1 mutants are only mildly sensitive to NAM than wild-type cells. Our results suggest the existence of a Pnc1-independent pathway that prevents excess NAM from inhibiting Hst3 and, thereby, causing hyper-acetylation of H3K56 and lethality. In addition, a Pnc1-independent biosynthetic pathway that generates NAD+ from NAM has never been reported in fungi. We are actively trying to determine how this pathway operates.

Poster Session

MEM-PW3019 - Depletion of the mitotic kinase Cdc5p in C. albicans results in the formation of elongated buds that may switch to the hyphal fate over time in a Ume6p-dependent manner

Amandeep Glory[1], Chloe Oostende[1,2], Anja Geitmann[2], Catherine Bachewich[1], Catherine Bachewich[1]
[1]Concordia University, Montreal, Canada, [2]Universite de Montreal, Montreal, Canada

Candida albicans is a common fungal pathogen of humans. Its ability to differentiate between yeast, hyphae and pseudohyphae is important for virulence. Hyphae are induced by environmental cues such as heat and serum. Filaments can also be produced by yeast cell cycle arrest, but the nature of these cells and mechanisms of their formation are not clear. For example, we previously demonstrated that depletion of the mitotic polo-like kinase Cdc5p resulted in formation of filaments that were distinct from hyphae in their initial transcription profiles, dependence on the spindle checkpoint factor Bub2p, and independence of the hyphal regulator Efg1p. However, some hyphal-specific genes were expressed at later growth stages. These cells may thus represent elongated buds that switch to the hyphal fate over time. In order to address this, we investigated aspects of the polar growth machinery, which can show hyphal-diagnostic features. During late but not early stages of Cdc5p depletion, the myosin light chain Mlc1p showed a Spitzenkorper-like localization in the tips of some filaments, and the Cdc42p GAP Rga2p became hyper-phosphorylated, like true hyphae. Further, hyphal-specific genes HWP1, UME6 and HGC1 were strongly expressed at the time that Rga2p was phosphorylated. HWP1 expression required Ume6p, and absence of Ume6p or Hgc1p influenced the maintenance, but not initiation, of filament morphology and integrity. Finally, polarized growth and UME6 expression in Cdc5p-depleted cells were independent of Hms1p, which is required for filament formation and UME6 expression during depletion of Hsp90p. Thus, polarized cells that form in response to depletion of Cdc5p may initially represent elongated buds, but switch to the hyphal fate over time through a mechanism that involves UME6 induction, possibly in response to maintenance of polarized growth. The results expand on the multiple strategies with which C. albicans can modulate growth mode and expression of virulence-influencing genes.

Poster Session

MEM-PW3021 - Dosage matters: Revealing the fitness effects of experimental gene duplication

Diana I. Ascencio[1], Adriana Espinoza[1], Alexander De Luna Fors[1]
[1]Laboratorio Nacional de Genómica para la Biodiversidad(Langebio), CINVESTAV-IPN, Irapuato Gto. México

Duplicate genes are widely distributed at high frequencies across eukaryotic genomes. Within the hemiascomycete yeasts, certain genes are often been retained in duplicate, while others are universally present as single-copy genes. What is the role of natural selection in determining such duplication frequencies? Since gene redundancy is known to be evolutionary unstable, it is likely that positive selection has been determinant for the immediate fixation of newly arisen duplicate genes. On the other hand, the duplication of dosage-sensitive genes could have been prevented by immediate fitness disadvantages of such duplication events. Here, we experimentally duplicate over 900 essential genes in Saccharomyces cerevisiae using centromeric (monocopy) constructs bearing these yeast genes controlled by their native promoter and terminator sequences. We measure the immediate phenotypic consequence of such genetic perturbation by quantifying the relative fitness of single-copy vs double-copy strains under normal or stressful laboratory conditions using a high-resolution phenotyping method. Our results show that 13% of the strains bearing an additional gene copy grow significantly slower than the wild-type, indicating that gene duplication of certain genes has an immediate deleterious fitness consequence. Surprisingly, experimental gene duplication results in an immediate fitness advantage for 9% of the strains, and this fraction increases to 12% when yeast is grown under osmotic stress. Gene duplications with a fitness disadvantage are enriched among genes that encode for proteins involved in important cell processes like structural organization and ribosome biogenesis. Our results suggests that natural selection of duplicated gene dosage influences the presence or absence of redundant gene copies in eukaryotic genomes, which may represent an initial step for the further diversification of gene function.

Poster Session

MEM-PW3023 - The parasitophorous vacuole formation: A fusional relation

Olivier Séguin[1], Albert Descoteaux[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada

Leishmania is the parasite responsible for leishmaniasis, a disease that puts over 350 million people at risk annually. These infection can cause many effects ranging from cutaneous lesions to the visceral progressive infection that may lead to death. Sadly, treatment methods are scarce and often based on chemotherapy that is both painful and expensive as well as inefficient. To survive in its human host, the parasite remodels the phagosome into a parasitophorous vacuole (PV). This PV can be either tight and individual for parasite such as Leishmania major or large and communal for Leishmania amazonensis and Leishmania mexicana. Defining the difference leading to the formation of an individual or communal PV is therefore an important step in the understanding of Leishmania survival in the host macrophage. Our hypothesis in that matter is that SNAREs, small membrane proteins responsible of membrane fusion, play a central role in the formation of the large communal PV. We previously showed that modification of the SNAREs VAMP8, Syntaxin 4 and VAMP3 by the parasite metalloprotease GP63 is vital to the parasite infection for species living in individual PVs, thus the study of such SNAREs as been undertaken and differential cleavage as already been found for VAMP8 and VAMP3. Thus, by permitting the membrane fusion of different vesicle, the SNAREs are an important part of the PV formation and their deletion may lead to inefficient infection and therefore to a better immune response from the host.

Poster Session

MEM-PW3025 - Mechanisms leading to the suppression of CD8 T cells expansion during Leishmania donovani infection

Akil Hamami[1], Tania Charpentier[2], Mélina Smans[3], Simona Stager[4]
[1]INRS - Institut Armand Frappier, Laval, Canada

Leishmania is a protozoan parasite transmitted by the bite of sandflies. This parasite is the causative agent of Leishmaniasis, a disease with a wide range of clinical manifestations. Visceral Leishmaniasis is considered the most severe form of Leishmaniasis. Experimental infection with L. donovani results in chronic infection in the spleen and in immune suppression. Thus far, the only cells that are known to be able to reduce the splenic parasite burden following immunotherapy are antigen-specific CD8+ T-cells. Yet, L. donovani appears to be able to evade this defense by limiting the expansion and effector functions of parasite-specific CD8+ T cell responses. Since strong clonal expansion is needed to control infection, we are particularly interested in dissecting the mechanisms by which L. donovani interferes with the expansion of parasite-specific CD8+ T-cells. In order to characterize antigen-specific CD8+ T cell responses during L. donovani infection, we designed an adoptive transfer system that employs OVA-transgenic parasites and TCR-transgenic OTI CD8+ T cells. Here, we show that L. donovani not only limits expansion of CD8+ T cells, but also induces mainly memory precursors rather than end-differentiated effector cells. Furthermore, blocking inhibitory molecules such as B7H1 and LAG3, or TGFβ blockade failed to increase expansion of antigen-specific CD8+ T cells. In contrast, IL10R blockade resulted in increased expansion and greater cytotoxic capacity of parasite-specific CD8+ T-cells. Our results suggest that early IL-10 production is partially involved in inhibiting CD8+ T cell expansion during experimental Visceral Leishmaniasis. Further inhibitory mechanisms will be discussed.

Poster Session

MEM-PW3027 - Growth and soil nutrient utilization by Suillus isolates and pine seedlings

Ling Yuan[1], Liyuan Peng[1], Jianguo Huang[1]
[1]*Chinese Society of Microbiology, Beijing, China*

Most of forest soils are acidic and P deficient in Sichuan, China. Suillus are main ectomycorrhizal fungi in those forests. Our present experiments were conducted with thirteen Suillus isolates and ectomycorrhizal pine seedlings to study the mobilization of phosphorus by the fungal isolates from soils and the growth and nutrition of mycorrhizal seedlings. Thirteen fungal species varied significantly in growth, phosphorus absorption and utilization from the soil, and efflux of oxalate and proton. Fungal isolates except S. bovinus S1J, S. granulatus XC2 and S. placidus 11 extracted Ca-P from soils. S. brevipes S12J, S. collinitus XC, S. luteus 12, S. proximus S3M, S. sibiricus JZG, S. pictus EMS and S. subluteus XC4 utilized Al-P and most of them also decreased Fe-P and O-P from soils. Moreover, Inorganic P in fungal culture solution was much higher than the control. Al-P in soils correlated negatively with oxalate efflux ($r = -0.731^*$, $n = 13$) and proton efflux ($r = -0.734^*$, $n = 13$). Similar results was also found between Fe-P and oxalate and between Fe-P and proton, indicating the importance of oxalate and protons in the process of soil P mobilization Fungal inoculation promoted the growth of pine seedlings at different levels. More N, P and K were absorbed by ectomycorrhizal seedlings than the control. Fungal species also varied greatly in the abilities to stimulate plant nutrient uptake. Fungal biomass in vitro correlated positively with seedling biomass ($r = 0.649^*$, $n = 13$), suggesting that external hyphae of mycorrhizas widespread in soils could enlarge the soil spaces for exploiting more nutrients for hosts. However, there was no significant correlation between fungal P absorption in vitro and plant P absorption ($r = -0.093$, $n = 13$). The result suggested that P absorption of fungal isolates could not reflect that of mycorrhizal plants.

Poster Session

MEM-PW3031 - In vitro activities of nine antifungal drugs and the combinations against Phialophora verrucosa

Yali Li[1], Zhe Wan[1], Ruoyu Li[1]

[1]Department of Dermatology and Venereology, Peking University First Hospital, Beijing, P.R. China, [2]Research Center for Medical Mycology, Peking University, Beijing, P.R. China, [3]Beijing Key Laboratory of Molecular Diagnosis of Dermatoses, Beijing, P.R. China

Antifungal therapies for Phialophora verrucosa infections are limited. The aim of this study was to determine the in vitro antifungal susceptibility of P. verrucosa to nine antifungal drugs and to identify effective drug combinations. Methods: Forty-six isolates, which included thirty-one clinical strains and fifteen environmental strains, were tested for single-drug susceptibility by CLSI broth microdilution M38-A2. Clinical strains were also used to evaluate potential synergy based on the chequerboard technique. Results: The MIC/MEC90s (μg/ml) across all strains were as follows: terbinafine, 0.25; posaconazole, 0.5; voriconazole, 1; itraconazole, 2; amphotericin B, 4; caspofungin and micafungin, 16; fluconazole and flucytosine, 64. Itraconazole plus caspofungin showed synergy against 100% of 31 clinical strains, followed by amphotericin B plus flucytosine (45.2%), itraconazole plus terbinafine or micafungin (25.8% and 12.9%). Synergy or antagonism was not detected for any other combination. Conclusions: P. verrucosa had low MICs to the newer triazoles but had relatively high MICs/MECs to amphotericin B, fluconazole, flucytosine, caspofungin, micafungin and terbinafine in vitro. Therapy itraconazole combined with caspofungin seems to be the most potent combination against P. verrucosa in vitro.

Poster Session

MEM-PW3000 - Chemical and antimicrobial studies of medicinal plant Costus speciosus (Koen.)

Manal Othman Alkattan[1], Suzan Khayyat[1]
[1]*King Abdulaziz University, Jeddah, Saudi Arabia*

Plants are alternatives to antibiotics and have fewer side effects than allopathic medicines. Costus speciosus is the only species in the genus Costus that is Indian ornamental plant which has long been used in traditional systems of medicine and is important for many pharmacology purposes. In the present study, the antimicrobial activities of Indian Costus extracts were tested on fungi, yeast and bacteria. Epoxidation of the extract was performed with m-chloroperbenzoic acid with CHCl3 in a nitrogen atmosphere. These extracts were analyzed by various instrumental analyses such as IR, NMR, GC-MS, thin layer chromatography (TLC) and preparative layer chromatography (PLC). The extracts and essential oil were tested for their antimicrobial activity. After treatment with essential oil extract, the change in the general shape of the fungal spores was examined by SEM analysis. The results showed that treatment by the essential oil led to hyphae disruptions and changes in the general shape of the fungal spores. The studies on the antimicrobial activity showed high growth inhibition for Microsporum gypseum, M.canis, Candida albicans, Pseudomonas aeruginosa and Staphylococcus aureus when oil extract concentrations were used. The 1.25 % of methanol and ethanol extracts yielded an inhibitory effect on the fungi, yeast and bacteria. However, the epoxidation of diosgenin resulted in more activity than the diosgenin extract against M. gypseum ,C. albicans and C. tropicalis.
Keywords: Indian Costus , medicinal plants, dermatophytes, antimicrobial activity.

Poster Session

MEM-PW3002 - Antifungal effect of silver nanoparticles syntetized using Mentha piperita against microscopic filamentous fungi

Alica Bobková[1], Alexander Vatľák[1], Attila Kántor[1], Simona Kunová[1], Katarína Rovná[1], Ľubomír Lopašovský[1]
[1]*Slovak University of Agriculture, Nitra, Slovak Republic*

The efficiency of antimicrobial effect of Ag nanoparticles extracts of Menta piperita as reducing agents towards the microscopic filamentous fungi was assessed. The effects of plant material on the process of Ag nanoparticles formation was investigated by methods of UV-VIS. These silver nanoparticles were tested for some antifungal activities using disc diffusion method. The tested Aspergillus niger, Penicillium expansum, Candida albicans and Geotrichum candidum cultures species of fungal were used. There was suspended an inoculum of each of the fungal strains in 5 ml potato dextrose broth and incubated at 37°C for 2 days. Because of this, the inoculum was spread over potato dextrose agar and Sabouraud dextrose agar medium with sterile glass spreader. Small circular paper discs (5 mm diameter) impregnated with known amount of silver nanoparticles was placed upon the surface of the inoculated plates separately. The plates were kept at room temperature for absorption of extract in the medium and then incubated at 28°C in the incubator for 24 hrs. The antifungal activity was evaluated by measuring the diameter of inhibition zone. The strongest antifungal effect of silver nanoparticles solution against Aspergillus niger and Candida albicans were found.Our results confirmed that these silver nanoparticles were found to be highly toxic and provide once again the pharmaceutical evidence of the medicinal plant Mentha piperita. Therefore, the use of silver nanoparticles should emerge as one of the novel approaches in many disease therapies, and applications of plant extract silver nanoparticles should be exploited for the medical treatment. The Poster was supported by the project: Development of International Cooperation for the Purpose of the Transfer and Implementation of Research and Development in Educational Programs conducted by the Operational Program: Education, ITMS code: 26110230085.

Poster Session

MEM-PW3004 - Effect of amikacin on the cell wall glycopeptidolipid synthesis and pathogenecity of Mycobacterium abscessus

So-Young Lee[1], Hee-Youn Kim[1], Bum-Joon Kim[1], Yoon-Hoh Kook[1]
[1]Seoul National Untiversity, College of Medicine, Seoul, Korea

Mycobacterium abscessus is the most common pathogen causing human infection among rapidly growing mycobacteria. In previous study, we observed that M. abscessus smooth colony cultured in a broth with amikacin at sub-minimum inhibitory concentration(MIC) had unusual growth pattern. We suggested that the pattern is correlated with bacterial morphologic change. In this study, we have demonstrated that amikacin induce morphological change and biofilm formation. Furthermore, possible mechanisms of these morphological changes were studied at molecular level. The morphological change was observed from smooth to rough(amikacin-induced rough colony) on a blood agar plate containing amikacin at sub-MIC. Through microscopic examination, it was revealed that amikacin induced smooth colony had direction and aggregated by forming cord-like structure, which is similar to rough colony. Amikacin also affected to reduce sliding motility and induce biofilm formation of smooth colony. The results of Thin layer chromatography(TLC) and Matrix Assisted Laser Desorption Ionization-Time Of Flight(MALDI-TOF) showed that glycogpetidolipid(GPL), a large amount of cell wall component in smooth colony, was not expressed in rough colony and decreased without change of the component in amikacin-induced rough colony. In RNA levels, the key genes of GPL synthesis which are pks, pks-associated genes, genes involved in GPL transport(gap, gap-like) and production(sap, ecf) were less expressed in both rough and amikacin-induced rough colony than smooth colony. In vitro and in vivo infection assay revealed that cytokines by amikacin-induced rough colony affected the decrement of growth rate. We also observed that rough colony did not affect host immune response, whereas smooth colony induced inflammatory cytokines, except for TNF-α. Their intracellular growth, however, had no difference. On the basis of the above results, we verified that exposure to amikacin induced cell wall GPL and morphologic change, which affected growth rate in host by stimulating immune response.

Poster Session

MEM-PW3006 - Inhibition of histone deacetylation as a novel antifungal therapy

Sarah Tsao[1], Li Zhang[1], Mike Tyers[1], Alain Verreault[1]
[1]*IRIC-Université de Montréal, Montreal, Canada*

Candida albicans is a leading cause of systemic bloodstream infections in immunosuppressed individuals and is associated with high mortality rate. Current antifungal therapy is limited by the emergence of multidrug resistant clinical isolates or undesirable adverse effects. Therefore there is an urgent need for new antifungal therapeutic strategies. We previously showed that histone H3 lysine 56 acetylation (H3K56ac) plays an important role in the DNA damage response in *C. albicans*. Furthermore, we demonstrated that genetic inactivation or pharmacological inhibition with nicotinamide (NAM) of the histone deacetylase Hst3 results in H3K56 hyperacetylation, which leads to extensive DNA damage and cell death. H3K56ac is an abundant histone modification in fungi and Hst3 is a histone deacetylase with fungal-specific properties, making it a promising target for novel antifungal drug discovery. Furthermore, NAM is a form of vitamin B3 that is innocuous to humans but exhibits a weak fungicidal activity against *C. albicans*. The goal of this study was to discover small molecules that potentiate the effect of NAM and can be used in combination therapy with NAM. We performed a high-throughput, cell-based phenotypic screen of a library of 678 compounds preselected for bioactivity against a wild-type *C. albicans* strain in the absence or presence of NAM at a non-toxic concentration of 0.5 mM. The growth of *C. albicans* in 96-well plates containing individual chemicals was monitored every 15 minutes for 48 hours. Small molecule(s) that enhance NAM-dependent growth inhibition of *C. albicans* were considered as primary hit(s). From this chemical screen, we identified one compound that we termed NAM sensitizer 1 (NS1). When used alone, NS1 caused mild growth inhibition, but the combination of NS1 and NAM dramatically delayed *C. albicans* growth. We are currently trying to determine the physiological basis of the synergy between NS1 and NAM.

Poster Session

MEM-PW3008 - Characterization of Aspergillus nidulans α-glucan synthesis: roles for two synthases and two amylases

Shengnan Li[1], Susan Kaminskyj[1], Xiaoxiao He[1]
[1]*University of Saskatchewan, Saskatoon, Canada*

The cell wall is essential for fungal survival and growth. Fungal walls are ~90 % carbohydrate, mostly types not found in humans, making them promising targets for anti-fungal drug development. Echinocandins, which inhibit the essential β-glucan synthase, are already clinically available. In contrast, α glucan, another abundant fungal cell wall component has attracted relatively little research attention because it is not essential for most fungi. Aspergillus nidulans has two α-glucan synthases (AgsA and AgsB) and two α-amylases (AmyD and AmyG), all of which affect α-glucan synthesis. Gene deletion showed that AgsB was the major synthase. In addition, AmyG promoted α-glucan synthesis whereas AmyD had a repressive effect. The lack of α-glucan had no phenotypic impact on solid medium, but reduced conidial adhesion during germination in shaken liquid. Moreover, α-glucan level correlated with resistance to Calcofluor White. Intriguingly, overexpression of agsA could compensate for the loss of agsB at the α-glucan level, but not for phenotypic defects. Thus, products of AgsA and AgsB have different roles in the cell wall, consistent with agsA being mainly expressed at conidiation. These results suggest that α-glucan contributes to drug sensitivity and conidia adhesion in A. nidulans, and is differentially regulated by two synthases and two amylases.

Poster Session

MEM-PW3010 - Auger-architectomics expose novel drugs and their metabolic effects in yeast biosensors

Khumisho Dithebe[1], Chantel Swart[1], Pieter Van Wyk[2], Johan Kock[1]
[1]UNESCO-MIRCEN: Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa, [2]Centre for Microscopy, University of the Free State, Bloemfontein, South Africa

In this study, we used Auger-architectomics (http://en.wikipedia.org/wiki/Auger_architectomics) to determine the ultrastructure and atom composition of yeast biosensors while screening for mitochondrial inhibitors. The purpose was not only to reveal new inhibitors but also to image their metabolic effects. Yeast biosensors were subjected to conditions that inhibit mitochondrial activity and in turn favours fermentation metabolism. Cells were collected and prepared for nano scanning Auger microscopy (NanoSAM) coupled to scanning electron microscopy (SEM) while nano-etching with Argon. Transmission electron microscopy (TEM) was also performed to verify results. The sexual cells of the yeast *Nadsonia* were used as biosensors to determine the anti-mitochondrial activity of the antifungal fluconazole which unexpectedly revealed the presences of gas bubbles, containing CO_2, in the biosensor space. Similarly, a maze of CO_2 bubbles, occupying a significant part of the cells, was observed when the brewer's yeast was grown under conditions inhibiting mitochondrial activity. This phenomenon is ascribed to the inhibition of mitochondrial activity which thus stimulates the fermentative pathway for energy purposes. Enhanced CO_2 production is characteristic of yeast fermentation compared to low levels of CO_2 during mitochondrial respiration metabolism. Strikingly, during TEM analysis, CO_2 gas bubbles were discovered to compress cell organelles thereby deforming them. In this study, biosensors have been developed which are linked to Auger electron atom physics, SEM and Argon nano-etching. These biosensors are not only capable of screening for mitochondrial drugs but may also be applied to detect the metabolic effects of these compounds *in vivo*. This can be considered a major advantage when leads for further drug tests and development are selected.

Poster Session

MEM-PW3012 - Genome wide mapping of transcription factors in Candida albicans

Walters Tebung[1], Luis Roberto Rodríguez-Ortiz[1], Faiza Tebbji[1], Malcolm Whiteway[1]
[1]*Biology Department, Concordia University, Montreal, Canada*

Candida albicans is an opportunistic pathogen that can invade host tissues causing potentially lethal systematic mycoses. Candida species are the most common agents in human fungal infections with C. albicans accounting for more than half of these cases. A global study of the biology of this organism is mandatory and the regulation of gene expression is an important determinant to clarify diverse aspects of different processes such as mating and virulence. Transcription factors (TFs) play a central role in gene regulation acting either as activators or as repressors through binding to specific short DNA motifs. In order to assess the global spectrum of genes regulated by these TFs in C. albicans we are performing ChIP-Chip and calling-card analysis. First, we are investigating all zinc cluster transcription factors family using ChIP-Chip and bioinformatics tools to identify their targets. Interspecies functional preservation for each transcription factor will be verified by comparing genes controlled by these C. albicans transcription factors with those of the corresponding orthologs in the well-studied S. cerevisiae. As a second approach we are testing a "Calling Cards" methodology. This technology uses fusion of a specific TF to a transposase, which in turn targets the deposition of a transposon into the genome near the TF binding site. When a TF leaves its target site, the visit is recorded and the location can then be determined by DNA sequencing. To test for the feasibility of this method in C. albicans, we will use Tbf1 as a positive control because much is known about this TF from previous studies by our group. Results for specific TFs using both methodologies will be presented.

Poster Session

MEM-PW3014 - Negative regulation of C. albicans filamentous growth by Dig1p

Hannah Regan[1,2], Christine Scaduto[3], Matthew Hirakawa[3], Kearney Gunsalus[4], Yaolin Chen[2], Carol Kumamoto[4], Richard Bennett[3], Malcolm Whiteway[1,2]
[1]McGill University, Montreal, Canada, [2]Concordia University, Montreal, Canada, [3]Brown University, Providence, USA, [4]Tufts University, Boston, USA

Candida albicans is an opportunistic fungal pathogen capable of causing superficial and systemic infections in humans. The ability of C. albicans to switch between various morphological forms depending on its host environment is thought to contribute to its virulence. Filamentous growth states are associated with tissue invasion, biofilm formation, evasion of innate host defences and mating. Although the mechanisms of activation of filamentous growth pathways are well understood, less is known about which factors control the negative regulation of filamentation. In this study, we have identified a previously uncharacterized Orf that shares sequence similarity with Saccharomyces cerevisiae Dig1 and Dig2. Deletion of the gene encoding this Orf triggers invasive growth in C. albicans and so we have retained the yeast designation of Dig1 (for Down-regulation of Invasive Growth). Mutants lacking CaDIG1 form cultures of hyper-polarized cells, form robust biofilms, are highly invasive in vitro but not in vivo and are constitutively activated for the pheromone response. Deletion of key transcription factors that act downstream of Dig1p provide evidence to suggest that CaDig1 regulates filamentation and mating through multiple signalling pathways.

Poster Session

MEM-PW3016 - Salt stress response in the halotolerant yeast Debaryomyces hansenii

Saad A. Sharief[1], Ahmed F. Yousef[1], Mette H. Thomsen[1]
[1]*Masdar Institute of Science and Technology, Department of Chemical and Environmental Engineering, Abu Dhabi, UAE*

As the world heads closer to the impending world energy and water crisis, efforts have been directed to establishing novel sustainable sources of fuel. Much of the effort is currently being directed towards second generation biofuels, relying on lignocellulosic biomass as a source of carbon for ethanol production through fermentation. The pretreatment of second generation biomass is an important step in releasing accessible sugars for the fermenting organisms to convert to ethanol. The pretreatment of the biomass can lead to the production of a hostile growth medium full of growth inhibitors and other molecules that result in a decrease in biofuel yield. The abundance of “salty biomass”, such as Salicornia, in the United Arab Emirates is cause for new efforts directed at designing new engineering processes for “salty bioreactors”. Debaryomyces hansenii is one of the most halotolerant yeast species uncovered thus far. The genome sequence of two strains (CBS767 and MTCC234) have been determined, providing insight into the evolution of various yeast species. We have isolated a new strain of D. hansenii from salt brine at a cheese factory in Denmark. In this study, we will assess the response of this strain to salt stress through transcriptome analysis. These studies will help shed light on the molecular pathways and mechanisms underlying this yeast’s ability to grow in high salinity environments. The newly uncovered information will be of value for developing novel genetically engineering organisms for the purpose of producing various products from salty biomass.

Poster Session

MEM-PW3018 - Deletion of MIG1 causes metabolic changes and affects mitochondrial function in Cryptococcus neoformans

Mélissa Caza[1], Guanggan Hu[1], James W Kronstad[1]
[1]*University of British Columbia, Vancouver, Canada*

Introduction: *Cryptococcus neoformans* is the leading cause of fungal meningoencephalitis in immunocompromised people such as HIV/AIDS patients. Recently, mitochondria have been identified as important contributors to the virulence and drug tolerance of human fungal pathogens. Mitochondria are essential for metabolic functions such as heme biosynthesis and electron transport chain (ETC) activity, but they also contribute to the response to stress, cellular lipid homeostasis and cell wall biosynthesis. Aim: The aim of this study is to characterize the transcription factor Mig1 and its impact on metabolic functions fulfilled by mitochondria. Results: Deletion of *MIG1* in the *C. neoformans* H99 strain resulted in a growth advantage under hypoxic conditions suggesting enhanced adaptation to hypoxia. Quantitative real-time PCR revealed a differential regulation of genes involved in the TCA cycle (e.g. aconitase *CNAG_01137*) and in heme biosynthesis pathway of C. neoformans (*HEM3, HEM13*, and *HEM4*) in the *mig1* mutant compared to H99. Furthermore, a *mig1*mutant showed increased susceptibility to fluconazole and to the hypoxia-mimicking agent CoCl2, which targets sterol synthesis. The *ERG25* gene for ergosterol biosynthesis is indeed deregulated in a *mig1* mutant. Susceptibility to rotenone and LiCl2 were also observed in the *mig1* mutant indicating a role in ETC and cellular lipid homeostasis. On the other hand, a *mig1* mutation conferred increased resistance to rapamycin and cyclosporine A suggesting a role in the mTOR and calcineurin pathways, respectively. A *mig1* mutant showed reduced capsule, a key virulence trait. A *mig1* mutant didn't show any virulence defect in a mouse inhalation model of cryptococcosis, and an evaluation of the fungal load in different tissues is currently underway. Conclusions: Taken together, we find that Mig1 plays a role in remodeling metabolic functions fulfilled by mitochondria. This study therefore expands the regulatory network that contributes to the virulence of
C. neoformans.

Poster Session

MEM-PW3020 - Composition and putative targets of the G1/S transcription factor complex in Candida albicans

Yaolin Chen[1], Vinitha Chidipi[1], Sandra Webber[2], Martine Raymond[2], Catherine Bachewich[1]
[1]Department of Biology, Concordia University, Montreal, Canada, [2]Institute for Research in Immunology and Cancer, University of Montreal, Montreal, Canada

The G1/S transition is a critical control point for cell proliferation, and involves the essential transcription complexes SBF and MBF in Saccharomyces cerevisiae, or MBF in Schizosaccharomyces pombe. In the fungal pathogen Candida albicans, G1/S regulation is not yet clear. We previously characterized orthologues of Swi6p, Swi4p and Mbp1p, and found that absence of Swi4p or Swi6p similarly influenced cell proliferation, expression of some G1/S-associated genes, and morphogenesis. However, Mbp1p had little effect on growth. Cells lacking Swi6p and Swi4p or Swi4p and Mbp1p were viable, suggesting that additional factors contribute to G1/S regulation. In order to confirm the composition of the putative G1/S transcriptional complex in C. albicans, we tandem-affinity purified Swi4p, Swi6p and Mbp1p, and identified interacting factors using Orbitrap LC/MS. Swi6p-interacting factors included Swi4p and Mbp1p. In Swi4p and Mbp1p affinity-purified complexes, Swi6p or Swi6p and Nrm1p, respectively, were the most predominant. Additional peptides were detected at lower levels. Co-immunoprecipitations confirmed that Swi6p interacts with Swi4p and Mbp1p, but Swi4p and Mbp1p did not physically bind. Collectively, the data support the idea that Swi4p and Swi6p may be the major components of a G1/S transcription factor complex in C. albicans, but a separate complex composed of Mbp1p and Swi6p of unknown function also exists. We next investigated putative Swi4p targets using ChIP-chip. Swi4p was enriched at promoters of genes associated with several functions, including cell division. However, the most abundant group of targets included filamentous growth genes, including EFG1. Northern blotting showed that EFG1 expression was moderately induced in the absence of Swi4p, and absence of EFG1 reduced the extent to which swi4Δ/swi4Δ cells formed long filaments. Thus, Swi4p may contribute to the regulation of EFG1 and filamentous morphogenesis, as well as the G1/S transition in C. albicans, suggesting a link between the cell cycle and development.

Poster Session

MEM-PW3022 - Differential regulation of macrophage translation by leishmania donovani depending on its life cycle stage

Aude Zimmermann[1], Mirtha William[1], Guillermo Arango-Duque[1], Ola Larsson[2], Albert Descoteaux[1], Maritza Jaramillo[1]
[1]INRS-Institut Armand Frappier, Laval, Canada, [2]Karolinska Institutet, SE-17230, Stockholm, Sweden

Leishmania donovani (L. donovani) is a protozoan parasite responsible for visceral leishmaniasis. Finding therapeutic targets to combat this life-threatening disease is a challenge that we are trying to face by characterizing the molecular mechanisms involved in the regulation of host-encoded immune functions during leishmaniasis. Our previous study showed that Leishmania major, which causes cutaneous leishmaniasis, blocks the translation initiation machinery of its host cell, the macrophage, in order to survive. However, macrophage translational control by L. donovani has not been investigated. Polysome profile and Western blot experiments revealed two different effects of the infection on host translation, depending on the stage of the parasite's life cycle. We found that L. donovani promastigotes inhibit initiation of translation in infected mouse macrophages. Moreover, when the virulence factor lipophosphoglycan is deleted from the promastigotes, translation inhibition is further exacerbated. In an attempt to define the molecular mechanisms responsible for such effect, we monitored the activation of the main translation initiation factor eIF4E. We observed that eIF4E is dephosphorylated (i.e. inactivated) in macrophages infected with L. donovani promastigotes. In contrast, L. donovani amastigotes activate the mTORC1 and the MNK/phospho-eIF4E signaling pathways, which correlates with an increase in macrophage translation initiation. Altogether, our results demonstrate that L. donovani differentially controls macrophage translational activity depending on its life cycle stage. These effects could have consequences on the establishment (i.e. promastigotes) and the chronicity (i.e. amastigotes) of the infection.

Poster Session

MEM-PW3024 - Study on the role of Leishmania mexicana cystein protease B in phagosome maturation

Pierre-André Casgrain[1], Albert Descoteaux[1]
[1]INRS Institut Armand-Frappier, Laval, Canada

Leishmania is the protozoan parasite responsible of leishmaniasis, an illness that comprehends 3 different forms: cutaneous, mucocutaneous and visceral. This disease is present in 98 countries and with 2 million new cases reported each year. The parasite evades the immune system by surviving and multiplying in the macrophage. Following phagocytosis of pathogens, the newly-formed phagosome undergoes multiple steps to reach the phagolysosomal state and to be able to kill the parasite. After degradation of the parasite, the macrophage can present the antigen to lymphocyte by MHC class I or II. Phagosome maturation into a phagolysosome is due to fusion of it with other vesicles containing several microbicidal elements. These vesicles use soluble N-ethylmaleimide-sensitive-factor attachment protein receptors (SNAREs) to fuse with the phagosome. Earlier studies have shown that L. major and L. donovani use the lipophosphoglycan (LPG) and the metalloprotease GP63 to block the maturation of the phagosome and use it like a niche. Furthermore, it has been shown that these parasites can not survive inside the macrophage without LPG except for L. mexicana. It has been described that L. mexicana needs the cysteine protease CPB to infect BALB/c mice, but not LPG. CPB is recruited to the parasite-containing phagosome and is capable of cleaving several host proteins such us NF-kB and inhibiting the antigen presentation by degrading MHC class II molecules in the parasitophorous vacuole. The aim of this project is to determine whether CPB has a similar role to GP63 and LPG during the infection process as in blocking phagosome maturation. To do so, we are currently evaluating the parasitophorous vacuole composition as well as the effect of CPB on SNAREs. Results of this project will help to provide a better understanding of the mechanisms underlying L. mexicana survival and alteration of the host response.

Poster Session

MEM-PW3026 - Membrane-associated RING-CH1 (MARCH1) deficiency protects against *Leishmania donovani* infection in mice

Maxime Raymond[1], Akil Hammami[2], Tristan Galbas[1], Christine Matte[2], Albert Descoteaux[2], Cheolho Cheong[3, 4], Simona Stäger[2], Jacques Thibodeau[1]

[1]Laboratoire d'immunologie Moléculaire, Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal,Canada, [2]Institut National de la Recherche Scientifique, Institut Armand Frappier, Université du Québec Laval, Canada, [3]Laboratory of Cellular Immunology and Physiology, Institut de Recherches Cliniques de Montréal, Canada, [4]Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Canada

Aim: The membrane-associated RING-CH1 (MARCH1), an E3 ubiquitin ligase, has been shown to dampen antigen presentation and to be readily induced by IL-10 in monocytes and macrophages. Moreover, IL-10 was identified as a key mediator of parasite evasion during *Leishmania* infections. Also, pro-inflammatory monocytes "Ly6C hi" have been shown to play a critical role in the early response to *Leishmania* spp. infections in mice. Here we set out to investigate the role of MARCH1 in the resolution of *Leishmania* infections. Methodology: We infected MARCH1-/- mice with *Leishmania donovani* promastigotes and followed the parasite burden at day 7, 14 and 28 post infection. Using OVA-transgenic *L. donovani* and adoptive transfer of OT-I CD8 T cells, we studied T cell responses in the spleen. Flow cytometry was used to characterize the different cell populations implicated in parasite clearance in the spleen. We also performed histological analysis. Results: Here we show that MARCH1-KO mice have a significantly decreased parasite burden in the spleen when compared to their wild-type counterpart. Surprisingly, this effect was not observed in the liver. We show that this effect is not due to an enhanced T cell response but rather of a pro-inflammatory state in the MARCH1-KO mice that correlates with an increased proportion of Ly6C hi monocytes in the blood and the spleen of knockout mice. Conclusion: These results show that MARCH1 enables *Leishmania* to persist in the spleen of mice due to a decreased pro-inflammatory state.

Poster Session

MEM-PW3028 - Biodiversity of ectomycorrhizal (EcM) fungi associated with tropical lowland forest in Colombia, Amazonia

Aida Vasco-Palacios[1,2], Ana Esperanza Franco-Molano[2], Leho Tedersoo[3], Teun Boekhout[1]

[1]CBS Fungal Biodiversity Centre, Utrecht, The Netherlands, [2]Taxonomy and Ecology of Fungi, Institute of Biology, Antioquia University, Medellin, Colombia, [3]Natural History Museum and Institute of Ecology and Earth Sciences, University of Tartu, Estonia

The ecological importance of tropical ectomycorrhizal (EcM) fungi is poorly understood and relatively little is known about their biodiversity, community structure and functional roles in tropical ecosystems. Recently, studies in the tropics reported a high biodiversity of macrofungi in diverse ecosystems and many undiscovered fungal species. Attempts have been made to document the diversity of ectomychorrizal fungi associated with ectomycorrhizal host trees in lowland tropical rainforest in Colombia. More than 70 morphospecies of putative EcM fungi have been collected in two different types of forests. The first forest is dominated by Pseudomonotes tropenbosii, a neotropical endemic Dipterocarpaceae tree which is only known from small populations in the Colombian Amazon. The second is a “White Sand” forests dominated by Aldina sp. and Dicymbe sp., two trees that belongs to the Fabaceae family. These sandy soils where these two different ecosystems occur are very nutrient-poor and acid. We collected EcM roots tips, basidiocarps belonging to EcM taxa and as soil samples for metagenomics studies. The more abundant taxa collected were Boletaceae (20 species), Russula (19 species), Clavulinaceae (6 species), Cantharellaceae (6 species), and Agaricales (Amanita 6 species, Cortinarius 4 species). Many of these species may represent new species (e.g, Austroboletus tropenbosii, Sarcodon sp. nov, Coltriciella spp nov.). Several species collected from the “White sand” forest have been reported from similar ecosystems in Guyana by Henkel and co-workers. Fungal species occurring in EcM root tips were identified using the Internal Transcribed Spacer of the ribosomal DNA (ITS) and identified as Sebacina type A. The fungal community structure is currently studied by 454 pyrosequencing of soil samples. Some species that were identified from ITS sequences amplified from root tips were not collected as mushrooms showing that diversity of EcM fungi will increase with combined molecular and collection approaches.

Poster Session

MEM-PW3030 - Attenuation of vacuole-mediated potassium resistance by the Putative C_2H_2 zinc finger transcription factor aslA in *Aspergillus nidulans*

Dong Soo Park[1], Yeong Man Yu[1], Yong Jin Kim[1], Pil Jae Maeng[1]
[1]*Chungnam National University, Daejeon, Korea*

We analyzed the function of *aslA* gene encoding a putative C_2H_2-type zinc finger transcription factor (TF) in context of potassium stress resistance and vacuolar morphology in the filamentous *Aspergillus nidulans*. The mutant lacking *aslA* showed increased mycelial growth and decreased branching at high potassium concentrations. Deletion of *aslA* also caused elevated expression of the genes encoding putative endosomal and vacuolar cation/proton exchangers, *nhxA* (AN2288), *vnxA* (AN6986) and *vcxA* (AN0471), as well as those encoding vacuolar proteins, *cpyA* and *vpsA*, under potassium stress conditions. Interestingly, vacuolar fragmentation induced by potassium stress was blocked by *aslA* deletion, resulting in retaining enlarged vacuoles. In the presence of bafilomycin, an inhibitor of vacuolar H^+-ATPase, the mutant phenotype was suppressed in terms of growth rates and vacuolar morphology. These results together suggest that the C_2H_2-type zinc finger TF AslA represses the expression of the genes encoding the ion pumps involved in vacuolar sequestration of proton powered by vacuolar H^+-ATPase as well as the proteins that function in vacuolar biogenesis.

Poster Session

VIR-PW2001 - Bat adenovirus survey in Central Europe

Marton Vidovszky[1], Claudia Kohl[2], Sandor Boldogh[3], Tamas Gorfol[1], Gudrun Wibbelt[4], Andreas Kurth[2], Balazs Harrach[1]

[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, [2]Robert Koch Institute, Centre for Biological Security, Berlin, Germany, [3]Aggtelek National Park Directorate, Aggtelek, Hungary, [4]Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany

In the past decade, bats emerged as important reservoirs of numerous viruses including those with zoonotic potential. More recently, several novel adenoviruses (AdVs) have also been detected in bats. We carried out a survey on the bat fauna of a couple of Central European countries. From over 900 extant species of the order Chiroptera, about 28 and 25 are native to Hungary and Germany, respectively. More than 350 bat samples originating from 27 bat species (25 from Hungary and 17 from Germany) were screened for AdVs using a consensus nested PCR that targets the viral DNA polymerase gene. The majority of the Hungarian samples consisted of excrements collected from roosting areas of known bat species, but a few samples were taken from live individuals transiently captured for population studies. All German samples were from the internal organs of dead animals. About the 28% of the samples were found positive by PCR. After sequence analysis, twenty-five novel and three previously published putative bat AdVs were found. Identical AdV sequences were often derived from individuals of the same species especially within each country. The phylogeny inference indicated the clear separation of AdVs detected in bats of species belonging to the Vespertilionidae or Rhinolophidae families, respectively. The close common ancestry of vespertilionid AdVs and equine AdV-1 was confirmed. Moreover, the canine AdVs, well-known for their exceptionally broad host range, did also cluster in the clade of the vespertilionid bat AdVs. These findings deserve further scrutiny. Further characterization of the newly detected viruses is also planned.

Poster Session

VIR-PW2003 - Partial genome sequence of an adenovirus, representing a newly discovered lineage, from read-eared slider (Trachemys scripta elegans)

Mária Benkő[1], Zoltán László Tarján[1], Balázs Harrach[1]

[1]*Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy od Sciences, Budapest, Hungary*

Presently, the family Adenoviridae is divided into five genera. Three of them (Mastadenovirus, Aviadenovirus and Ichtadenovirus) encompass viruses that are assumed to have cospeciated with one major vertebrate class (mammals, birds and fish, respectively). Members of the two other genera occur in more diverse hosts. Siadenoviruses have been found in birds, a frog, and a particular virus type in several captive turtles of different species. The genus Atadenovirus includes viruses derived from domestic and wild ruminants, birds, a marsupial and a large number of representatives of squamate reptiles. This lineage is hypothesized to have coevolved with the order Squamata, whereas the host origin of the siadenoviruses is unknown. An interesting group of adenoviruses (AdVs) have been detected in a number of turtles belonging to the Testudinoidea superfamily recently. Phylogeny reconstructions with short sequences derived from the PCR products (from the viral DNA polymerase and the hexon genes) indicated that these AdVs form a separate lineage that probably merits the establishment of a novel, sixth genus. The tentative genus name Testadenovirus has been proposed. Here, we report the sequence of the central genome part of one putative testadenovirus found in a dead individual of the red-eared slider (Trachemys scripta elegans). Attempts to isolate the virus were unsuccessful. We performed a nested long PCR with specific primer pairs designed from the two partial gene sequences. The large fragment (of over 10 kb) encompasses seven full and two partial genes. Phylogeny inference based on several genes further supported the genus-level separation of the new AdV lineage. However, the genus-specific genes and transcription units are usually contained in the genome termini of AdVs. Acquisition of these sequences, supposedly encoding numerous hitherto unknown "testadenoviral" proteins, is in progress. The support by the Hungarian Scientific Research Fund (grant OTKA NN107632) is acknowledged.

Poster Session

VIR-PW2005 - Novel adenoviruses detected in racing pigeons

Mónika Z. Ballmann[1], Balázs Harrach[1]
[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Two different diseases (adenovirosis type I and II) are thought to be caused by adenoviruses in racing pigeons (Columba livia f. domestica). The two disease types have been described by the divergent clinical signs but no data are available for the eventual difference between the adenoviruses involved. On primary chicken cells, several fowl adenovirus types as well as a pigeon adenovirus (pigeon adenovirus 1, PiAdV-1) have been isolated from racing pigeons (Hess et al., 1998). Up to now, only one sequence, the partial hexon gene of PiAdV-1 has been published. According to sequence analysis, it belongs to the genus Aviadenovirus, while members of the other two genera (Siadenovirus and Atadenovirus) which can also occur in birds, have not been reported yet from pigeons. During our 5-year survey, 216 pigeons (from 79 different lofts) were screened by a nested PCR method that targets the DNA-dependent DNA-polymerase gene of adenoviruses (Wellehan et al., 2004). The PCR products were sequenced and/or analyzed by a restriction enzyme assay. For comparative purposes, partial hexon sequences were amplified from the positive samples. We found 109 positive samples, from which 45 amplicons were sequenced. In 33 cases, aviadenoviruses were identified. The sequence of 12 PCR products proved to be from siadenovirus. According to the sequence analyses, 4 different types of aviadenoviruses could be differentiated. One of them, the PiAdV-1, was detected just in one case. Out of the 12 siadenoviruses, 11 were identical, and one is considerably different. Fowl adenoviruses were not found, although our method is a sufficiently sensitive technique for their detection, too. This is the first report on the presence of siadenoviruses and aviadenoviruses, other than PiAdV-1, in pigeons. Full genome analysis of the most frequently found aviadenovirus (proposed to be PiAdV-2) is in progress. Support: Hungarian Scientific Research Fund (OTKA NN107632).

Poster Session

VIR-PW2007 - Propagation of viruses infecting waterfowl on continuous cell lines immortalized by adenoviral genes

Zoltán Zádori[1], Renáta Tóth[1], Ádám Bálint[2], Ádám Dán[2], Ingo Jordan[3], István Mészáros[1]
[1]Institute for Veterinary Medical Research,Centre of Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, [2]Veterinary Diagnostic Directorate, National Food Chain Safety Office, Budapest, Hungary, [3]ProBioGen AG, Berlin, Germany

Avian cell lines (AGE1.CR, AGE1.CS) were immortalized by the E1A and E1B genes of the human adenovirus serotype 5 (Ad5). In addition to these genes a cell line was established to express the pIX structural gene of Ad5 (AGE1CR.pIX). Duck circovirus (DuCV), duck hepatitis A virus 1 (DHAV-1), goose parvovirus (GPV) and goose hemorrhagic polyomavirus (GHPV) are economically harmful pathogens of waterfowl, and do not or poorly replicate in established cell lines. AGE1.CR, AGE1.CR.pIX and AGE1.CS cells were tested for their suitability to isolate and diagnose these viruses. Immunofluorescence (IF) and qPCR investigations verified that all cell lines are permissive for each of the four viruses, however AGE1.CR.pIX proved to be the most productive and most sensitive for viral infection. It can be a robust alternative to primary cells and embryonated eggs for viral detection and isolation. E1A and E1B genes are known to be able to induce cell cycle progression while pIX is a multifunctional protein which alone and in combination with E1A may be involved in modulation of antiviral processes. GPV, GHPV and DuCV are small DNA viruses that due to the lack of their own DNA polymerases depend on the host machinery for replication. However, the presence of an active cellular replication machinery cannot explain by alone permissivity of the anatine continuous cell lines to viral replication since pre-senescent primary cells and other continuous cell lines are not able to support the replication of these viruses. Interference with signaling pathways of innate immunity and antiviral genes by the adenoviral proteins might contribute to the observed permissivity. This notion is supported, by the replication of RNA viruses like DHAV-1 and influenza in these cell lines.

Poster Session

VIR-PW2009 - Time to treatment access when pharmacists prescribe oseltamivir

John C. Akitt[1], Joanne Morris[1], Anil K. Gupta[2]
[1]Trial Management Group Inc., Toronto, Canada, [2]Albion Finch Medical Centre, Toronto, Canada

Background: The earlier that oseltamivir treatment is started, the greater its efficacy. It was hypothesized that if pharmacists were authorized to prescribe oseltamivir for influenza, patients could receive treatment earlier than if a physician visit was required - thereby enhancing treatment efficacy. Methods: This study, which was conducted in Canada, received approval from a central ethics committee. Written informed consent was obtained from all subjects. Two patient groups were studied. Group 1 consisted of patients arriving at the pharmacy with an oseltamivir prescription from a non-study physician. Group 2 patients presented at the pharmacy with symptoms/signs diagnosed as influenza by the pharmacist, and were judged to be suitable for oseltamivir treatment. Patients screened at the pharmacy from both groups were transferred to a nearby participating clinic. Influenza signs/symptoms were assessed and a nasal swab specimen collected for confirmation of the diagnosis. Eligible group 2 subjects were prescribed oseltamivir. At the end of treatment on day 5, nasal swab specimens were collected for oseltamivir resistance testing and symptoms/signs were reassessed. Adverse events were elicited on days 5 and 21. Results: Despite conducting the study over two influenza seasons, only 40 subjects (14% of the calculated sample size) were enrolled in the provinces of Ontario, Quebec and Newfoundland. No statistical testing was performed; however, a trend towards subjects diagnosed by pharmacists having quicker access to oseltamivir treatment was observed. Diagnostic accuracy, symptom response and occurrence of adverse events appeared similar; no resistance to oseltamivir was reported. Conclusion: While no statistical analysis could be performed, a trend towards patients diagnosed by pharmacists having faster access to oseltamivir was observed. If this difference is real, pharmacist prescribing of oseltamivir could increase treatment efficacy. ClinicalTrials.gov Identifier: NCT01456234 Key words: Oseltamivir, pharmacist, prescribe, treatment, time

Poster Session

VIR-PW2011 - Synergistic anti-viral effects of different specific IgA antibodies against different structural proteins of measles virus

Dihan Zhou[1], Huimin Yan[1]
[1]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

The mucosal immune system provides the initial immunological barrier against most pathogens which invade organism through mucosal system, and secretory IgA antibodies mediate primary defense functions in mucosal immune system. We have demonstrated that measles virus matrix protein specific IgA antibody can interact with newly synthesized matrix protein during IgA transport through the epithelial cells, and neutralize virus replication intracellularly, implying that besides surface proteins which were traditionally regarded as targets antigens, highly conserved non-surface proteins can also be effective target antigens. M protein plays an important role in the assembly process of the measles virus, studies have pointed out that the M protein was able to interact with RNP which was comprised of RNA genome and the N, P, L proteins, and associate with the rafts together. M protein also interact with other structural proteins involved in virus assembly. We found that there is synergistic effect between the M protein and N protein specific IgA antibodies. Our studies also showed that synergistic effect exists between 4 major structural proteins' specific IgA antibodies with different efficiencies. According to our results, we may advance an optimized immunization scheme for measles recombinant polypeptide mucosal vaccine, and give some academic support for the mucosal vaccines design of measles virus mutant and other virosis.

Poster Session

VIR-PW2013 - Effect of oseltamivir treatment on cognitive and mood decrements associated with influenza: a randomised, controlled study

John C. Akitt[1], Joanne Morris[1], Michael F. J. O'Mahony[2]
[1]*Trial Management Group Inc., Toronto, Canada,* [2]*London Road Diagnostic Clinic and Medical Centre, London, Canada*

Background: Decrements in cognition and mood have been reported in individuals with upper respiratory tract infections. The objective of this study was to assess the effect of oseltamivir phosphate (Tamiflu®) in alleviating these deficits in patients with influenza. Methods: This multicenter, randomised, parallel-group, open-label study was conducted at 19 Canadian primary care centres over the 2010/11 and 2011/12 influenza seasons. The study was approved by a central ethics committee. Written informed consent was obtained from all subjects. Adults with influenza were randomised to treatment with oseltamivir phosphate (75 mg BID for 5 days) + standard of care (SoC) for influenza, or SoC alone. Subjects completed online cognitive tests and assessments of mood, symptoms and overall health over 14 days. The primary endpoint was the change from baseline to Day 4 in simple reaction time (SRT), a measure of attention. Results: Of 215 patients screened, 122 were randomised. At Day 4, a statistically significant difference between treatments in change from baseline was detected for SRT and working memory with the oseltamivir + SoC group showing greater improvement (SRT LS Means Diff -30.4 ms, 95% CI -60.7 to –0.1, p = 0.0492; working memory LS Means Diff 3.78 correct answers, 95% CI 1.14 - 6.42, p = .0054). No treatment differences were observed for processing speed or mood. Interpretation: Oseltamivir + SoC was superior to SoC alone in alleviating decrements in attention and working memory in subjects with influenza. This is the first study to evaluate the effects of an antiviral medication such as oseltamivir on cognitive and mood deficits experienced by subjects with confirmed influenza. ClinicalTrials.gov Identifier: NCT01249833

Poster Session

VIR-PW2015 - Actinobacillus pleuropneumoniae (App) blocks Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) replication prior to its genome replication and transcription

Yenney Hernandez Reyes[1], Chantale Provost[1], Jérémy Ferreira-Barbosa[1], Josée Labrie[1], Mario Jacques[1], Carl A. Gagnon[1]
[1]*Faculté de médecine vétérinaire, Université de Montréal, Canada*

Porcine reproductive and respiratory syndrome (PRRS) is the most economically important infectious disease of swine production. Current management strategies are inadequate for long term control of PRRS, which justify the search of novel strategies to control PRRS. A recent study, performed in our laboratory, has demonstrated that the cell culture supernatant of App inhibits PRRSV infection in the newly discovered SJPL permissive cell line, but its inhibition effect was almost insignificant in MARC-145 infected cells. Interestingly, this phenomenon was also observed in the primary target cells of PRRSV in vivo: porcine alveolar macrophages (PAM). Following this finding, the objective of this study was to elucidate how App inhibits PRRSV replication in PAM cells. First, PAM were treated with App supernatant before and after infection with PRRSV strain IAF-Klop. At different time post-infections (pi), viral genome replication and transcription were measured in the presence of App. In addition, relative protein expression levels of PAM treated with App was also evaluated using the KINEX™ Microarrays. The expression levels of selected proteins were subsequently, confirmed by immunofluorescence (IFA) and westernblot assays. PRRSV genome replication and transcription was detected at 8 hours pi, however in the presence of the bacterial supernatant the PRRSV genome replication and transcription was not observed, indicating that App antiviral effect against PRRSV take place early in PRRSV replication cycle. Proteomic results revealed an increase of cofilin (a protein that regulates actin cytoskeleton dynamics) in App treated PRRSV infected cells. Subsequently, a diminution of actin filaments in the presence of App was demonstrated by IFA. Interestingly, a microfilament-disrupting compound (Cytochalasin D) induce the same effect on PRRSV replication than App supernatant suggesting that App antiviral effect against PRRSV take place via the activation of cofilin and thus actin depolymerisation, which probably affect PRRSV endocytosis.

Poster Session

VIR-PW2017 - Antiviral activity of kuraridin isolated from Sophora flavescent against reovirus replication and absorption

Hyung-Jun Kwon[1], Young Bae Ryu[1], Young Min Kim[1], Kyoung-Oh Cho[2], Mun-Chual Rho[1], Woo Song Lee[1], Su-Jin Park[1]

[1]Korea Research Institute of Bioscience and Biotechnology, Jeongeup-Si, Korea, [2]College of Veterinary Medicine, Chonnam National University, Gwangju, Korea

Background: We evaluated the ability of MeOH extract and EtOAc fraction, and kuraridin isolated from the roots of Sophora flavescent (S. flavescent) in anti-reovirus activity. Upon finding that MeOH extract and EtOAc fraction, and kuraridin possessed anti-reovirus activity, we evaluated whether these properties were attributable to direct inhibition of the binding of reovirus to cells and/or to inhibition of viral replication by means of time-of-addition experiments; pre-treatment, simultaneous treatment, and post treatment. Results: In pre-treatment assay, the MeOH extract and EtOAc fraction, and kuraridin did not show significant antiviral activity. Using the simultaneous assay, we found that MeOH extract and EtOAc fraction, and kuraridin directly inhibited reovirus binding, with activity being dependent on the type of virus. The 50% effective inhibitory concentrations (EC50) were 86.1-197 and 46.3-195.9 ug/mL against type 1-3 reoviruses by the MeOH extract and EtOAc fraction and 15.3-176.9 uM against type 1-3 reoviruses by kuraridin, respectively. The MeOH extract and EtOAc fraction, and kuraridin exhibited completely hemagglutination inhibition activity. Moreover, the post treatment assay showed that MeOH extract and EtOAc fraction inhibited viral replication with EC50 values of 15.6-112.1 and 13.8-81.4 ug/mL against type 1-3 reoviruses. Consequently, kuraridin exhibited EC50 value of 14.0-62.0 uM against type 1-3 reoviruses. In all assays, MeOH extract, EtOAc fraction, and kuraridin showed a stronger inhibitory effect at type 3 reovirus (T3D and KPR113 strain) than type 1 (T1L strain) and type 2 (T2J strain) reoviruses. Quantitative real-time PCR showed that the kuraridin suppressed strongly viral RNA synthesis of reovirus replication step in late stage. Interestingly, kuraridin was attributable to inhibition of both viral absorption and viral replication.

Poster Session

VIR-PW2019 - The small peptide derived from viral FLIP of KSHV disrupts enveloped RNA viruses

Jong-Soo Lee[1], Ho-Jin Moon[1], Chul-Joong Kim[1], Jae U Jung[2]
[1]*College of Veterinary Medicine (BK21 Plus program), Chungnam National University, Daejeon, Republic of Korea,*
[2]*University of Southern California, Keck School of Medicine, Los Angeles, USA*

The Virocidal activities of Ka2-helix peptide against Influenza A virus (IAV) in vitro and in vivo are investigated and the mechanism of action are suggested. Ka2-helix peptide which can induce the robust autophagy, derived from viral FLIP (vFLIP) of Kaposi's sarcoma-associated herpesvirus (KSHV) and treatment of Ka2 peptide fused with TAT peptide inhibited a significant enveloped RNA viruses which containing IAV replication and transmission by inactivating infectious particles. Moreover, Ka2 peptide protected mice against challenge with lethal doses of the highly pathogenic influenza A H5N1 virus. Mechanically, we found that Ka2 peptide can destabilizes viral membranes based on their lipid composition. These results suggest Ka2 peptide has potential value as a reagent to the antiviral strategies. [This research was supported by National Agenda Project grant from KRCFST (KGM 0821113) and Grant no. (K12050) from the Korea Institute of Oriental Medicine (KIOM)].

Poster Session

VIR-PW2021 - The mechanism of HIV-1 resistance to CCR5 inhibitors

Odalis Asin Milan[1,2], Yi Wei[1], Mohamed Sylla[1], Annie Chamberland[1], Farida Vaisheva[3], Alpha Haidara[1], Pascale Kouassi[1,2], Mohamed El Far[1], Cecile Tremblay[1,2,4]

[1]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CR-CHUM), Montréal, Canada, [2]Department of Microbiology Infectiology and Immunology. Faculty of Medicine. University of Montreal, Canada, [3]Department of Pharmacology. McGill University. Montreal, Canada, [4]Laboratoire de Santé Publique du Québec/Institut National de Santé Publique du Québec, Canada

The most common genetic basis for HIV resistance to CCR5 inhibitors involves the acquisition of sequence changes in the envelope V3 loop that renders the gp120 capable of recognizing the inhibitor-CCR5 complex without losing its ability to interact with the free coreceptor. Mutations in other regions of gp160 has been shown to play a role in viral resistance. Knowledge of HIV-1 tropism is essential to assess the potential use of novel CCR5 inhibitors, they are active only against R5. We first developed a phenotypic test to identify the viral tropism both clinical and research settings. Second we studied how mutations in the variable regions of gp120 protein of HIV-1 may contribute to resistance to CCR5 inhibitors. Finally, we employed in vitro drug-interaction system to assess whether CCR5 inhibitors could be used together or subsequently within the same therapeutical regimen. To perform a tropism assay, pseudoviruses were used to infect U87CD4CCR5+ and U87CD4CXCR4+ cells. Our clonal assay was sensitive and could differentiate the truly dual-tropic viruses and detected the X4 and R5 tropic viruses when they represented 0.4 % or greater in a mixture of viral populations. CC1/85 and BAL resistant viruses were generated in the presence of sub-inhibitory concentrations of maraviroc (MVC) and vicriviroc (VCV). The mutations associated with a resistant phenotype were determined. We selected three mutations in the variable domains of gp120; V169M, L317W and I408T to construct mutant envelopes with either single or combinations of mutations to assess the contribution of mutations to MVC and VVC resistance. All mutants remained R5-tropic. The mutant harbouring the I408T mutation had the most significant impact on susceptibility to MVC. The in vitro interactions of CCR5 inhibitors showed that combinations of CCR5 inhibitors have interactions ranging from synergy to antagonism and these combinations should be considered with caution.

Poster Session

VIR-PW2023 - Arenaviruses hijack partially activated SKI-1/S1P precursors present early in the secretory pathway to avoid perturbing enzyme cellular functions

Joel R. Palma[1], Dominique Burri[1], Joel Oppliger[1], Laura Cendron[2], Patrizia Polverini[2], Marco Salamina[2], Nabil George Seidah[3], Stefan Kunz[1], Antonella Pasquato[1]

[1]Institute of Microbiology, University Hospital Center of Lausane and University of Lausanne, Lausanne, Switzerland, [2]Department of Biomedical Sciences, University of Padua, Padua, Italy, [3]Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Université de Montréal, Montréal, Canada

Arenaviruses are pathogens able to cause severe and lethal diseases in humans. Currently no licensed vaccines are available to prevent arenaviral infections and current therapeutic options are limited . Thus, understanding of the virus-host interaction is relevant for the development of novel inhibitors. During the life cycle of arenaviruses in the host cell, the membrane viral glycoprotein precursor GPC must be matured before it is incorporated into the viral particles and is able trigger fusion with the host membrane upon cell entry. This maturation step crucially depends on Subtilisin Kexin Isozyme-1 (SKI-1) / Site-1 Protease (S1P), a cellular protease with crucial roles in cellular homeostatic functions. Here we used arenavirus GPC as a molecular probe to dissect the mechanisms underlying SKI-1/S1P maturation. SKI-1/S1P maturation occurs via sequential autocatalytic processing of its N-terminal prodomain at sites B'/B and the here newly identified C'/C sites. We found that autoprocessing results in heterodimer intermediates comprised of the catalytically active subunit associated with prodomain fragments of different length. Inhibition of auto-processing modulated enzymatic activity in a substrate-specific manner. Using a novel cell-based sensor for SKI-1/S1P activity, we pinpointed eight amino acid residues at the cleavage site to be necessary and sufficient to define the dependence of some substrates on SKI-1/S1P maturation, as well as the subcellular location of SKI-1/S1P cleavage. In contrast to SKI-1/S1P's cellular substrates, the recognition sequence in viral envelope GPs determines processing in early compartments of the secretory pathway, where immature forms of SKI-1/S1P locate. The pathogens may have evolved to hijack partially activated enzyme precursors present early in the secretory pathway to avoid perturbing SKI-1/S1P's cellular functions.

Poster Session

VIR-PW2025 - Prevalence of LCMV in patients with renal carcinoma

Katarina Laposova[1], Lubomira Lukacikova[2], Ingrid Oveckova[1], Jan Breza[3], Silvia Pastorekova[1], Jaromir Pastorek[1], Jana Tomaskova[1]

[1]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Center for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovakia, [3]Department of Urology, Derer's University Hospital, Bratislava, Slovakia

Lymphocytic choriomeningitis virus (LCMV) is a rodentborne arenavirus. In immunocompetent persons it mostly causes mild or asymptomatic infections, but aseptic meningitis and encephalitis can also occur. Increasing evidence indicates that LCMV posses a great danger to immunosuppressed individuals. Lately we have found out that hypoxia markedly enhances expression of LCMV genes via HIF-dependent mechanism and improves virus transmission in persistently infected human cell lines. These findings imply a possibility of LCMV reactivation in human diseases linked with hypoxia. The aim of our present study was to elucidate a prevalence of anti-LCMV antibodies and prevalence of LCMV in the group of renal carcinoma patients. It is known that mutations within the VHL gene specific for clear cell renal carcinomas lead to constitutive HIF activation and that during a persistent infection virus-antibody complexes deposit in renal glomeruli. For this purpose we have developed a sensitive diagnostic test for detection of anti-nucleoprotein (NP) IgG antibodies in sera based on ELISA method. Our results revealed 37,7% prevalence of these antibodies in 62 tested sera. Moreover, we also examined tissue samples which included specimens of tumors and healthy parts of kidneys obtained by partial nephrectomy. In several of them we detected viral NP by immunoprecipitation, thus confirming a persistent infection. Notably, the nucleoprotein was more often found in tumor tissues what could be explained by the presence of hypoxia in these tissues. The presence of NP in these samples was also validated by mass spectrometry. By this study we proved that patients with renal carcinoma have increased levels of LCMV-specific antibodies in comparision to healthy population. Concurrently, the evidence of persistent infection in humans confirm that LCMV is an underestimated human pathogen important for clinical medicine. This project was supported by grant VEGA 2/0128/11.

Poster Session

VIR-PW2027 - Development of the detection system to identify antibodies against Schmallenberg virus in ruminant sera

Justas Lazutka[1], Kestutis Sasnauskas[1], Kerstin Wernike[2], Aiste Bulavaite[1], Aliona Spakova[1]
[1]Institute of Biotechnolgy, Vilnius, Lithuania, [2]Friedrich-Loeffler-Institut, Insel Riems, Germany

Schmallenberg virus is a novel positive-stranded RNA virus from family Bunyaviriade, genus Orthobunyavirus. It is transmitted by biting midges and infects ruminants resulting in fever, diarrhea or reduced milk production, while fetuses can develop severe malformations and die shortly after birth. Since its discovery in 2011 in Germany, Schmallenberg virus has been detected in animals in 20 European countries, which has led to import bans from several countries around the world on live ruminants, their genetic material and dairy products. As the virus spreads quickly it is essential to develop fast and reliable its infection detection methods. We decided to develop a serological Schmallenberg virus detection system based on its nucleocapsid protein which has highly conservative amino acid sequence between different Schmallenberg virus isolates. Saccharomyces cerevisiae yeast was transformed with the plasmid coding for the nucleocapsid protein with a poly-histidine tag at the N terminus of this peptide. We have purified the synthesized protein at a yield of 2 mg form 1 g of yeast cells. Later, four different monoclonal antibodies against purified nucleocapsid protein were produced in mice. They were all shown to react with Schmallenberg virus in infected Baby Hamster kidney cells. One of the antibodies was shown to recognize five different isolates of Schmallenberg virus. Other three antibodies recognized only one out of five isolates. We have also synthesized and purified form yeast the Gn glycoprotein of Schmallenberg virus and a fusion protein, consisting of nucleocapsid protein and truncated Gn glycoprotein. Our current work allows us to further develop Schmallenberg virus detection system by utilizing enzyme linked immunosorbent assay (ELISA), western- or dot-blot methods. Based on these techniques we are hoping to produce a cheap, fast and reliable alternative to time consuming real-time PCR, virus neutralization tests or commercial ELISA Scmallenberg virus detection kits.

Poster Session

VIR-PW2029 - Distribution of Toscana virus in blood donors, South France

Nadege Brisbarre[1], Isabelle Leparc-Goffart[2], Sebastien Plumet[2], Philippe De Micco[1]
[1]UMR 7268 ADES/Aix-Marseille Université/EFS/CNRS, Marseille, France, [2]IRBA, Marseille, France

Toscana Virus (TOSV) is a Phlebovirus (family Bunyaviridae) of growing interest as a human pathogen since it may cause acute meningitis and meningoencephalitis. This arbovirus (arthropod borne virus) is transmitted by sandflies (Phlebotomus spp.) that are widely distributed around the Mediterranean basin. TOSV has been identified in numerous Mediterranean countries. Most of TOSV infections are asymptomatic or with mild symptoms. This suggests a potential risk for transmitting the virus through blood transfusion or organ transplantation. In France, cases of TOSV infection have been reported in residents and in its vector as well. A seroprevalence study, conducted in 2007 on southeastern french blood donors reported seroprevalences ranging from 6% to 13% with a commercial ELISA test (Diesse). Interestingly, a recent study showed that cross reactivity with close Phleboviruses, that co-circulate in these regions, may affect this serologic test since the antigen used harbors highly conserved regions among related phleboviruses. In order to better understand the effective distribution of TOSV in South of France, we first compared performances of a new ELISA test against the test used in 2007 on a negative plasma cohort (n=198) originated from an area with currently no TOSV vector. Positive hits of both ELISA tests were verified by seroneutralisation assay. Results highlighted differences in tests specificity, particularly for false positives. In a second approach, we investigated TOSV seroprevalence among blood donors (n=2031) across the whole French Mediterranean basin. Seroprevalence values ranged from 0% to 9.3% among territorial departments. A gradient was observed, linked potentially to the presence of the vector. Finally, we examined the presence of TOSV RNA in IgG negative plasma samples. No positive results for TOSV RNA were found in tested samples. Molecular and serological approaches along with results of this study are presented.

Poster Session

VIR-PW2031 - Phylogenetic analyses of SFTSVs in Japanese SFTS patients

Tomoki Yoshikawa[1], Shuetsu Fukushi[1], Hideki Tani[1], Aiko Fukuma[1], Momoko Ogata[1], Masayuki Saijo[1], Masayuki Shimojima[1]

[1]*Special Pathogens Laboratory, Department of Virology I, National Institute of Infectious Diseases, Tokyo, Japan*

Severe fever with thrombocytopenia syndrome (SFTS) is a newly discovered tick-borne disease characterized by fever, gastrointestinal symptoms, elevated levels of liver enzymes in serum, and a high case fatality rate, and has been prevalent in three countries, China, South Korea, and Japan. The causative agent of SFTS is SFTS virus (SFTSV), a member of the genus Phlebovirus of the family Bunyaviridae. The phylogenetic analyses indicated that SFTSV detected from Japanese patients who became illness between 2005 and 2012, form an independent cluster from Chinese and Korean SFTSV strains. The fact means that SFTSV has been present in Japan for some time (Takahashi et al., JID, 2014). The genome of bunyaviruses is a negative sense RNA comprising three segments, S, M, and L. Viral genome reassortment, which is the process of exchanging the gene segments, have been reported in all genera of the family Bunyaviridae. In SFTSV, so far, only two Chinese strains, AHL and HZM strains, are known reassortants: AHL strain, S segment from Chinese lineage 2 but M and L segments from Chinese lineage 3; HZM strain, S segment from Chinese lineage 1B but M and L segments from Chinese lineage 2 (Ding et al., Virus Res, 2013). In 2013 in Japan, a total of 40 patients were diagnosed as having SFTS. Phylogenetic analyses of SFTSV detected in the 40 patients led to three findings: the 1st was that Japanese SFTSV except one strain, designated SPL087A, could be divided into two Japanese lineages, the 2nd that the SPL087A belongs to the Chinese cluster, and the 3rd that SPL087A is a reassortant because the M segment was from Chinese lineage 2, while S and L segments were from Chinese lineage 3. The findings suggest that SFTSV is circulating in small areas but can be transmitted oversea.

Poster Session

VIR-PW2033 - Severe fever with thrombocytopenia syndrome virus in domestic and wild animals in Japan

Shigeru Morikawa[1], Masanobu Kimura[1], Shuetsu Fukushi[1], Aiko Fukuma[1], Yoshihiro Kaku[1], Eunsil Park[1], Hideki Tani[1], Tomoyuki Yoshikawa[1], Koichi Imaoka[1], Masayuki Shimojima[1], Masayuki Saijo[1], Ken Maeda[2]
[1]National Institute of Infectious Diseases, Tokyo, Japan, [2]Yamaguchi University, Yamaguchi, Japan

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease, with fatality rate ranging from 2 to 30%, first identified in China in 2009, then found in Japan and South Korea in 2013. In Japan, a total of 40 laboratory-confirmed SFTS cases with fatality rate of 32.5% were reported in 2013. All patients were distributed in the western region of Japan. SFTS virus (SFTSV), a member of the Phlebovirus genus in the family Bunyaviridae, has been identified as the causative agent of SFTS. SFTSV belongs to. SFTSV is transmitted by ticks, especially Haemahysalis longicornis and some other tick species. In China, a wide range of domesticated animals, such as goats, sheep, cattle, dogs, pigs and chickens, were shown to be seropositive in the endemic region. Serological surveillance data in China indicates that ruminants play a crucial role in the ecological cycle of SFTSV. In Japan, little is known about animals infected with SFTSV. To understand the ecological cycle of SFTSV at the tick-animal interface, serological surveillance of SFTSV in wild deer, wild boars and domesticated dogs was conducted. Antibodies to SFTSV were detected by ELISA using SFTSV-infected and uninfected Huh7 cell lysates as antigens and by immunofluorescent antibody test using acetone-fixed SFTSV-infected cells. Anti-SFTSV antibody prevalence was 24% (177/731) in wild deer, 10% (66/629) in dogs, and 5% (25/542) in wild boars. Antibody-positive animals were found in the northern to western regions of Japan, while the antibody positive rate was higher in the SFTS-endemic region.

Poster Session

VIR-PW2035 - Development of IFA and ELISA to detect antibodies against SFTSV

Aiko Fukuma[1], Shuetsu Fukushi[1], Hideki Tani[1], Tomoki Yoshikawa[1], Satoshi Taniguchi[1], Momoko Ogata[1], Masayuki Shimojima[1], Shigeru Morikawa[2], Masayuki Saijo[1]

[1]Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan, [2]Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo, Japan

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease with a case-fatality rate ranging from 2% to 30%, caused by SFTS virus (SFTSV). SFTSV is a tick-borne phlebovirus and belongs to family Bunyaviridae. SFTS was first identified in China in 2009, and recently reported in Japan and South Korea. Serological tests with high sensitivity and specificity are required to investigate the SFTS prevalence and evaluate risk factors for SFTSV infection in the endemic area. Previous studies demonstrated that recombinant nucleocapsid (rN) protein based-serological assays are useful for the diagnosis of many viral hemorrhagic fevers. In this study, immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) were developed using both SFTSV infected cells and SFTSV rN expressing cells as antigens. The sensitivity and specificity of these assays were examined using healthy human sera and convalescent-phase SFTS patients' sera collected in Japan. IFAs using either of SFTSV infected cells or SFTSV rN expressing HeLa cells, and ELISAs using either of SFTSV infected cell-lysates or purified SFTSV rN expressed using recombinant baculovirus detected IgG-antibodies against SFTSV with high specificities. However, sensitivities of the IFA and ELISA using SFTSV rN were lower than those using SFTSV infected cells. These results indicate that serological assays using the antigens prepared from SFTSV infected cells are suitable not only for the diagnosis of but also for the surveillance of SFTS in humans.

Poster Session

VIR-PW2037 - Genetic diversity and phylogenetic analysis of Hantavirus harbored by Myodesregulus in Korea

Jin Goo Lee[1], Se Hun Gu[1,2], Sook-Young Lee[1], Jeong Ah Kim[1], Seung Ho Lee[1], Won Keun Kim[1], Luck Ju Baek[1], Richard Yanagihara[2], Jin-Won Song[1]

[1]Korea University, Seoul, Republic of Korea, [2]University of Hawaii at Manoa, Honolulu, Hawaii

About 5% of cases of haemorrhagic fever with renal syndrome (HFRS) occurringannually in Korea have been found to exhibit a fourfold or higher antibody titre to Puumalavirus (PUUV) than to Hantaan virus (HTNV) by double-sandwich IgM ELISA, suggesting theexistence of a PUUV-related hantavirus.A genetically distinct hantavirus, designated Muju virus (MUJV), was found inlung tissue of royal voles(Myodesregulus), captured in widely separated geographical regions in Korea, 1996. Among 101 royal voles, taxonomically verified by mitochondrial DNA (mtDNA) analysis, captured in six sites, Korea during 2008–2011, 3 strainsof MUJVwere fully sequenced to ascertain if it represented a genetically distinct hantavirus species. Entire genome sequence analysis of the1,831-nucleotide small (S), 3,652-nucleotide medium (M) and 6,544-nucleotide large (L)segments of MUJV, as well as the amino acid sequences of each segments, showed that MUJV strains from different capture sites in Korea were genetic variants of PUUV, harbored by the bank vole (My. glareolus). Distinct geographic-specific clustering of MUJV was found in different provinces in Korea, and phylogenetic analyses revealed that MUJV and PUUV shared a common ancestry.

Poster Session

VIR-PW2039 - Use of pseudotyped vesicular stomatitis virus for measurement of neutralizing antibodies to Crimean-Congo hemorrhagic fever virus

Yuto Suda[1,2], Hideki Tani[2], Masayuki Saijo[2], Taisuke Horimoto[1], Masayuki Shimojima[2]
[1]Graduate School of Agricultural and Life Sciences, University of Tokyo, Japan, [2]Department of Virology I, National Institute of Infectious Diseases, Japan

Objective Crimean-Congo hemorrhagic fever (CCHF) is a fatal tick-borne disease reported in Africa, Eastern Europe, Middle East, and Asia. CCHF is caused by CCHF virus (CCHFV) that is a member of the genus Nairovirus in the family Bunyaviridae and requires BSL-4 containment when manupulated. Pseudotyping of viral glycoproteins onto vesicular stomatitis virus (VSV) that requires BSL-2 containment have facilitated studies on the viral entry and measurement of neutralization antibodies to the viruses. However, the production of VSV bearing CCHFV envelope protein has not been reported. In this study, we generated VSV-based pseudotype for CCHFV to measure neutralization activities against CCHFV. Materials and methods CCHFV IbAr10200 strain glycoprotein (GP) cDNA was cloned into pCAGGS and used to generate VSV pseudotype. Infectivity of the VSV pseudotype was measured in VeroE6 cells as expression of a reporter gene which was inserted into the genome of VSV in place of the G gene. Using the generated pseudotype, we measured neutralization activities of human serum samples collected at a CCHF-endemic area in China. Results obtained were compared with those of ELISA using recombinant nucleoprotein of CCHFV. Results The pseudotype produced using full-length GP showed a low titer. Deletion of GP cytoplasmic tail made the titer of the pseudotype higher. Neutralization test using the VSV-pseudotype showed 100% specificity and 59% sensitivity when compared with ELISA. Conclusion The neutralization test using the generated pseudotype can be a surrogate for a neutralization assay using infectious CCHFV.

Poster Session

VIR-PW2041 - Mixed infection of phytopathogenic fungi Alternaria sp. with new partitivirus and new victorivirus

Tatiana Sarkisova[1], Karel Petrzik[1], Vladislav Curn[2], David Novotny[3], Ondrej Lenz[1]
[1]Biology Centre AS CR, Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic, [2]University of South Bohemia, Fac. Agri., Ceske Budejovice, Czech Republic, [3]Crop Research Institute, Prague, Czech Republic

Alternaria alternata, A. arborescens, and A. tenuissima (Phylum: Ascomycota, Family: Pleosporaceae) are the most common fungi associated with core rot and mouldy core decay, especially in Golden Delicious, Wagener, Gravenstein, Rubin, and other apples varieties. Thus, as important plant pathogens they are under phytosanitary control in many countries. On the other hand, hypovirus phenotype induced by mycoviruses could be an alternative for the biological control. Over 80 isolates of Alternaria alternata, A. arborescens and A. tenuissima from CRI fungi collection (Prague, Czech Republic) were screened for the presence of virus-like double-stranded RNA that sign presence of RNA viruses. Mixed infection of two different mycoviruses sharing either no homology either tight phylogenetic relatedness between was detected in A. arborescens isolates. Colony morphology and growing rates of these isolates were evaluated. Partial sequence of app. 2 kbp segment showed 34% of identity with RNA dependent RNA polymerase of Pepper cryptic virus 1 and thus, represents a new member in the genus Partitivirus. The genome of the 6 kbp segment showed a 55% identity to Aspergillus foetidus slow virus 1 and represents an unclassified new virus in the genus Victorivirus. Virus with almost identical genome to that victorivirus has been found in one entomopathogenic Beauveria bassiana strain and in symbiotic fungi of Cladonia sp. Lichen, too. Ribavirin treatment was performed to eliminate one of the viruses and cure fungal strains. Transmission double-stranded RNA elements between mycelia were performed by a vegetative compatibility using horizontal transmission. Protoplast fusion was used to overcome an incompatibility barrier. This work was supported with LH13136 Kontakt II project of the Czech MEYS.

Poster Session

VIR-PW2043 - Virulent mycoviruses up-regulate RNA silencing-related genes in a phytopathogenic fungus, Rosellinia necatrix

Hajime Yaegashi[1], Takeo Shimizu[1], Tsutae Ito[1], Satoko Kanematsu[1]
[1]*NARO Natinal Institute of Fruit Tree Science, Morioka, Japan*

RNA silencing is an antiviral defense mechanism widely conserved among eukaryotes. In fungi, it is believed that three kinds of proteins, Dicer-like proteins (DCLs), Argonaute-like proteins (AGLs), and RNA-dependent RNA polymerases (RdRps), play central roles in the RNA silencing pathway. In a phytopathogenic fungus, Rosellinia necatrix, we confirmed that one DCL gene (DCL-2), two AGL genes (AGL-1, -2), and four RdRp genes (RdRp-1, -2, -3, -4) are expressed in mycelia. In this study, we investigated expression levels of these RNA silencing-related genes in R. necatrix strains infected with various double-stranded RNA mycoviruses. Three R. necatrix W97 strains infected with a latent mycovirus, a partitivirus (RnPV1), a quadrivirus (RnQV1), or a victorivirus (RnVV1), and two R. necatrix W97 strains infected with a virulent mycovirus, a mycoreovirus (RnMyRV3) or a megabirnavirus (RnMBV1), had their DCL-2, AGL-1, -2, and RdRp-1, -2, -3, -4 gene expression levels analyzed by semi-quantitative reverse transcriptional polymerase chain reaction. The results showed that the expression levels of the tested genes in W97 strains infected with latent mycoviruses (RnPV1, RnQV1, or RnVV1) were comparable to those of a virus-free W97 strain. However, the expression levels of DCL-2, AGL-2, RdRp-1, and RdRp-2 in W97 strains infected with virulent mycoviruses (RnMyRV3 or RnMBV1) were two- to four-fold higher than those of a virus-free W97 strain. Our data suggest that the RNA silencing machinery in R. necatrix is up-regulated in response to virulent mycovirus infection.

Poster Session

VIR-PW2045 - Innate sensing of membrane perturbation during enveloped viral entry

David Hare[1], Susan Collins[1], Karen Mossman[1,2]
[1]Department of Pathology and Molecular Medecine, McMaster University, Hamilton, Canada, [2]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada

Innate sensing of virus is considered to occur by detection of pathogen-associated molecular patterns such as double-stranded RNA. This paradigm is changing with the observation that danger signals such as membrane perturbation during enveloped virus entry can directly induce antiviral IFN-stimulated genes (ISGs), in the absence of IFN production. This sensing is independent of known TLRs and RLRs, although it remains to be discovered how cells might sense a physical danger signal such as membrane fusion. We've recently discovered that Ca2+ and reactive oxygen species (ROS) play signaling roles and that purified fusogenic p14-FAST proteoliposomes are capable of direct induction of ISGs. The source and location of Ca2+ and ROS induced by viral particle entry remain to be determined. Moreover, preliminary data suggest that not all membrane perturbation events are sufficient to induce ISGs. We speculate that the culmination of multiple "danger signals" is necessary to fully signal an antiviral response.

Poster Session

VIR-PW2047 - Mx protein and autophagy eliminate Betanodavirus RNA-dependent RNA polymerase

Yu-Chi Wu[1], Shau-Chi Chi[1]
[1]*National Taiwan University, Taipei, Taiwan*

Nervous necrosis virus (NNV) belongs to Betanodavirus genus of Nodaviridae family and affects marine and freshwater fish, resulting in great loss in the aquaculture industry. NNV is a non-enveloped RNA virus, containing two-segmented, single-stranded, positive-sense genomic RNAs without poly A tails. The target organs of NNV are the brain and eye. In our previous study, a barramundi brain (cBB) cell line was established, and NNV-infected cBB cells were induced the expression of barramundi Mx (BMx) protein, one of the interferon-inducible antiviral proteins. The expression level of NNV RNA-dependent RNA polymerase (RdRp) peaked at 24 hpi, and then decreased from 48 to 72 hpi. When BMx protein expression was inhibited by siRNA, the NNV RdRp expression level increased. BMx protein was found to interact with NNV RdRp, and it colocalized with NNV RdRp at the perinuclear area. Subsequently, NNV RdRp colocalized with lysosomes at 48 hpi when the NNV RdRp began to degrade. Mx protein is not permanent protein in the cells. Therefore, we speculated that Mx protein would be degraded finally, and the viral proteins bound with Mx protein would be also eliminated together. To prove the hypothesis, the expression and degradation of BMx protein were analyzed in the poly I:C-transfected cBB cells. The expression level of BMx protein increased within 3 day post-transfection and then decreased. The degradation of BMx protein could be suppressed when the autophagy and the acidification of lysosomes were respectively inhibited by 3MA or ammonium chloride. Similarly, the degradation of NNV RdRp in the NNV-infected cBB cells could be retarded by treatment of 3MA or ammonium chloride. These results revealed that BMx protein would interact with NNV RdRp, and then the complexes of BMx/NNV RdRp would be eliminated by autophagy.

Poster Session

VIR-PW2049 - Inhibition of virus-induced Interferon beta gene expression by mutants of NS5A and their effect on HCV replication

Giridhar Akkaraju[1], James Gleaton[1], Coralee Toal[1], Wenjing Wu[1]
[1]Texas Christian University, Fort Worth, USA

Hepatitis C Virus (HCV) is a liver pathogen that establishes a long-term infection in the humans leading to chronic hepatitis, cirrhosis and hepatocellular carcinoma. As with many chronic viruses, HCV has evolved mechanisms to escape immune detection by the host. One HCV protein, NS3/4A, has been shown to inhibit signaling pathways leading to the interferon beta (IFN beta)-promoter mediated expression of type I interferons (IFNs). Normally, infection by RNA viruses induces the synthesis and secretion of interferon α/β (IFN α/β), which in turn leads to the establishment of an antiviral state in the within the cell and in the surrounding cellular milieu. Activation of IFNβ gene expression requires the activation of three transcription factors (ATF-2, IRF3/7 and NF-kappa B) and the formation of an enhanceosome on the IFN beta promoter. The HCV NS3/4A, a protease required for the maturation of the virus polyprotein, has been shown to interfere with IFN beta promoter activation by cleaving and detaching MAVS- a mitochondrial membrane-bound signal transduction protein, from the mitochondria. We have previously shown that HCV protein NS5A, also interferes with virus infection-induced activation of the IFN beta gene promoter independent of NS3/4A. However, the mechanism by which NS5A inhibits IFN beta gene activation is not yet understood. In this study we analyze the effect of NS5A expression on activation and translocation of the transcription factors required for IFN beta gene expression. Additionally we analyze the role of two mutants of NS5A on the replication of a cell culture adapted HCV particle. Quasipsecies of HCV containing mutations in NS5A that confer the ability to inhibit IFN beta induction, may be a factor contributing to the persistence of HCV in the host.

Poster Session

VIR-PW2051 - SUMOylation related motifs from rhesus macaque TRIM5α are important for the activation of innate immunity pathways

Marie-Edith Nepveu-Traversy[1], Ann Demogines[2], Sara Sawyer[2], Lionel Berthoux[1]
[1]Université du Québec à Trois-Rivières, Trois-Rivières, Canada, [2]University of Texas at Austin, Austin, USA

Specific recognition of incoming viral capsids by the antiretroviral factor TRIM5α disrupts progression of the virus life cycle and also triggers the induction of an antiviral state involving the activation of transcription factors NF-κB and AP-1. Additionally, TRIM5α promotes the formation of K63-linked ubiquitin chains, thought to be important for innate immune activation, through the E3 ubiquitin ligase activity of a RING motif found at the N-terminus of TRIM5α. Other TRIM5α motifs were predicted to be relevant to the sumoylation pathway, specifically a putatively sumoylated lysine (K10) just upstream of the RING domain, and putative SUMO interaction motifs (SIMs) in the C-terminal PRYSPRY domain. Here, we analyzed the role of lysine 10 and the SIMs in the activation of innate immunity by TRIM5α. Mutating lysine 10 significantly decreased activation of NF-κB and AP-1, and reduced generation of K63-linked ubiquitin. We identified a novel putative SIM (435VIIC438) in the rhesus macaque TRIM5α PRYSPRY, which we named SIM4. Mutating SIM4 abrogated activation of NF-κB and AP-1, while having no effect on the capacity of TRIM5α to trigger the formation of K63-linked ubiquitin chains. We have analyzed the SIM4 motif from 36 primate species and found significant sequence variation. We also found this motif to be polymorphic in two macaque species. Substituting Rhesus macaque SIM4 with these naturally occurring variants did not suppress the capacity of TRIM5α to activate NF-κB and AP-1. Finally, mutating K10 and SIM4 reduced the co-localization of TRIM5α and SUMO-1 in nuclei. In conclusion, lysine 10 modulates the ubiquitin ligase activity of the nearby RING domain to promote K63-linked ubiquitination, while SIM4 is important for the activation of NF-κB and AP-1, but does not modulate K63-linked ubiquitin formation. Thus, we have identified TRIM5α motifs linked to SUMOylation that differentially modulate the activation of innate immunity.

Poster Session

VIR-PW2053 - Rotavirus strain-specific modulation of cytokine responses in intestinal epithelial cells

Izabel Martini Di Fiore[1], Jessica Pane[1], Gavan Holloway[1], Barbara Coulson[1]
[1]Department of Microbiology and Immunology, The University of Melbourne, Melbourne, Australia

Rotavirus, the major etiologic agent of life-threatening infant gastroenteritis, primarily infects intestinal epithelial cells. In response to rotavirus infection, these cells can induce the production of pro-inflammatory and antiviral cytokines, each cytokine relying on activation of a specific set of transcription factors. The viral non-structural protein 1 (NSP1) subverts these innate immune responses in a strain-specific manner, inhibiting activation of either the nuclear factor kappa B (NF-κB) or the interferon regulatory factor-3 (IRF3) transcription factors. In the present study we have investigated diverse rotavirus strains, including RRV, CRW-8, SA11-4F and SA115S, for their ability to interfere with IRF3 or NF-κB and differentially modulate cytokine expression in the HT-29 intestinal cell line. Western blotting analysis showed effective IRF3 degradation following infection with RRV or SA11-4F. However both viruses lacked the ability to stabilize the inhibitor of NF-κB protein, IκB, resulting in NF-κB-signaling pathway activation. In contrast, CRW-8 efficiently stabilized IκB, blocking NF-κB activation, but was unable to degrade IRF3. Infection with SA11-5S, a rotavirus that encodes a defective NSP1, interfered with neither transcription factor. Similar levels of the NF-κB-dependent cytokine IL-8 were detected following infection with RRV, SA11-4F or SA11-5S. However, RRV and SA11-4S induced low levels of IRF3-dependent cytokines (IFN-β and RANTES), in comparison to the levels stimulated by SA11-5S. Conversely, infection with CRW-8 resulted in diminished levels of IL-8, but similar quantities of IRF3-dependent cytokines, compared to SA11-5S infected cells. Therefore, the interference of each rotavirus strain with either IRF3 or NF-κB was accompanied by reduced expression of a specific group of cytokines. This suggests that cytokine modulation during infection of intestinal cells is driven by viral NSP1. Moreover, this effect possibly plays a role in delaying both innate and adaptative immune responses against rotavirus in a strain-specific manner.

Poster Session

VIR-PW2055 - Fecal shedding of vaccine-derived Rotavirus strains after vaccination with RotaTeq® and Rotarix™ in South Korean infants

Sunyoung Jeong[1], Van Thai Than[1], Inseok Lim[2], Wonyong Kim[1]
[1]Department of Microbiology, Chung-Ang University College of Medicine, Seoul, South Korea., [2]Department of Pediatrics, Chung-Ang University College of Medicine, Seoul, South Korea

In 2007and 2008, 2 rotavirus vaccines (RotaTeq® [RV5] and Rotarix™ [RV1]) were introduced in South Korea. Genotyping of human rotaviruses were identified by routine ELISA, followed by RT-PCR and genome sequencing. Out of the 1,421 samples with acute gastroenteritis, rotaviruses were successfully genotyped in 386 samples (27.2%). Of these, 346 isolates (89.6%) were identified as wild-type rotavirus G and P genotypes. The most prevalent G and P types were G1P[8] (31.5%), followed by G3P[8] (23.3%), G4P[6] (10.9%), G2P[4] (10.3%), and G9P[8] (6.5%). Interestingly, a rare G11P[25] strain was detected for the first time in South Korea. Vaccine-derived rotavirus strains were identified in 40 samples (10.4%) of which 39 samples contained G and/or P genotypes from the RotaTeq® and one sample from Rotarix™ vaccine. Rotavirus-related acute gastroenteritis in Korean children was significantly reduced after the introduction of vaccines. However, fecal shedding of vaccine strains in infants following the vaccination is increased. These data are the first to feature an authentic distribution of two rotavirus vaccine-derived strains in populations. Tracing the clinical and environmental routes of the rotavirus vaccine strains revealed unexpected complexity, surveillance study should be supported by genome sequencing for at least the G1, G2, G3, G4, and P[8] genotypes that exclude the current rotavirus vaccines.

Poster Session

VIR-PW2057 - Amino acid substitutions in σ1 and μ1 proteins of a Vero-cell-adapted reovirus are required for optimal virus binding and disassembly

Véronique Sandekian[1], Guy Lemay[1]
[1]*Université de Montréal, Montréal, Canada*

Mammalian orthoreovirus (reovirus) is a natural oncolytic virus that is currently tested in phase III clinical studies. However, there is still a significant research effort to obtain novel virus variants better adapted to kill cancer cells while sparing non-transformed cells. As previously reported, viral persistence could be useful to select viruses further adapted as oncolytic agents (Kim et al., 2011). L929 cells are typically chosen for establishment of viral persistence in cell culture. This has led to the selection of reovirus mutants, although very few of those have been characterized in details. In the present study, reovirus was adapted to Vero cells which are inefficient in reovirus uncoating and are also unable to produce interferon as an antiviral response, unlike the L929 cell line. The Vero cell-adapted reovirus (VeroAV) exhibits amino acids substitutions in σ1 and μ1 proteins. This contrasts with uncoating mutants from persistently infected L929 cells, and various other cell types, that harbor amino acid substitutions in the σ3 protein. VeroAV remained sensitive to an inhibitor of lysosomal proteases; furthermore, in the absence of selective pressure for its maintenance, the virus has partially lost its ability to resist interferon. An increased ability of the virus to bind at the Vero cell surface was observed and is likely associated with an increased affinity toward cell-surface sialic acid residues. In addition, the kinetics of μ1 disassembly from the virions appears to be altered. The plasmid-based reverse genetics approach confirmed the importance of σ1 amino acids substitutions in VeroAV's ability to efficiently infect Vero cells, although an adequate sequence context of μ1 is required to fully express the phenotype. This approach of combining in vitro selection of reoviruses with reverse genetics to identify pertinent amino acid substitutions appears promising in the context of eventual reovirus modification to increase its oncolytic potential.

Poster Session

VIR-PW2059 - Molecular epidemiology and genetic evolution of the whole genome of G3P[8] human rotavirus in Wuhan, China, from 2000 through 2013

Yuanhong Wang[1], Beibei Pang[1], Souvik Ghosh[2], Xuan Zhou[1], Manqing Liu[1], Weifeng Tang[1], Jinsong Peng[1], Quan Hu[1], Dunjin Zhou[1], Nobumichi Kobayashi[2]

[1]Wuhan Centers for Disease Prevention and Control, Wuhan, China, [2]Sapporo Medical University School of Medicine, Sapporo, Japan

Background: Rotaviruses are a major etiologic agent of gastroenteritis in infants and young children worldwide. Since the latter of the 1990s, G3 human rotaviruses referred to as “new variant G3” have emerged and spread in China, being a dominant genotype until 2010, although their genomic evolution has not yet been well investigated. Methods: The complete genomes of 33 G3P[8] human rotavirus strains with identical or different RNA patterns in polyacrylamide gel detected in Wuhan, China, from 2000 through 2013 were analyzed. Phylogenetic trees of concatenated sequences of all the RNA segments and individual genes were constructed together with published rotavirus sequences. Results: Genotypes of 11 gene segments of all the 33 strains were assigned to G3-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1, belonging to Wa genogroup. Phylogenetic analysis of the concatenated full genome sequences indicated that all the modern G3P[8] strains were assigned to Cluster 2 containing only one clade of G3P[8] strains in the US detected in the 1970s, which was distinct from Cluster 1 comprising most of old G3P[8] strains. While main lineages of all the 11 gene segments persisted during the study period, different lineages appeared occasionally in RNA segments encoding VP1, VP4, VP6, and NSP1-NSP5, exhibiting various allele constellations. In contrast, only a single lineage was detected for VP7, VP2, and VP3 genes. Remarkable lineage shift was observed for NSP1 gene; lineage A1-2 emerged in 2007 and became dominant in 2008-2009 epidemic season, while lineage A1-1 persisted throughout the study period. Conclusion: Chinese G3P[8] rotavirus strains have evolved since 2000 by intra-genogroup reassortment with co-circulating strains, accumulating more reassorted genes over the years. This is the first large-scale whole genome-based study to assess the long-term evolution of common human rotaviruses (G3P[8]) in an Asian country.

Poster Session

VIR-PW2061 - Whole genomic analysis of G3P[6], G4P[6] and G4P[8] Rotavirus strains in Wuhan, China

Nobumichi Kobayashi[1], Xuan Zhou[2], Yuan-Hong Wang[2], Souvik Ghosh[1], Man-Qing Liu[2], Bei-Bei Pang[2], Wei- Feng Tang[2], Jin-Song Peng[2], Quan Hu[2], Dun-Jin Zhou[2]

[1]Sapporo Medical University, Sapporo, Japan, [2]Wuhan Centers for Disease Prevention and Control, Wuhan, China

Rotavirus A is a major etiological agent of diarrheal disease in human and animals. We previously reported a G4P[6] human rotavirus (HRV) strain which was considered to be transmitted from pig. In the present study, whole genome sequences of four HRV strains with G4 and/or P[6] detected In Wuhan, China, from sporadic patients (children) with diarrhea (RVA/Human-wt8/CHN/E931/2008/G4P[6], RVA/Human-wt/CHN/R1954/2013/G4P[6], RVA/Human-wt/CHN/R946/2006/G3P[6], andRVA/Human-wt/CHN/E2484/2011/G4P[8]) were determined and analyzed phylogenetically to estimate the origin of individual RNA segments. Genotypes of all the gene segments for both strains E931 and R1954 were assigned to G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1, while strains R946 and E2484 to G3-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1 and G4-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1, respectively. Phylogenetic analysis revealed that all genes of E2484 except for VP7 gene, VP7 gene of R946, and VP6 genes of all the four strains belong to clusters of HRVs. In contrast, VP3 gene of R1954, and VP2 genes of E931, R946 and R1954 clustered with those of porcine rotaviruses and porcine-like HRVs. Similarly, VP7 genes (E931, R1954 and E2484), VP1, VP4, and NSP1 genes (E931, R946 and R1954), NSP2 gene (E931), NSP3 genes (R946 and R1954), and NSP4 gene (E931, R1954) were closely related to porcine rotaviruses and HRVs which were believed to be derived from porcine rotaviruses. These findings indicated that all the 4 HRV strains are human-porcine reassortants having porcine rotavirus-VP7 gene in the backbone of typical Wa-like HRV (E2484), or VP6 gene and some other genes from HRV in the genetic background of porcine rotavirus (E931, R946 and R1954). The G4P[6] HRV strains are suggested to be originated from porcine rotaviruses which were transmitted to human, and subsequently emerged via reassortment with HRV, acquiring increased ability of adaptation to humans. Further epidemiological study may be necessary to monitor prevalence of these human-animal reassortant rotaviruses.

Poster Session

VIR-PW2063 - Rotavirus molecular epidemiology in a country with low rotavirus vaccination coverage: the case of Slovenia

Mateja Poljsak-Prijatelj[1], Andrej Steyer[1], Marko Kolenc[1], Martin Sagadin[1]
[1]*Institute of Microbiology,Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

In Slovenia Rotavirus vaccines Rotarix (GlaxoSmithKline Biologicals) and RotaTeq (Merck Sharp&Dohme) became available in 2007. Vaccination costs against rotavirus are not yet reimbursed, which is probably the reason for the low vaccination rate till 2012 (up to 27%) and there is no increasing trend observed. In this study we present rotavirus genotype distribution among children hospitalized for rotavirus gastroenteritis in Slovenia. During the vaccine introduction period, from 2007-2013, rotavirus genotype pattern was changed, with G1P[8] prevalence decreasing from 74.1% to 10% between 2007/08 and 2010/11 seasons, replaced by G4P[8] and G2P[4] with up to 52.0% prevalence. Lately, also G9P[8] appeared to be more prevalent, found in 11.0% of samples. In addition, detailed molecular analysis at the nucleotide and amino acid level was done for VP7 and VP4 segments, encoding proteins with the main neutralizing epitopes. Comparable analysis of VP7 within G1, G2, G4 and G9 genotypes revealed significant differences for rotavirus strains circulating before and during the vaccination period. The most evident changes were detected for G1P[8] rotavirus strains in both, VP7 and VP4 proteins. The G1P[8] rotavirus strains which circulated before vaccination period were clustered in phylogenetic tree within the Rotarix-like VP7 and VP4 lineages with 97.5-97.9% and 97.5% of VP7 and VP8* nucleotide sequence identity, respectively. However, from 2007 on, majority of G1P[8] strains evolve into distant genetic lineage with lower nucleotide (88.1-94.0% for VP7 and 86.6-91.1% for VP8*) and amino acid (93.8-95.2% for VP7 and 85.3-94.6% for VP8*) similarities to the vaccine Rotarix strain. This change resulted also in different deduced amino acid profile at the major VP7 and VP8* antigenic epitopes. Further studies are needed to confirm whether this changes resulted in neutralization characteristics with antibodies elicited with rotavirus vaccination.

Poster Session

VIR-PW2065 - Human herpesvirus 8 variants circulating in Argentina in patients with associated diseases and organ donor

Celeste Perez[1], Daniela Feliciotti[1], Tous Monica[1]
[1]*INEI-ANLIS, Buenos Aires, Argentina*

Genetic variants as well as infection rates of human herpesvirus 8 (HHV-8) are diversely distributed in different geographical regions. Serological prevalence rates for HHV-8 infection in Argentina vary from 1.9% to 10.8% in different areas. In order to asses HHV-8 genotypic variants in Argentina, DNA samples from biopsies of 23 patients with HHV-8 associated diseases: 21 Kaposi s sarcoma (KS) (10 epidemic, 6 classic and 5 iatrogenic KS), one primary effusion lymphoma (PEL), one multicentric Castleman disease (CD) and blood from one organ donor were studied. Presence of HHV-8 was assesed by amplification of the ORF 26 fragment, then the ORF K1 region including the hypervariable fragments VR1 and VR2 was amplified, sequenced and used for phylogenetic analysis. Genotyping of the ORF K1 region showed that 18 samples belonged to C subtype - C1, C2 and C3- (6 classic SK , 5 iatrogenic SK , 6/10 epidemic SK and 1 CD), 3 were A subtype – A1 and A3 - (2/10 epidemic SK, 1 PEL), meanwhile 3 were B1 subtype (2/10 epidemic SK and the organ donor). Our results reveal a predominance of C subtype in all HHV-8 associated diseases as well as the circulation of A and B subtypes in Argentina.

Poster Session

VIR-PW2067 - WITHDRAWN: The temporal and spatial evolution of Cauliflower mosaic virus

Ryosuke Yasaka[1], Huy D. Nguyen[1,2], Simon Y. W. Ho[3], Sebastián Duchêne[3], Savas Korkmaz[4], Nikolaos Katis[5], Hideki Takahashi[6], Adrian J. Gibbs[7], Kazusato Ohshima[1,2]

[1]Laboratory of Plant Virology, Saga University, Saga, Japan, [2]Kagoshima University, Kagoshima, Japan, [3]School of Biological Sciences, University of Sydney, Sydney, Australia, [4]Department of Plant Protection, University of Canakkale Onsekiz Mart, [5]Plant Pathology Laboratory, Aristotle University of Thessaloniki, Thessaloniki, Greece, [6]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan, [7]Emeritus Faculty, Australian National University, Canberra, Australia

Studies of the population genetics of plant viruses are important for understanding the evolution of virus-host interactions, because plant viruses sometimes adapt rapidly to new or resistant hosts. Most evolutionary studies of plant viruses have focused on those with single-stranded RNA (ssRNA) genomes, partly because many plant viruses have such genomes. Cauliflower mosaic virus (CaMV) is a plant pararetrovirus with a double-stranded DNA genome. It is the type member of the genus Caulimovirus in the family Caulimoviridae. CaMV is transmitted by sap inoculation and in nature by aphids in a semi-persistent manner. To investigate the patterns and timescale of CaMV migration and evolution, we sequenced and analyzed the genomes of 67 isolates of CaMV collected mostly in Greece, Iran, Turkey, and Japan together with nine published sequences. We identified the open-reading frames (ORFs) in the genomes and inferred their phylogeny. After removing recombinant sequences, we estimated the substitution rates, divergence times, and phylogeographic patterns of the virus populations. We found that recombination has been a common feature of CaMV evolution, and that ORFs I-V have a different evolutionary history from ORF VI. The ORFs have evolved at rates between 1.71 and 5.81 x 10-4 substitutions/site/year, similar to those of viruses with RNA or ssDNA genomes. We found four geographically confined lineages. CaMV probably spread from a single population to other parts of the world around 400-500 years ago, and is now widely distributed among Eurasian countries. Our results revealed evidence of frequent gene flow between populations in Turkey and those of its neighboring countries, with similar patterns observed for Japan and the USA.

Poster Session

VIR-PW2069 - Phylodynamics and molecular evolution of human bocavirus in Taiwan

Hsin-Fu Liu[1,2], Yung-Cheng Lin[2], Jih-Hui Lin[3], Yu-Chen Lin[2], Hsiu-Chuan Chan[2]
[1]Dept. of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, [2]Institute of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan, [3]Center for Research and Diagnostics, Centers for Disease Control, Taipei, Taiwan

Human bocavirus (HBoV) is a non-enveloped single-stranded DNA virus belonging to the Parvoviridae family. So far, there are 4 HBoV genotypes been identified and are clinically associated with respiratory tract infections and gastroenteritis. We have previous reported HBoV-1 in children with lower respiratory tract infection. In this study, we focused on their phylodynamics and compared the molecular evolution of HBoVs. Phylogeny was reconstructed by the neighbor-joining, maximum likelihood, and Bayesian inference based on the whole HBoV genomes. The substitution model parameters, evolutionary rates, time to the most recent common ancestor (tMRCA), and population dynamics were estimated using the Bayesian Markov Chain Monte Carlo (MCMC) approach implemented in the BEAST package. The MCMC chains were run for sufficient time to achieve convergence (ESS>200). Site-specific selection pressure was also investigated. Phylogenetic trees showed that isolates from Taiwan were all classified with previously published HBoV-1 reference in NCBI database. The substitution rate of the 2nd codon was slightly higher than that of synonymous codon. The estimated mean evolutionary rate of HBoV was 6.13×10-4 substitutions/site/year. The tMRCA of HBoV was determined at 415.26 years and the divergent time of HBoV1-4 genotypes was 22.6, 63.22, 42.13 and 55.78 years respectively. The phylodynamic pattern of HBoV-1 was steady before 1995 and slightly increased until 2007. In Conclusion, genetic diversity of HBoV-1 strains from Taiwan is lower than other isolates from the other countries. However, since evolutionary characteristics of HBoVs are rapid and comparable to that of RNA viruses, keep monitoring of this human pathogen is important for the control of bocavirus infection.

Poster Session

VIR-PW2071 - Characterization of ORF 94 from isolates of White Spot Syndrome Virus obtained in the state of Nayarit, Mexico during the period 2010-2012

Norberto Vibanco-Perez[1], Ma de Jesus Duran-Avelar[1], Jose Francisco Zambrano-Zaragoza[1], Ricardo Juarez-Vazquez[2], Jaime Sanchez-Meza[1], Ricardo Perez-Enriquez[2]

[1]UA Ciencias Quimico Biologicas y Farmaceuticas Universidad Autonoma de Nayarit Tepic, Nayarit Mexico, [2]Centro de Investigaciones Biologicas del Noroeste, La Paz, Baja California Sur, Mexico

White spot syndrome virus (WSSV) is a pathogen that has emerged globally affecting shrimp populations. The complete genomes sequence of three isolates tandem repeats (VNTRs) associated with the three open reading frames ORF94, ORF75 and ORF125, have been proposed as potential markers for epidemiological studies of WSSV. ORF94 has repeat units (RUs) of 54 bp with a single nucleotide polymorphism (SNP) at position 48 (T or G). In this work we analyzed isolates of WSSV from Litopenaeus vannamei for the period 2010-2012 in three shrimp producing areas of the State of Nayarit, Mexico. South zone (Municipality of San Blas) Center zone (Municipalities of Tuxpan and Santiago) and north zone (Municipalities of Acaponeta and Tecuala).The presence of WSSV in shrimp of 16, 15 and 18 farms was confirmed in the years 2010, 2011 and 2012 respectively. By PCR using specific primers, the region ORF 94 of WSSV was amplified, sequenced and determined the number of repeat units (RUs) of 54 bp, characteristics of this region of the viral genome , Also was determined the polymorphism at position 48 of each UR , thereby obtaining a characteristic pattern in which genotyping was based . Eight genotypes of WSSV was found in 2010, eleven in 2011 and six in 2012, of which only one was found in 2010 and in 2011 , none of the rest was the same in the three years studied . This suggests a dynamic of infection from different backgrounds and / or a very high mutation rate of resident strains.

Poster Session

VIR-PW2073 - A protein phosphatase 1-binding motif is critical for the anti-apoptotic function of the Rubella virus capsid protein

Steven Willows[1], Tamara Arnold[1], Charles Holmes[1], Tom Hobman[1]
[1]University of Alberta, Edmonton, Canada

Rubella virus (RV) is a single stranded, positive-sense RNA virus in the family Togaviridae. RV is highly teratogenic when contracted during the early stages of pregnancy and causes a range of birth defects collectively known as congenital rubella syndrome. In addition to its function in nucleocapsid assembly, the capsid protein plays a number of non-structural roles including modulation of viral RNA synthesis, inhibition of mitochondrial import and blocking apoptosis. Recently, we identified an interaction between RV capsid and protein phosphatase 1 (PP1), a highly conserved serine-threonine phosphatase with many cellular functions. Formation of capsid-PP1 complexes was demonstrated by two independent methods: coimmunoprecipitation and microcystin-sepharose pulldown. A putative PP1 binding site (RIRF) in the C-terminus of capsid was changed to RARA by site-directed mutagenesis and the properties of the mutant capsid were investigated. First, we noted that the RARA mutant exhibited altered localization compared to wildtype capsid. Normally, capsid associates with the endoplasmic reticulum and a small but significant pool binds to the outer mitochondrial membrane. However, the RARA mutant localized almost exclusively to the mitochondrial membranes. Mitochondrial clustering, a hallmark of RV infection, was highly exacerbated in cells expressing this mutant. Moreover, unlike wildtype capsid, RARA did not protect against apoptotic stimuli. These data may indicate that interaction with PP1 is critical for the ability of capsid to block programmed cell death during infection. Ongoing studies are focused on determining how the interaction of capsid with PP1 affects apoptotic. Funded by Canadian Institutes of Health Research, Alberta Innovates Health Solutions, and Canada Research Chairs program.

Poster Session

VIR-PW2075 - Challenges in probing cellular factors for chikungunya virus replication

Atsushi Tanaka[1,3], Uranan Tumkosit[1], Sumalee Chanama[2], Atchareeya A-nuegoonpipat[2], Pornsiri Somasa[2], Naokazu Takeda[1,3], Areerat Sa-ngasang[2]

[1]Thailand-Japan Research Collaboration Center, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, [2]Arbovirus section, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, [3]Reaserch Institute for Microbial Diseases, Osaka University, Osaka, Japan

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes fever, rash, myalgia, and arthralgia. Molecular mechanisms of CHIKV infection and replication in human cells have been poorly characterized. In this study, we challenged to determine the cellular factors that exert negative impact on viral replication. A cDNA library (U251MG/cDNA library) derived from CHIKV-susceptible human astrocytoma cell line, U251MG, was established using a retrovirus vector. Each 2x10^6~ of U251MG and U251MG cells further transduced with U251MG/cDNA library were inoculated with CHIKV at moi 0.05-0.1. Cell colonies survived at 5 weeks after the inoculation were picked and propagated. Viability of the surviving cell clones after CHIKV infection were investigated by WST-1 assay detecting the decreased cell proliferation, and susceptibility of the clones to CHIKV was determined by the same assay as well as an infectivity assay with a retrovirus vector expressing EGFP pseudotyped with CHIKV envelop proteins (pMX-EGFP[CHIKV E3-E1]). Ten clones were established from U251MG cells further transduced with the cDNA library but not parental U251MG cells. Eight clones were 2 to 11 times resistant to CHIKV infection when compared with parental U251MG cell line, indicating that the transduction of retrovirus vector into U251MG chromosomal DNA was responsible for the resistance. Some of resistant clones showed decreased susceptibility against both wild type CHIKV and pMX-EGFP[CHIKV E3-E1]. As these clones have only retrovirus vector but not inserted DNAs, chromosomal DNA encoding certain function in CHIKV replication might be impaired by integration of the vector.

Poster Session

VIR-PW2077 - Specific epitopes and critical residues of classical swine fever virus E2 glycoprotein that contribute to antigen variations between the vaccine C-strain and group 2 isolates

Chao Tong[1], Ning Chen[1], Xun Liao[1], Wenqi Xie[1], Yan Zhang[1], Xiaoliang Li[1], Weihuan Fang[1]
[1]Institute of Preventive Veterinary Medicine, Zhejiang University, Hangzhou 310058, China

Glycoprotein E2 is an important envelope protein of classical swine fever virus (CSFV) and can induce neutralizing antibodies and confer protective immunity in pigs. The E2 genes of CSFV C-strain and recent subgroup 2.1 field isolates are genetically divergent. To investigate the antigenic variations between C-strain and group 2 isolates, we raised pig antisera against the C-strain and a subgroup 2.1 strain QZ-07 as well as monoclonal antibodies (mAb) against E2 of different strains. A two-way neutralization analysis using the pig anti-CSFV sera revealed that heterologous neutralization was less effective, especially at the early days post-vaccination or -infection. Site-directed mutagenesis was performed to systematically substitute amino acids in C-strain E2 protein with those at the same positions in subgroup 2.1 proteins. We found that substitutions D705N, L709P, G713E, N723S and S779A caused significant increase in binding efficiency, while moderate increase was observed with D725G, N729D, N777S, T780I, D847E, M854V, T860I and N863K substitution. Using the same method, we identified two linear epitopes 707IXPXGXGG714 and 774DGXNP779 recognized by mAbs 2B10 and 2B6, respectively. These two linear epitopes are not conserved between group 1 and group 2 strains. The mAbs 1E7 and 6B8 against group 1 C-strain and 4F4 and 4G6 targeted to group 2 strain QZ-07 recognized conformational epitopes and bound to both group 1 and 2 viruses. However, they were less efficient in neutralizing heterologous viruses. The residues G711 and G713, located in the epitope recognized by mAb 2B10, were also critical for conformational mAbs 1E7 and 6B8 binding. These results demonstrate that CSFV vaccine C-strain and group 2 strains circulating in China differ in the antigenicity of E2 glycoproteins. These findings might be useful for the development of serological differential assays and improvement of immunogenicity of novel CSFV vaccines.

Poster Session

VIR-PW2079 - Conformational Modifications of gB from Herpes Simplex Virus Type 1 Analyzed by Synthetic Peptides

Massimiliano Galdiero[1,3], Marco Cantisani[2,3], Annarita Falanga[2], Mariateresa Vitiello[1], Novella Incoronato[1], Luigi Russo[1], Alfonso De Simone[4], Rita Berisio[5], Stefania Galdiero[2,3,5]

[1]Department of Experimental Medicine, II University of Naples, Naples, Italy, [2]Department of Pharmacy, University of Naples Federico II, Naples, Italy, [3]Centro Interuniversitario di Ricerca sui Peptidi Bioattivi, University of Naples Federico II, Naples, Italy, [4]Division of Molecular Biosciences, Imperial College, London, UK, [5]Istituto di Biostrutture e Bioimmagini, CNR, Napoli, Italy

Entry of enveloped viruses requires fusion of viral and cellular membranes. This process is generally driven by conformational changes of viral glycoproteins. The crystallized trimeric form of glycoprotein B of Herpes simplex virus has been described as a post fusion conformation and several hypothesis suggest that gB undergoes switch between the pre and post fusion conformation. The essential role of the coiled coil region in the assembly of gB post-fusion state suggests that the central helix may be an interesting target for the design of inhibitors of viral fusion and may serve as a key factor for understanding HSV membrane fusion mechanism. We focused on the long helix spanning residues 500-544 of the gB ectodomain and tested a set of peptides of different length derived from this sequence in order to determine the minimal sequence able to elicit inhibition of viral infectivity. By means of several biophysical techniques, including fluorescence and circular dichroism spectroscopies, surface plasmon resonance, and molecular dynamic simulations, we provide evidence of a possible different pre fusion conformation. In fact, during a conformational rearrangement, some epitopes are lost or formed, which supports the hypothesis for the search of a minimal peptidic sequence able to interact with the virus in its prefusogenic conformation. Those peptides represent good candidates for further design of peptidomimetic antagonists capable of blocking the fusion process.

Poster Session

VIR-PW2081 - Antiviral and virucidal activities of polyphenols extracted from Japanese apricot

Kazuko Tsujimoto[1], Sakiko Tsukishiro[1], Mitsunori Nishide[1], Minoru Yoshida[1], Keiko Ikeda[2], Yukiko Suzuki[2], Takahiko Mitani[3], A. Hajime Koyama[1]

[1]Wakayama Shin-Ai Women's Junior College, Wakayama, Japan, [2]Sch. Health & Nursing, Wakayama Medical Univ., Wakayama, Japan, [3]Wakayama Industry Promotion Foundation, Wakayama, Japan

Previously we have characterized antiviral and virucidal activities of our daily dietary products and observed that many common vegetables and fruits show these activities. Recently, we obtained polyphenols in the salt-extracts of Japanese apricot (Nanko-mume; Prunus mume Sieb et Zucc, a special product of Wakayama in Japan) and found antibacterial activities in the extracts. We have here further examined antiviral activities of these polyphenols against viruses with different virion structures and replication strategies; i.e., herpes simplex virus type 1 (HSV-1, an enveloped DNA virus), influenza A virus (IAV, an enveloped RNA virus) and poliovirus type 1 and feline calicivirus (PV-1 and FCV, a non-enveloped RNA virus). These viruses were propagated in Vero (for HSV and PV), MDCK (for IAV) or CRFK (for FCV) cells and the amounts of infectious viruses were determined by a plaque assay. The polyphenols inhibited the multiplication of all tested viruses, when added to the culture media of the infected cells. The inhibition depended on their concentrations. IAV and FCV were the most sensitive and PV-1 was the least; at 4 mg/ml polyphenols, the yield of FCV decreased, with mild cytotoxicity, to 0.04 relative to the value in the absence of the polyphenols. No correlation was observed between the viral sensitivities and the viral replication strategies. In addition to these antiviral activities, the polyphenols showed a remarkable virucidal activity at acidic pH; the infectivity of enveloped viruses, but not non-enveloped viruses, was impaired by the incubation with the agent at the concentrations lower than those required for the antiviral activity; for example, the infectivity of IAV decreased to less than 0.0001 at 3 mg/ml. These results suggest a potential pharmacological use of the polyphenols as an antiviral drug for the superficial infection or medicine for the public health, such as gargles against IAV infection.

Poster Session

VIR-PW2000 - Molecular typing of human adenovirus isolates originating from Sweden

Györző Kajan[1], Niklas Arnberg[1]
[1]*Umeå University, Umeå, Sweden*

Adenoviruses can be used in medical applications such as in the treatment of cancer and infectious diseases, or for gene therapy purposes: vectoring is a very active and developing field in virology. To find potential vector candidates, new human adenovirus types, we typed Swedish human adenovirus isolates originating from Uppsala and Umeå using molecular methods. Three PCR systems were applied - targeting the genes of the viral DNA polymerase, the penton base and the hexon - to be able to detect recombinant adenoviruses as well, two of these three PCRs were designed by us. The resulting PCR products were sequenced. Introductory results show, that around 80% of the samples are typeable - the strain gave a positive result with all the three PCR systems used. After analysing only 10% of the samples, based only on the partial hexon gene sequences, no new human adenovirus types were found. More than two third of the strains cluster to the species Human adenovirus C. The work was carried out in the frame of the AD-VEC consortium which is financed by the European Union through the Seventh Framework Programme (grant agreement no.: 324325).

Poster Session

VIR-PW2002 - Bovine Adenovirus-3 protein pVIII modulates formation of functional 80S ribosome by interacting with eukaryotic translation initiation Factor-6

Amit Gaba[1, 2], Lisanework E Ayalew[1, 2], Suresh K Tikoo[1,2,3]
[1]Vaccine and Infectious Disease Organization-International Vaccine Centre(VIDO-InterVac), Saskatoon, Canada, [2]Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada, [3]Vaccinology and immunotherapeutics program, School of Public Health, University of Saskatchewan, Saskatoon, Canada

Viruses depend entirely on their host cell translation machinery for synthesis of their own proteins. To ensure that viral proteins are produced, viruses have developed various strategies to recruit host ribosomes to viral mRNAs. These strategies involve interaction of viral proteins with cellular proteins that regulate or are part of the host protein synthesis machinery. Identification and characterization of virus host interactions that regulate protein synthesis in virus infected cells will not only reveal key steps in viral life cycle but will also help to develop strategies to combat viral infection. Our initial analysis of yeast two hybrid assay indicated that protein pVIII of bovine adenovirus (BAdV) -3 interacts with cellular protein Eukaryotic translation initiation factor-6 (eIF6). We have further confirmed the interaction of pVIII of BAdV-3 with cellular protein eIF6 by using bimolecular fluorescence complementation (BIFC), co-immunoprecipitation assay and GST-pulldown assay. Eukaryotic initiation factor-6 (eIF6) is an essential cellular protein that is necessary for 60S ribosome biogenesis and assembly. eIF6 possesses a unique anti association activity because of its ability to bind to 60S subunit, which prevents interaction of 40S ribosomal subunit with 60S subunits. Further characterization of pVIII-eIF6 interaction suggested that C-terminus of pVIII (amino acids 147 to 174) of BAdV- 3 interacts with N-terminus of eIF6 (amino acids 44 to 97). Our initial results also suggest that interaction of pVIII with eIF6 may prevent release of eIF6 from 60S subunit thereby preventing joining of 40S and 60S subunits, which is required for the formation of mature 80S ribosome. We speculate that this impaired ribosome assembly may be responsible for inhibition of cellular mRNA translation observed late in adenovirus infected cells.

Poster Session

VIR-PW2004 - Quantitative detection of human Adenoviruses in two wastewater treatment plant effluents in Amathole district municipality, Eastern Cape Province, South Africa

Olayinka Osuolale[1], Anthony Okoh[1]
[1]*University of Fort Hare, Alice, South Africa*

Enteric viruses are important pathogens which can be found in contaminated waters and have previously been detected in environmental waters. Human adenoviruses were monitored because of their high prevalence and persistence in aquatic environments. In this study, we quantified adenoviruses in two wastewater plants final effluents by real-time PCR. Samples of final effluents were collected between September 2012 and August 2013. Adenoviruses were detected in 67% of wastewater effluent samples from Dimbaza treatment plant and 92% from Amalinda treatment plant. Concentrations of adenovirus in the effluent from both plants were 4.86×10^3 genome copies/liter and 7.83×10^4 genome copies/liter, respectively. This research demonstrates that wastewater effluents from the two treatment plants contain high levels of viruses and may not be suitable for any re-use purposes. The discharge of the effluent into surface water will also negatively impact the water elevating the level of the virus in the water. High concentrations of adenovirus in these effluents may be due to inefficient removal during wastewater treatment and to the high persistence of these viruses in the environment. With this, the presence of adenoviruses in effluent discharged to surface waters may represent a public health risk if treatment process and disinfection is not shown to be adequate. Quantitative detection of adenoviruses as indicators in the final effluent may provide a means of better understanding the risk associated with effluent in contaminated water.

Poster Session

VIR-PW2006 - Rapid generation of ORF2 and ORF11 deleted fowl adenovirus 9 (FAdV-9) as expression vector

Yanlong Pei[1], Bryan Griffin[1], Li Deng[1], Jondavid de Jong[1], Éva Nagy[1]
[1]Department of Pathobiology, University of Guelph, Guelph, Canada

Fowl adenoviruses (FAdVs) have the largest genomes of any fully sequenced adenovirus, and are widely considered as excellent platforms for vaccine development and gene therapy. As such, there is a strong need for stream-lined protocols/strategies for the generation of recombinant genomes. Current genome engineering strategies rely upon plasmid based homologous recombination in E. coli BJ5183. This process is time-consuming, involves multiple cloning steps, and low efficiency recombination. Here we describe a novel system for the rapid generation of recombinant fowl adenovirus genomes using the lambda RED recombinase system in E. coli DH10B. In this strategy, PCR based amplicons with 50 nt homologous arms, a unique Swa I site and chloramphenicol resistance cassette, are introduced into the FAdV-9 genome in a highly efficient and site-specific manner. To demonstrate the efficacy of this system we were able to generate FAdV-9ORF2, and FAdV-9 ORF11 marked and unmarked deletion FAdV-9 genomes, and re-introduce either ORF2 or 11, or an EGFP expression cassette via the unique Swa I sites, in approximately 3 weeks. All recombinant genomes were fully infectious in CH-SAH cells and EGFP gene was expressed in infected CH-SAH cells.

Poster Session

VIR-PW2008 - Assessment of novel acylguanidine-based small molecules as broad-spectrum viroporin inhibitors and antiviral agents

Ian Tietjen[1], Philip Mwimanzi[2], Scott C. Miller[1], Aniqa Shahid[2], Zabrina Brumme[2,3], Mark Brockman[2,3], David Fedida[1]
[1]Dept. of Anesthesiology, Pharmacology, and Therapeutics, UBC, Vancouver, Canada, [2]Faculty of Health Sciences, Simon Fraser University, Burnaby, Canada, [3]British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada

Viroporins are virus-encoded ion channels essential for replication, making them potentially attractive targets for new antiviral agents. Here, we describe a series of novel acylguanidine-based small molecules with broad-spectrum activity against viroporins. To first confirm viroporin function, we implemented a mammalian cell assay using a pH-sensitive fluorescent dye that localizes to intracellular vesicles. Consistent with known viroporin-regulated transport of protons and alkalinization of vesicles, transient expression of Hepatitis C virus genotype 1b p7 [HCV-1b p7] quenched fluorescence signal by 30.4% compared to mock-transfected cells ($p<0.05$). Treatment of cells with established viroporin inhibitors restored fluorescence with dose-dependence; for example, 1, 3, and 10µM rimantadine respectively increased fluorescence in HCV-1b p7-expressing cells by 42.0%, 85.4%, and 117.0% compared to untreated controls ($p<0.05$). We next used this assay to screen novel acylguanidine-based small molecules for anti-viroporin activity. Our lead compound, SM111, restored fluorescence in cells expressing viroporins from Influenza A, Hepatitis C, Bovine Viral Diarrhea Virus, and Dengue; for example, SM111 (10µM) increased fluorescence in HCV-1b p7-expressing cells by 62.4% compared to untreated controls ($p<0.05$). The HIV-1 accessory protein vpu has also been described to display viroporin activity. We assessed the anti-HIV activity of SM111 using a multi-cycle in vitro replication assay and observed that SM111 inhibited HIV strain NL4-3 replication by >75% at 100µM concentration with minimal cellular toxicity. Notably, SM111 also inhibited replication of recombinant HIV strains encoding patient-derived HIV polymerase sequences displaying major resistance mutations to nucleoside and non-nucleoside reverse transcriptase inhibitors, supporting a mechanism of action that is distinct from currently available antiretroviral agents. Taken together, we suggest that SM111 and related compounds are promising lead molecules for broad-spectrum antiviral drug development, including anti-HIV inhibitors with novel mechanisms of action.

Poster Session

VIR-PW2010 - *Pseudomonas* secondary metabolites can inhibit La Crosse virus infection and kill developing mosquito larvae

Justin Anderson[1], Nathaniel Frisch[1], James Walker[1], Andrew Cheeseman[1], Misty Queen[1], Zachary Neitzey[1]
[1]Department of Biology, Radford University, Radford, USA

Mosquito-borne viruses continue to cause significant morbidity and mortality worldwide, and many are expanding their ranges. Few are preventable by vaccines or treatable with antiviral drugs, and new methods to treat and/or prevent diseases caused by these viruses must be developed. Bacteria of the genus *Pseudomonas* produce a number of secondary metabolites, such as the phenazine pigments that include pyocyanin secreted by *Pseudomonas aeruginosa*. These pigments have demonstrated activity, through the production of reactive oxygen species (ROS), against other bacteria, fungi, and higher organisms, but they have not been tested against viruses or mosquitoes. The bunyavirus La Crosse virus (LACV), which causes pediatric encephalitis in the Eastern United States, serves as our model. We have isolated several strains of *Pseudomonas* from the digestive tracts of *Aedes albopictus* mosquitoes, and have investigated whether these bacteria, along with known species, can affect virus transmission. Initially, bacteria were reared in a pigment-promoting medium until pigment production peaked. Cells were removed by centrifugation and filtration, and culture supernatants were incubated with LACV prior to infection of cultured Vero cells. Inhibition of LACV infection ranged from 0-100% when supernatant was used at 90% concentration, and this inhibition was dose dependent. *Pseudomonas aeruginosa* supernatant completely inhibited viral infection when diluted to 11.25%. Pyocyanin, purchased commercially, also significantly inhibited LACV infection, and this inhibition could be ablated by blocking ROS with N-acetyl-L-cysteine. We also tested culture supernatants for their ability to kill third- or fourth-instar *Aedes albopictus* larvae according to World Health Organization criteria. *Pseudomonas aeruginosa* and *Pseudomonas chlororaphis* supernatants killed all mosquito larvae within 48 hours, while isolates from mosquitoes tended to induce little mortality. Elucidation of the mechanism by which these metabolites kill the virus and mosquito larvae may lead to novel means to interrupt the transmission of LACV, and potentially other mosquito-borne viruses.

Poster Session

VIR-PW2012 - Potent antiviral efficacy of Morpholino against Chikungunya virus replication

Shirley Lam[1], Chen Huixin[1], Chen Caiyun Karen[1], Chu Jang Hann Justin[1]
[1]Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Chikungunya virus (CHIKV) infection in humans has recently re-emerged as a significant infectious disease due to various outbreaks in geographical regions worldwide. As an arbovirus, CHIKV is transmitted to the human host by Aedes mosquito. Following infection, CHIKV induces Chikungunya fever associated with debilitating and persistent arthralgia and arthritis. Currently, there is no specific vaccine or effective antiviral available. Anti-CHIKV Phosphorodiamidate Morpholino Oligomers (CPMO) was investigated for its efficacy to inhibit CHIKV replication in a human cell line. CPMO1 and CPMO2 were constructed to target CHIKV genomic RNA at the translation initiation region of non-structural and structural polyprotein. The target sequences were highly conserved against seven geographical CHIKV strains. Anti-CHIKV efficacy of CPMO was assessed by viral plaque assay, real-time PCR, Western blotting, transmission electron microscopy (TEM) and in a CHIKV mouse model. CPMOs displayed no cellular toxicity at various concentrations tested for virus inhibition assays. Pre-treatment of HeLa cells with CPMO1 achieved significant decrease in CHIKV titre. CHIKV RNA production was reduced at early infection phase and CHIKV structural E2 protein showed a complete knockdown at post-infection. TEM images showed an absence of CHIKV-induced cytopathic effect relative to wild-type and scrambled CPMO1 controls. Furthermore, CPMO1 was likely to be CHIKV-specific as there was no cross-reactivity inhibition against SINV or DENV replication. Mice serum LDH level remained significantly low at various CPMO1 doses, suggesting the lack of in vivo toxicity. When administered prophylactically in mice, CPMO1 conferred strong protection from CHIKV disease by significantly reducing viremia and viral load in several organs of the infected mice. In conclusion, we present a novel finding that CPMO exerts a strong inhibition against CHIKV replication in vitro and in vivo. This highlights its potential as a promising antiviral strategy for Chikungunya infection.

Poster Session

VIR-PW2014 - Effective intranasal therapeutics and prophylactics with monoclonal antibody against lethal infection of H7N7 influenza Virus

Fang He[1], Rajesh Kumar[1], Jimmy Kwang[1]
[1]Temasek Life Sciences Laboratory, Singapore, Singapore

Recurrence of highly pathogenic avian influenza (HPAI) virus subtype H7 in humans and poultry continues to be a serious concern to public health. No effective prevention and treatment are currently available against H7 infection. One H7 monoclonal antibody, named “62, was selected and characterized. Mab 62 presented efficient neutralization activity against all six representative H7 strains tested, including the H7N9 strain from the recent outbreak in China. The epitope of 62 identified on H7 HA1 exists in all the human H7 strains, including the recent H7N9 strains from China. Mab 62 when administered passively, pre or post challenge with 5 MLD50 (50% mouse lethal dose) HPAI H7N7 influenza viruses could protect 100% of the mice from death. The efficacy of intranasal administration of the Mab was evaluated versus the intraperitoneal route. In the therapeutic study, body weight loss and virus load were reduced in intranasally inoculated mice, as compared to the intraperitoneal group. Intranasal administration results in early clearance of the virus from the lungs and completely prevents lung pathology of H7N7. The study confirmed that intranasal administration of Mab 62 is either an effective prophylactic or therapeutic means against H7 lethal infection. The results of epitope analysis suggest the potential of Mab 62 to be used for the efficacious prevention and treatment against the recent human H7N9 strains.

Poster Session

VIR-PW2016 - The anthracycline compounds are potent inhibitors of Enterovirus 71 replication

Szu-Hao Kung[1], Po-An Deng[1], Shin-Yu Hou[1], Fang-Yen Kung[1], Cheng-Wen Lin[2]
[1]National Yang-Ming University, Taipei, Taiwan, [2]China Medical University, Shenyang, China

The human enteroviruses (HEVs) form a large genus containing many pathogens that can be life-threatening. Among them, EV71 has emerged as the most common nonpoliovirus HEV associated with poliomyelitis-like paralysis. However, the treatment of HEV infections remains a significantly unmet medical need. A drug screening was conducted for a library of small molecules consisting of 1280 compounds with defined bioactivities for hits with anti-EV71 activity. The screening platform was based on a genetically-engineered cell line that displays fluorescence resonance energy transfer (FRET), and the FRET biosensor cells showed a real-time and quantifiable impairment of FRET upon EV71 infections. The screening identified idarubicin and mitoxantrone, both are anthracycline compounds that are US Food and Drug Administration (FDA)-approved for the treatment of certain tumors. IDA and MTX significantly protected the infected cells from the cytopathic effects and cell death caused by EV71 infections, with the 50% effective concentrations at 0.42- and 2.24- μM, respectively. The 50% cytotoxicity concentrations of both compounds are greater than 100 μM, making their specific indexes fairly high. The compounds effectively intervened with the viral life-cycle at the stages of viral translation initiation, viral protein synthesis, and viral genome replication, but not viral proteolysis processes. The antiviral activity of both compounds can be extended to other HEV species including Coxsackievirus-A16, -B1 and Echo 30, suggesting that they target viral and/or cell factors commonly required in the replications of many HEV species. Both compounds are well-known topoisomerase II inhibitors; however, the antiviral activity of both compounds was manifest by other anthracycline structural analogues and was independent of cytotoxic or topoisomerase inhibitory effects. The finding of the structural importance of anthracycline compounds in anti-HEV activity and the new use of anthracycline compounds at a non-cytotoxic concentration may provide new perspectives for therapeutic strategies for controlling EV71 infections.

Poster Session

VIR-PW2020 - In Vitro and In Vivo inhibition of virus replication by water-soluble extract from Phellodendri cortex and Salvia miltiorrhiza

Tae-Hwan Kim[1], Prasanna Weeratunga[1], Myun-Soo Kim[2], Hong-Ik Kim[2], Jong-Soo Lee[1]
[1]College of Veterinary Medicine (BK21 Plus program), Chungnam National University, Daejeon, Republic of Korea, [2]Vitabio Corporation, Daejeon, Republic of Korea

In order to identify new potential antiviral agents, we have screened 200 natural oriental herbal medicines and we found that the herbal extracts of Phellodendri cortex and Salvia miltiorrhiza (Danshen) displayed significant antiviral activity against diverse viruses. The water extracts of Phellodendri cortex and Dansheninhibited a significant influenza A virus, vesicular stomatitis virus, Newcastle disease virus, Herpes Simplex Virus, Coxsackie virus and Enterovirus-71 replication in vitro and also protected mice against challenge with lethal doses of the highly pathogenic influenza A which containing H1N1, H5N2, H7N3 virus in vivo. Mechanically, we found that the extracts of Phellodendri cortex and Danshen induced the type I interferon and pro-inflammatory cytokines both in mRNA & cytokine levels on murine macrophage cells. Especially, extracts of Phellodendri cortex also has the potential virocidal activity. Consequently, our results suggest that the extracts of Phellodendri cortex and Danshen have the components which can induce the type I interferon stimulation and can be a potential antiviral reagent against wide range of viruses by inducing possible cellular antiviral state. [This study was supported by the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea(Grant No. 112013031)].

Poster Session

VIR-PW2022 - Study of the type I interferon production and the response to this cytokine in a Junin virus persistently infected human adenocarcinomic alveolar cell line

Eugenia Cuervo[1], Luis Alberto Scolaro[1]
[1]Departamento de Química Biológica, FCEyN, UBA, Buenos Aires, Argentina

Junin virus (JUNV) is the etiological agent of the Argentine hemorrhagic fever and a member of the Arenaviridae family. JUNV is able to cause both, acute and persistent infections in cell cultures. We have characterized an A549 cell culture persistently infected with the strain XJCl3, named A3. A3 cells produce low viral titters, show no cytophatic effect and are resistant to superinfection. A3 cells express large amounts of viral mRNAs and proteins. We proved that A3 cells are deficient in the production of type I interferon (IFN) after infection or transfection with liposomes. We analized the A3 cells behavior in response to various IFN inducing stimuli. We used poly(I:C) (dsRNA synthetic analog), superinfection with JUNV or pre-treatment with IFN. Either superinfection with JUNV or poly(I:C) (10 µg/ml) treatment induced a minor increase, comparatively to A549 cells, in the expression of RIG-I (Retionic acid-inducible gene 1) at the level of mRNA and protein synthesis. The same happened for the mRNAs of the genes MDA5 (Melanoma differentiation-associated protein 5), IFNB (Interferon beta gene), TLR3 (Toll-like receptor 3) and Viperin. The RIG-I expression pattern, analyzed by immunofluorescence showed a diffuse cytoplasmic pattern in non-stimulated A549 cells and a marked granular pattern in infected or poly(I:C) treated cells. In the A3 cells case, the induction of this granular pattern was less pronounced. However, treatment of A3 cells with IFNα(104 UI/ml) showed an increase of RIG-I expression, associated to a marked granular pattern, and a protective effect when A3 cells were challenged with an heterologous virus. We conclude that JUNV persistent infection negatively modulates the IFN synthesis of these cells in response to infection or poly(I:C) treatment. This modulation involves the RIG-I receptor, among other probable targets like MDA5 or TLR3. At the same time, A3 cells maintain a typical antiviral response to IFN treatment.

Poster Session

VIR-PW2024 - Impact of LCMV infection on the activity of HIF-regulated signal transduction pathways

Jana Tomaskova[1], Ingrid Oveckova[1], Miriam Zatovicova[1], Katarina Laposova[1], Katarina Polcicova[1], Lubomira Lukacikova[2], Silvia Pastorekova[1], Jaromir Pastorek[1]

[1]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Centre for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

The outcome of viral infections can be significantly affected by the components of tissue physiology and cellular microenvironment. Low oxygen tension exerts significant effect on the replication of several DNA and RNA viruses in cultured cells. During persistent infection, when the virus exploits the host cell without disturbing its vital functions, microenvironmental hypoxia can uncouple the delicate virus-cell relationship and escalate virus pathogenesis. Previously, we demonstrated that chronic hypoxia enhances lymphocytic choriomeningitis virus (LCMV) replication in hypoxia inducible factor (HIF) -dependent manner. In this study, we investigated the biological impact of LCMV infection on the activity of HIF-regulated signal transduction pathways in cells cultured under low or atmospheric oxygen tensions. Western blot and real-time quantitative reverse transcription-PCR analyses revealed that LCMV infection stabilized HIF1alpha protein under hypoxic conditions (2% O2) in human cell lines and enhanced its transcriptional activity. These results were also supported by the signal transduction reporter array, which revealed increased activity of HIF1 transcription factor in infected cells not only in hypoxia, but also in normoxia. Moreover, we observed influence of LCMV infection on the activity of MAPK-, PI3K/Akt- and NF-κB- signalling pathways. Since the presence of viral nucleoprotein or Z protein alone led to the stabilization of HIF1alpha protein, it is possible that LCMV proteins indirectly increase the transcriptional activity of HIF1alpha by modulating the above-mentioned pathways. Thus, the upregulation of HIF might be a part of a more general viral strategy established during infection.
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Poster Session

VIR-PW2026 - Cell-based assay to monitor arenavirus GPC maturation by SKI-1/S1P

Joel Ramos da Palma[1], Joel Oppliger[1], Dominique J. Burri[1], Stefan Kunz[1], Antonella Pasquato[1]
[1]University Hospital Center and University of Lausanne, Lausanne, Switzerland

Arenaviruses are important emerging pathogens capable to cause lethal diseases in humans. No licensed vaccine is available to prevent arenaviral infections and current therapeutic options are limited. Development of new effective drug treatments is therefore highly relevant for public health. A crucial step in the arenavirus life-cycle is proteolytic processing of the viral envelope glycoprotein precursor (GPC), which is strictly required for productive infection. Cleavage is mediated by the cellular protease Subtilisin Kexin Isozyme-1 (SKI-1) / Site-1 Protease (S1P), whose inhibition represents a promising approach to block arenavirus infection. Classical biochemical studies on SKI-1/S1P processing of arenavirus GPC-derived sequences involved homogeneous biochemical assays using soluble recombinant enzyme and chromogenic substrate peptides. However, evidence is accumulating that SKI-1/S1P processing of arenavirus GPCs may be regulated at the level of subcellular location. Here, we developed a novel molecular sensor for SKI-1/S1P based on chimeric proteins that contain the processing sites of viral GPCs and allow monitoring of cleavage in a robust, rapid, and cost-effective format. Sensors mimicking GPC cleavage sites of different members of the Arenaviridae family have been generated and characterized. Our data suggest that nine residues encompassing the processing site are necessary and sufficient to recapitulate the phenotype of the full length GPC maturation. This sensitive cell-based assay allows multiple applications including the identification of previously unknown viral GPC cleavage sites, the investigation of the subcellular compartment of maturation, and definition of the contribution of neighboring residues to processing efficiency. The robustness of this innovative analysis allows its implementation in a high throughput screening assay format to identify new SKI-1/S1P inhibitors that could be used to treat arenaviral infections and other kind of diseases, including metabolic disorders.

Poster Session

VIR-PW2028 - Identification of targets for therapeutic intervention against Crimean-Congo Hemorrhagic Fever Virus

Olena Shtanko[1], Raisa Nikitina[2], Alexander Chepurnov[2], Robert Davey[1]
[1]Texas BioMedical Research Institute, San Antonio, USA, [2]Institute of Clinical Immunology, Novosibirsk, Russia

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne bunyavirus causing outbreaks of severe hemorrhagic disease in humans, with a fatality rate reaching 30%. The virus is endemic to many parts of the world, including Russian Federation, Eastern Europe, the Middle East, Asia, and Africa. There is no widely accepted vaccine or drug treatment available. Viruses rely on host cell proteins for replication, although few such proteins have been identified for CCHFV. Here, we describe our work toward (i) identification of cellular proteins required for entry and replication of CCHFV; and (ii) prediction of and testing clinically-approved drugs targeting the identified host proteins. We generated a novel recombinant virus expressing the red fluorescent protein mKate2 (CCHFV-mKate2). Using a high-throughput approach, cells were transfected with a library of small interfering RNAs (siRNAs) depleting host proteins that are clinical drug targets (7,765 genes). The cells were then challenged with CCHFV-mKate2. Infected cells were imaged and counted by high-content analysis. Host genes networks critical for infection were identified and used to predict drugs inhibiting CCHFV infection. These compounds were tested for inhibition of wild-type virus infection in cell culture. Among drugs efficiently inhibiting virus, ethacrynic acid was particularly potent with an EC50 of <1 μM. Our strategy demonstrates a fast and reliable approach to identify novel therapeutics to treat highly pathogenic viruses. This work was supported by DOD/DTRA HDTRA1-12-1-0002; Project FRBAA09-6H-2-0043; AIC CTB119922900.

Poster Session

VIR-PW2030 - Passage of Hantaan virus strain AA57 in Vero E6 cells affects pathogenicity in mice

Hiroaki Kariwa[1], Masahiro Maki[1], Takahiro Seto[1], Takahiro Sanada[1], Kumiko Yoshimatsu[2], Jiro Arikawa[2], Kentaro Yoshii[1]

[1]Graduate School of Vet. Med., Hokkaido Univ., Sapporo, Japan, [2]Graduate School of Medicine, Hokkaido Univ., Sapporo, Japan

Hantaviruses belong to the genus Hantavirus within the family Bunyaviridae. Several rodent-borne hantaviruses are the causative agents of hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) in humans. Since there are few suitable animal models for hantavirus infection, little is known about the mechanisms of the pathogenicity of the disease. In this study, Hantaan virus AA57 strain was passaged in Vero E6 cells 30 times, and the viruses were characterized in cultured cells and a mouse model. The viruses passaged 20 times (P20) and 30 times (P30) grew significantly more than the virus passaged 3 times (P3) in Vero E6 (African green monkey) and A549 (human) cells. Morbidity and mortality in mice infected with P20 or P30 were significantly lower than in those infected with P3. While a peak of the virus titer (8.8 × 104 ffu/gram) was observed 5 days post-inoculation in the lungs of P3-infected mice, only a few virions were detected in mice infected with P30 throughout the experiments. In the nucleocapsid protein of P20 and P30 which is encoded by the S segment, one amino acid substitution was detected at the amino acid position 43 from Ala to Thr. Comparing P3 with P20 and P30, there were four amino acid substitutions at positions 200 (Lys → Thr), 772 (Glu → Asn), 1,662 (Gly → Glu) and 2,096 (Asp → Glu) in viral polymerase encoded by the L segment. Moreover, there were several substitutions in the 5’ non-coding region of each segment (S, M, and L), and one substitution in the 3’ non-coding region of the S segment. These results indicate that mutations by the passage of AA57 in Vero E6 cells induced high virus multiplicity in primate cells and reduced multiplicity in mice, resulting in lower virulence.

Poster Session

VIR-PW2032 - Analyses of cell entry of severe fever with thrombocytopenia syndrome virus using pseudotype vesicular stomatitis virus system

Hideki Tani[1], Masayuki Shimojima[1], Shuetsu Fukushi[1], Tomoki Yoshikawa[1], Aiko Fukuma[1], Satoshi Taniguchi[1], Momoko Ogata[1], Shigeru Morikawa[2], Masayuki Saijo[1]

[1]Department of Virology I, National Institute of Infectious Diseases, Tokyo, Japan, [2]Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo, Japan

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging fatal hemorrhagic fever caused by a newly identified bunyavirus, SFTS virus (SFTSV). Despite the medical importance of this disease, there are no vaccines and effective therapies against SFTS. Although studies on the virus-host cell interactions are expected to contribute in development of antiviral strategies, the interaction has not been examined well for SFTSV. In this study, we have developed a pseudotype vesicular stomatitis virus (VSV) bearing the Gn/Gc glycoproteins (Gn/Gc) of SFTSV (SFTSVpv) and analyzed host cell entry of SFTSV. A pseudotype VSV bearing the Gn/Gc of Rift Valley fever virus (RVFVpv), another member of the bunyavirus family, was also constructed as a control. Both SFTSVpv and RVFVpv generated in 293T cells exhibited high infectivities in various mammalian cell lines. A pH-dependent endocytosis of SFTSVpv was confirmed by the use of lysosomotropic agents. Infectivities of SFTSVpv were neutralized by a serial dilution of the convalescent patient sera. The entry of SFTSVpv was increased in Raji cells expressing DC-SIGN, one of the receptor candidates for SFTSV. Furthermore, we found that DC-SIGN-related (DC-SIGNR) and liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin) also enhanced the entry of SFTSVpv. The results were further confirmed by the observation that authentic SFTSV growth was increased in Raji cells expressing these molecules. The results strongly indicated that several C-type lectins are involved in the entry of SFTSV. These results also indicate that the SFTSVpv developed in this study can be used not only to study SFTSV glycoproteins in the entry process but also to develop neutralization test for diagnosis of SFTS.

Poster Session

VIR-PW2034 - Severe fever with thrombocytopenia syndrome virus in ticks in Japan

Akihiko Uda[1], Hiroki Kawabata[1], Shuetsu Fukushi[1], Yoshiharu Kaku[1], Masayuki Shimojima[1], Shuji Ando[1], Ken Maeda[2], Hiromi Fujita[3], Masayuki Saijo[1], Shigeru Morikawa[1], Tomoyuki Yoshikawa[1], Aya Niikura[1], Sawabe Kyoko[1]
[1]National Institute of Infectious Diseases, Tokyo, Japan, [2]Yamaguchi University, Yamaguchi, Japan, [3]Mahara Institute of Medical Acarology, Tokushima, Japan

First identified in China in 2009, severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-born infectious disease, with a fatality rate ranging from 2 to 30%,. SFTS patients were also found in Japan and South Korea in 2013. SFTS virus (SFTSV), the causative agent of SFTS, belongs to the Phlebovirus genus in the family Bunyaviridae. Phylogenetically, Japanese SFTSV isolates form an independent genotype to Chinese SFTSV isolates. In Japan, 52 laboratory-confirmed SFTS cases with a fatality rate of 36.5% were reported between 2005 and 2013. All patients resided in the western region of Japan. In Japan, Haemaphysalis longicornis and Amblyomma testudinarium are known to be associated with SFTS patients. However, little is known about tick species infected with the virus in Japan. Thus, molecular detection of SFTSV in ticks in Japan was conducted with a novel real-time RT-PCR with TaqMan MGB-probe. RT-PCR detects 10 RNA copies and 1 FFU of SFTSV. Generally, 5 ticks were pooled and RNAs were isolated and subjected to RT-PCR. Among tick species so far tested, SFTSV RNAs were detected in Haemaphysalis longicornis, Amblyomma testudinarium and several other tick species. SFTSV-positive ticks were widely distributed in Japan, not only in the SFTS-endemic region. SFTSV RNAs were detected in 15.2% of 1,655 tick pools (3,812 ticks). Further analysis is required to determine if all the tick species carrying the virus are at risk for humans, since only two tick species are known to be associated with human disease.

Poster Session

VIR-PW2036 - Clinical and molecular epidemiological analysis of hemorrhagic fever with renal syndrome patients in Korea

Ji Yun Noh[1], Sook-Young Lee[1], Jeong Ah Kim[1], Ki-Joon Song[1], Luck Ju Baek[1], Richard Yanagihara[2], Jin-Won Song[1]
[1]Korea University, Seoul, Republic of Korea, [2]University of Hawaii at Manoa, Honolulu, USA

Hantaan (HTN) virus, the etiologic agent of clinically severe hemorrhagic fever with renal syndrome (HFRS), was first isolated in 1976 from lung tissues of striped field mice (Apodemusagrarius) captured in Songnae-dong, Gyungki-do, Korea. HTNV has accounted for about 70% of the HFRS cases in Korea, followed by SEOV in 20%. The remaining 10% of HFRS cases are presumably caused by other hantaviruses, such as Soochong virus and Muju virus.Laboratory diagnosis of HFRSprimarilydecidedby serological methods. Afewreports are available about the clinical and molecular epidemiological analysis of HFRS in Korea. Medical records of HFRS patients,admitted to a hospital in Korea during the 10-year period,2002 to2012, were reviewed. Sera from patients were tested for HTNV and Seoul virus (SEOV) by RT-PCR and IFA. Among 35 HFRS patients(mean age was 44.2±14.7 years), 29 were men (82.9%) and 6 were women (17.1%). Acute renal failure developed in 27 patients (77.1%), and 12 patients (34.3%) were admitted to the intensive care unit (ICU). Multivariate analysis showed that conjunctival injection and initial serum albumin less than 3 g/dLwere risk factors for ICU admission. Of 35acute-phase sera, 11 (31.4%) were positive for HTN viral RNA. Severe HFRS was characterized by the clinical triad of fever, renal insufficiency and abdominal pain or gastrointestinal symptoms.The sequence andphylogenetic analysis, HTNV was the principalcause of HFRS requiring hospitalization in Korea. Ourdata provide valuable clinical clues about HFRS over a 10-year period and clearly demonstrate that HTNV is the primary cause of HFRS in Korea.

Poster Session

VIR-PW2038 - Inkoo virus infection among patients with febrile illness or patients with neurological symptoms

Niina Putkuri[1], Antti Vaheri[1,2], Olli Vapalahti[1,2,3]
[1]Haartman Institute, Department of Virology, Faculty of Medicine, University of Helsinki, Finland, [2]HUSLAB, Helsinki University Central Hospital, Helsinki, Finland, [3]Department of Veterinary Biosciences, Faculty of Veterinary Medicine, University of Helsinki, Finland

Genus orthobunyavirus (family bunyaviridae) includes a large group of mosquito-borne viruses found all over the world which many are important human and veterinary pathogens. Until recently, Inkoo virus (INKV) has been the only representative of the genus in Finland. Nowadays, Schmallenberg virus and Möhkö isolate of the Chatanga virus are known to circulate in Finland. However, ongoing research and laboratory diagnostics of INKV infections have been neglected for decades. Many of the California encephalitis group viruses are well-known human pathogens and we know INKV often infects human (high seroprevalence among Finnish population). Still the association of Inkoo virus infection with clinical disease has not been confirmed, although occasional cases of meningitis and encephalitis have been diagnosed. In this study, we focused on the frequency of the acute INKV infections and the clinical picture of the patients. We screened 8818 sera samples for INKV IgM antibodies from patients with febrile illness or neurological symptoms collected during summertime between 2001 and 2012. The positive samples were confirmed with plaque reduction neutralization test and the patient histories were collected. We found the frequency of the acute infection to be under 1%. Majority of the patients were not hospitalized. However, those patients admitted were children. Hospitalized patients suffered from fever, flu-like symptoms, nausea/vomiting, disoriented/drowsiness, small changes in EEG, headaches and seizures. Ages of the not hospitalized patients were between 28-54 years and they visited doctor most often once, indicating very mild symptoms. In conclusion, it seems INKV infection rarely causes severe neurological infection in Finland. Patients are mostly asymptomatic or have a febrile disease and do not seek to medical care during the infections.

Poster Session

VIR-PW2040 - Development of a mouse model of hemorrhagic fever with renal syndrome

Kenta Shimizu[1], Kumiko Yoshimatsu[1], Takaaki Koma[1], Jiro Arikawa[1]
[1]Hokkaido University Graduate School of Medicine, Sapporo, Japan

Hemorrhagic fever with renal syndrome (HFRS) caused by hantavirus infection is characterized by fever, renal dysfunction and hemorrhage. Immune responses and vascular hyperpermeability are thought to be related to the pathogenesis. However, lack of animal model that mimics the human disease hampers elucidation of the mechanism in vivo. Here we developed a disease model showing renal hemorrhage like in HFRS patients by using BALB/c mice. A clone of Korean hemorrhagic fever virus (KHFV cl-5) that was derived from a HFRS patient serum were obtained by plaque cloning and intravenously inoculated into 6 weeks old female BALB/c mice. KHFV cl-5 caused transient bodyweight loss in mice at 6~9 days post-inoculation (dpi) in a dose-dependent manner. Pathological examination demonstrated prominent hemorrhage of renal medulla in KHFV cl-5 infected mice at 9~12 dpi. Uric protein level was elevated at 6 dpi. Uric blood was detected from 6 dpi, peaked at 9 dpi and decreased at 12 dpi. Pretreatment of inoculum with immune serum of Hantaan virus that is belonged to the same serotype as KHFV cl-5 inhibited the manifestation of symptoms. Infected severe combined immunodeficiency mice with a defect of functional T and B cells showed continuous bodyweight loss and died without renal hemorrhage. In contrast, infected nude mice with a defect of T cells showed no symptom, suggesting that T cells have a role in developing renal hemorrhage. This is a first animal model of HFRS with renal hemorrhage.

Poster Session

VIR-PW2042 - Phomopsis longicolla hypovirus 1 and induced hypovirulence of its host, Phomopsis longicolla

Igor Koloniuk[1], Karel Petrzik[1], Said Ghabrial[2]
[1]Institute of Plant Molecular Biology, Biology Centre of ASCR, Czech Republic, [2]Plant Pathology Department, University of Kentucky, USA

Phomopsis longicolla Hobbs 1985 (teleomorph = Diaporthe) is a fungal pathogen and the major cause of phomopsis seed decay of soybean in majority of soybean-growing countries. Differences in the pathogen aggressiveness were reported for several isolates of P. longicolla. Recently, we reported a new virus, a member of Hypoviridae family, tentatively named Phomopsis longicolla hypovirus 1 (PlHV1/ME711), isolated from P. longicolla strain ME711. This fungal culture was originally derived from a slow-growing sector in a culture of P. longicolla strain (KY) obtained from soybean seed lot in Kentucky [1] and maintained in culture for 25 years by serial passages. This culture exhibited abnormal colony growth [2] and hypovirulence. A genomic organization of PlHV1/ME711 is typical to those of the proposed genus Betahypovirus [3]. It was highly unexpected to find PlHV1/ME711 in the hypovirulent isolate of P. longicolla as other known betahypoviruses do not debilitate their hosts. After sequence analysis of dsRNA from the P. longicolla, KY strain another nonrelated not yet described hypovirus was found to be present along with PlHV1/ME711. We describe here firstly coexistence of two hypoviruses in one host and their possible influence on the phenotype and induced hypovirulent traits of their host. 1. Gleason ML, Ghabrial SA, Ferriss RS (1987) doi: 10.1094/Phyto-77-371 2. Koloniuk I, El-Habbak MH, Petrzik K, Ghabrial SA (2014) doi: 10.1007/s00705-014-1992-8 3. Yaegashi H, Kanematsu S, Ito T (2012) doi: 10.1016/j.virusres.2012.02.008

Poster Session

VIR-PW2044 - A novel ssRNA virus isolated from a phytopathogenic filamentous fungus, Rosellinia necatrix with similarity to hypo-like viruses

Rui Zhang[1], Shengxue Liu[1], Sotaro Chiba[1], Hideki Kondo[1], Satoko Kanematsu[2], Nobuhiro Suzuki[1]
[1]IPSR, Okayama Universsity, Kurashiki, Japan, [2]IFTS, NARO, Morioka, Japan

The white root rot fungus, Rosellinia necatrix is an important soil-borne fungal pathogen to perennial crops and emerges recently as a fungal host for studying virus/host and virus/virus interactions. Extensive screens of over 1000 Japanese field isolates for mycoviruses showed relatively high (20%) incidence of virus infections in the fungus. Here we report a biological and molecular characterization of attributes of a novel positive-sense RNA virus isolated from a field isolate (N10) of R. necatrix that is tentatively designated as Rosellinia necatrix fusarivirus 1 (RnFV1). A recently developed technology using zinc ion allowed us to transfer RnFV1 to two mycelially incompatible R. necatrix strains. A biological comparison of the virus-free and isogenic recipient fungal strains suggested that RnFV1 infects asymptomatically and thus has no potential as a virological control agent for the phytopathogenic host fungus. The virus has an undivided positive-sense RNA genome of 6, 266 nucleotides excluding the poly (A) tail. The genome possesses two non-overlapping ORFs: a large (ORF1) and a small ORF (ORF2) that encode polypeptides with functions in RNA replication and with unknown function, respectively. Amino acid sequence similarities were found between RnFV1 putative proteins and counterparts of a previously reported mycovirus, Fusarium graminearum mycovirus 1 (FgV1). Interestingly, several related sequences were detected by BLAST searches of independent transcriptome assembly databases; one of which probably represents an entire virus genome. Phylogenetic analysis based on conserved RNA-dependent RNA polymerase showed that RnFV1, FgV1 and these similar sequences are grouped in a cluster distinct from related hypoviruses. A taxonomic proposal was made that a new genus termed Fusarivirus be created to include RnFV1 and FgV1.

Poster Session

VIR-PW2046 - Autophagy activity in Pacific oyster during ostreid herpesvirus 1 infection

Pierrick Moreau[1], Kevin Moreau[2], Amélie Segarra[1], David Rubinsztein[2], Tristan Renault[1]
[1]Ifremer, LGPMM, La Tremblade, France, [2]Cambridge Institute for Medical Research, Cambridge, UK

The publication of the Pacific oyster genome in 2012 has opened up new opportunities to study innate immunity in a marine bivalve species. A comparison between human and oyster genomes indicates that many proteins are conserved between the two species, especially proteins involved in autophagy. Autophagy is an important degradation pathway that has been involved in several pathologies including infectious diseases. Numerous lines of evidence suggest that autophagy plays a key role in the clearance of many viruses. However, some viruses can exploit autophagy for their own benefit. Work has been undertaken through the research of genes involved in autophagy in the Pacific oyster genome (ATG1: Serine/threonine-protein kinase ATG1; ATG8/LC3: Microtubule-associated protein 1A/1B-light chain 3). Gene expression and protein detection have been analysed using real time PCR and western blot, respectively. ATG1 and ATG8 expression was upregulated during experimental viral infection and western blot analysis showed an increase of LC3 protein during the infection, suggesting an activation of autophagy. Ammonium chloride treatment was associated with increased oyster mortality whereas less mortality was reported after carbamazepine treatment in experimentally infected animals. These results suggest that autophagy plays a key role protecting oysters from viral infection. Interestingly, different autophagy activities were also observed between different oyster families presenting different susceptibilities to OsHV-1 infection. Our data describe for the first time the autophagy pathway in a marine mollusc species, the Pacific oyster, Crassostrea gigas, and suggest a protective role of autophagy against OsHV-1 infection.

Poster Session

VIR-PW2048 - Insights into structural determinants for nuclear domain 10 disruption by the major immediate early protein IE1 of human cytomegalovirus

Myriam Scherer[1], Stefan Klingl[2], Madhumati Sevvana[2], Viktoria Otto[1], Joachim Stump[3], Heinrich Sticht[3], Yves Muller[2], Thomas Stamminger[1]

[1]Institute for Clinical and Molecular Virology, Erlangen, Germany, [2]Division of Biotechnology, Erlangen, Germany, [3]Division of Bioinformatics, Institute of Biochemistry, Erlangen, Germany

Recent research identified the nuclear domain 10 (ND10) components PML, hDaxx, and Sp100 as components of an intrinsic immune response that suppress the initiation of human cytomegalovirus (HCMV) replication. This antiviral defense, however, is efficiently counteracted by the viral immediate-early protein IE1. To gain further insights into the mechanism by which IE1 disrupts ND10 during HCMV infection, we set out to determine the three-dimensional structure of IE1. Here we present the crystal structure of a central, 45kDa subdomain of the rhesus macaque cytomegalovirus IE1 protein (RhIE1) at 2.3 Å resolution. RhIE1 exhibits an elongated all alpha-helical structure that displays no obvious homology to known protein folds. In vitro and in vivo approaches revealed that function and structure are conserved between IE1 proteins of primate cytomegaloviruses. Consistently, a reliable model for IE1 of HCMV was generated, based on the crystal structure of the RhIE1 subdomain. In order to investigate the functional importance of the IE1 subdomain for ND10 disruption, we engineered a recombinant HCMV expressing this truncated IE1 variant. Analogous to full- length IE1, the IE1 subdomain localized to the dot-like ND10 structure and was sufficient to induce de-SUMOylation of PML upon infection with the recombinant virus. Intriguingly, this event resulted in the release of ND10-associated proteins into the nucleoplasm, e.g. Sp100, but not of PML itself. We propose that ND10 disruption by IE1 is an at least two-step process involving PML de SUMOylation and disassembly of PML aggregates, which are mediated by distinct domains of the IE1 protein.

Poster Session

VIR-PW2050 - Myxomaviral Serpin Reactive Center Loop (RCL) peptides display independent coagulation and immune modulating activities

Sriram Ambadapadi[1], Donghang Zheng[1], Alexandra Lucas[1]
[1]*University of Florida, Gainesville, USA*

Background - Serpins regulate coagulation and inflammation, binding serine proteases in a suicide inhibitory complex. Target proteases cleave the serpin reactive center loop (RCL) scissile P1-P1' bond and resulting serpin-protease complexes lose both protease and serpin function. Inhibition requires near full-length serpin sequence. Myxomavirus Serp-1, inhibits thrombolytic and thrombotic proteases while mammalian neuroserpin (NSP) inhibits only thrombolytic proteases. Both serpin proteins markedly reduce arterial inflammation and plaque in rodent models after single dose infusion and Serp-1 has been successfully tested in a Phase 2a clinical trial. Serp-1 treatment also improves survival and lung congestion in lethal MHV68 herpes infection in interferon gamma receptor knock out mouse models. We postulated that proteolytic cleavage of the RCL produces active peptide derivatives with expanded functions. Methodology/Principal Findings - Eight peptide sequences based upon predicted RCL cleavage sites for Serp-1 and NSP were synthesized and tested for inhibitory function in vitro and in vivo using cell activation and aortic allograft models. In engrafted aorta, peptides containing R or RN, not RM, with 0 - +1 charge, significantly reduced plaque. Conversely, one hydrophobic peptide, lacking R or RN with -4 charge, induced early thrombosis and mortality. S-1, a Serp-1 RCL peptide, had greater inhibitory activity for aortic allografts deficient in the mammalian serpin plasminogen activator inhibitor-1 (PAI-1-/-) than for wild-type. Selected peptides modified mononuclear cell activation and gene expression. One peptide with anti-inflammatory activity in mouse transplants is being tested in the MHV68 infection model and to date has improved survival. Conclusions - Serpin reactive center RCL metabolites with R or RN sequences and 0 - +1 charge have anti-inflammatory activity and a negatively charged, hydrophobic peptide lacking RN is pro-thrombotic. Naturally processed reactive center peptides derived from a Myxomaviral serpin, Serp-1, have the potential to extend inhibitory and immune modulating functions of serpins.

Poster Session

VIR-PW2052 - Laboratory-based rotavirus surveillance, Brazil, 2007-2012

Adriana Luchs[1], Audrey Cilli[1], Simone Guadagnucci Morillo[1], Rita de Cassia Compagnoli Carmona[1], Maria do Carmo Sampaio Tavares Timenetsky[1]
[1]Enteric Disease Laboratory, Adolfo Lutz Institute, Sao Paulo, Brazil

A new scenario emerged with rotavirus (RV) vaccination era. Continued genotype surveillance is needed for better understanding the RV spread in the broad community. The aim of this study was describe the results of 5-years surveillance of G- and P-type RV strains from individuals with acute gastroenteritis in Brazil. From June 2007 to June 2012, a total of 6,196 fecal specimens were collected from children ≤5 years of age, school-age children (6–18 years), adults (19–59 years), and elderly patients (≥60 years). All specimens were screened for RV using commercial ELISA, and RV positive samples were genotyped by RT-PCR. RV was detected in 19.1% (1181/6196); incidence peaked in September. RV was detected less frequently (19.5%) among children ≤5 years than in older children (6-18 years) (40.6%). Genotype distribution showed a different profile for each year: G2P[4] from 2007 to 2010, G9P[8] in 2011, and G12P[8] in 2012. G8 genotype was identified during 2009-2011. Mixed infections (G1+G2P[4], G2+G3P[4]+P[8], G2+G12P[8]) and uncommon strains (G1P[4], G2P[6], G3P[3]) were also identified throughout the study period. G4P[8] was not detected. The peak of RV infections moved from June-August to September. Widespread vaccination may reduce the number of susceptible individuals, delaying the timing of RV activity and altering its seasonal pattern across the country. A tendency of RV-related cases to affect older children after vaccine implementation has been observed worldwide, probably due to herd immunity. G2P[4] emergence most likely follows a global trend dictated by the oscillatory fluctuations of RV genotypes seemingly unrelated to vaccination. In fact, G9P[8] re-emerged as the prevalent genotype in 2011. G12 apparently is emerging in Brazilian population as observed worldwide. The rapidly changing RV genotype patterns detected during this study do illustrate a more dynamic wild-type population thus suggesting that vaccine pressure may be speeding up the selection process.

Poster Session

VIR-PW2054 - Establishment of evanescent wave fiber-optic immunosensor method for detection bluetongue virus

Huiqiong Yin[1], Peipei Jin[1], Jun Liu[1], Rui Wang[1], Jingang Zhang[1]
[1]Institute of Transfusion Medicine, the Academy of Military Medical Sciences.

Bluetongue virus (BTV) is an arthropod-borne virus that infects both domestic and wild ruminants. Due to the wide distribution of BTV, reliable and rapid detection methods are needed. In this study, evanescent wave fiber-optic immunosensor (EWFI) method was established for detection of BTV. Firstly, cladding (1 cm) on the proximal end was removed from the fiber to expose the core. BTV VP7 protein was immobilized on the surface of the exposed fiber-optic core. Then the monoclonal antibody 3E2 specific to BTV VP7 were added to form a immuno-complex, and the goat ant-rat IgG conjugated with Cy3 was captured. evanescent wave was generated after the 635 nm pulse (excitation source) reached the fiber probe. The evanescent wave was changed into electrical signals through photodiode, and the signals were read through computer finally. The preliminary results suggested that a detection limit of 10ng/ml was measured for the monoclonal antibody 3E2, which is equal to the sensitivity of ELISA. The sensitivity of EWFI method will be improved further through optimazing the optimal concentration of VP7 and the second antibody (goat ant-rat IgG conjugated with Cy3). In addition, the EWFI regeneration method was established in this research. The results showed that The EWFI can be recycled at least ten times through TEA solution (pH 11.5) condition, and it can be recycled six times and two times through Glycine-HCl buffer (pH 2.0) and 0.5% SDS (pH 1.9) solution respectively. The EWFI assay was proved to be a sensitive, specific and rapid (15min) way for the detection of BTV antibody. It has a potential application for grassroots laboratory, emergency detection and spot detection.

Poster Session

VIR-PW2056 - Prevalence of rotavirus genotypes in South Korea in 1989–2009: Implications for a nationwide rotavirus vaccine program

Van Thai Than[1], Wonyong Kim[1]
[1]*Chung-ang University, Seoul, South Korea*

The epidemiology of human group A rotavirus was analyzed by examining genotypic data acquired from 1989 to 2009 in South Korea. This information was derived from all the available published articles on rotavirus studies in South Korea, retrieved from both the PubMed and KoreaMed databases. Four common G types (G1, G2, G3, and G4) and three common P types (P[8], P[4], and P[6]) accounted for approximately 93% and 99% of the rotavirus reports, respectively. The G9 type was frequently detected after 2000, and because of this prevalence, it is considered to be the fifth most important G type rotavirus after the G1–G4 genotypes. Less common G types of the virus such as G12, G11, and G10 were detected in some geographic settings, and it is important to consider the context of these subtypes and their epidemiological significance. The P[9] virus genotype was observed in the study and has been discussed in many other studies; however, the P[3], P[10] and P[25] genotypes were rarely detected in the epidemiological research. In general, the distributions of the G and P genotypes showed temporal and geographical fluctuations, and a nationwide rotavirus vaccine program that targeted these genotypes demonstrated effectiveness in protecting against the circulating rotavirus strains. However, further analysis is needed to determine the true long-term effectiveness of these vaccines; the analysis should also consider the unexpected effects of vaccinations, such as vaccine-induced diseases, herd immunity, and changes in host susceptibilities.

Poster Session

VIR-PW2058 - Identification of divergent amino acids residues affecting interferon sensitivity in reovirus type 3 Dearing laboratory stocks

Véronique Sandekian[1], Guy Lemay[1]
[1]*Université de Montréal, Montréal, Canada*

In the last few years, the development of a plasmid-based reverse genetics system for mammalian reovirus has revolutionized the field, allowing to systematically produce mutant viruses from the cloned viral genome. This is especially significant in the perspectives of optimizing the virus' oncotropism and oncolytic activity. Despite these progress, the virus originally used as the parental strain of reovirus type 3 Dearing might not completely recapitulate the phenotype of different laboratory virus stocks used by various investigators. In this study, it was observed that the virus used in the laboratory (referred to as wild-type “Sandekian”, T3D-S) was significantly less sensitive to interferon than the one rescued by reverse genetics (“Kobayashi”, T3D-K). Complete sequencing of the viral genome revealed that 7 out of 10 proteins, with the exception of σ2, σNS and σ3, harbored single or multiple amino acid differences. Using site-directed mutagenesis and reverse genetics, T3D-S outer capsid proteins σ1 and μ1, as well as inner capsid protein μ2, were found to confer only partial interferon resistance when introduced in the T3D-K background. To further identify all differences responsible for T3D-S resistance, site-directed mutagenesis was performed on the 4 remaining genes to systematically rescue viruses harboring various gene combinations. In parallel, the P4L-12 virus mutant, previously isolated based on its hypersensitivity to interferon, was completely sequenced and found to harbor amino acids substitutions in σ3, μ1, μNS, λ3 and λ2, compared to the original wild-type T3D-S. Further work is under progress to determine which of these substitutions are responsible for the P4L-12 phenotype, although these data already support the idea that multiple reovirus genes are involved in reovirus' sensitivity to interferon. This work also further highlight the importance of working with well characterized “wild-type” viruses, especially when comparing results between laboratories.

Poster Session

VIR-PW2060 - Isolation and genomic characterization of a novel orthoreovirus from a brown-eared bulbul in Japan

Rikio Kirisawa[1], Yoshitaka Ogasawara[1], Naoya Kikuchi[1], Hiromi Ueda[1]
[1]*Rakuno Gakuen University, Ebetsu, Japan*

In the genus Orthoreovirus, five species, Mammalian orthoreovirus, Avian orthoreovirus, Nelson Bay orthoreovirus, Baboon orthoreovirus and Reptilian orthoreovirus, have been identified and their genome consists of ten double-stranded RNA segments that form a 3/3/4 electrophoretic PAGE profile pattern. We isolated a novel orthoreovirus from the intestine with hemorrhage of a dead brown-eared bulbul in Japan. The virus formed syncytia in Caco-2 and Vero cells. Electron microscopy revealed non-enveloped capsids of approximately 70 nm in diameter, which are characteristic of reoviruses. Complete genomic sequences were determined using the traditional sequencing method. Sequence and phylogenetic analyses showed that the virus was similar to the two species Avian orthoreovirus and Nelson Bay orthoreovirus but existed between the two species' phylogenetic branches. The virus had the closest phylogenetic relationship to two strains, SSRV from a Steller sea lion in Canada and PsRV from a psittaciform bird in Europe. These results suggest that the novel virus might form a new group with the two strains in the genus Orthoreovirus.

Poster Session

VIR-PW2062 - Roles of GM1 ganglioside and N-acylneuraminic acids in mediating rotavirus infection

Fiona Fleming[1], Vi Dang[1], Gavan Holloway[1], Thomas Haselhorst[2], Paul Madge[2], Jaigeeth Deveryshetty[2], Helen Blanchard[2], Mark von Itzstein[2], Barbara Coulson[1]

[1]Peter Doherty Institute for Infection and Immunity at The University of Melbourne, Parkville, Australia, [2]Institute for Glycomics, Griffith University, Gold Coast Campus, Australia

N-acetyl- and N-glycolylneuraminic acids (Sia) are rotavirus cellular receptors recognized by the VP8* subunit of spike protein VP4. VP8* of human Wa rotavirus binds the internal (branched) N-acetylneuraminic acid on ganglioside GM1. Wa infection is increased by enhanced access to internal Sia (following removal of terminal Sia on main glycan chains with sialidase) and reduced by the GM1 ligand cholera toxin B (CTB). Here we show that sialidase treatment increased cellular GM1 expression and the infectivity of several other rotaviruses that like Wa were originally termed "sialidase-insensitive" (including human strain RV-3), typically rendering them susceptible to methyl α-D-N-acetylneuraminide treatment. CTB reduced the infectivity of these viruses. GM1 glycan inhibited Wa and RV-3 infectivity in both sialidase-treated and untreated cells. Furthermore, cell supplementation with GM1 increased Wa and RV-3 infectivity, whereas supplementation with ganglioside GM3 had no effect. Rotavirus usage of GM1 was mapped to VP4 using virus reassortants, and recombinant VP8* of Wa and RV-3 bound the GM1 pentasaccharide by Saturation Transfer Difference (STD) NMR. Cell binding by recombinant VP8* of Wa and RV-3, measured by flow cytometry, was reduced by CTB treatment. Similar VP8*-cell binding assays showed that Sia recognition properties of sialidase-sensitive rotaviruses also directly resulted from VP8*-cell binding, but most did not use GM1 for infection. In particular, monkey rotavirus RRV VP8* interacted minimally with aceramido-GM1 by STD NMR, and RRV infectivity was unaffected by GM1 glycan treatment. During rotavirus-cell attachment and entry, the subterminal N-acetylneuraminic acid on GM1 is commonly utilised by VP8* of sialidase-insensitive rotaviruses, whereas VP8* of sialidase-sensitive rotaviruses typically bind terminal N-acetyl- and/or N-glycolylneuraminic acids and recognise other gangliosides.

Poster Session

VIR-PW2064 - Real time detection of human group A Rotavirus in water

Janette Moreno-Gonzalez[1], Gilberto Erosa-de la Vega[1], Blanca Sanchez-Ramirez[1], Maria del Carmen Gonzalez-Horta[1], Francisco Javer Zavala-Diaz de la Serna[1], Melchor Roa-Huerta[2], Rocio Infante-Ramirez[1], Patricia Tamez-Guerra[3], Carmen Delgado-Gardea[1]

[1]Univesidad Autonoma de Chihuhua Facultad de Ciencias Quimicas, Chihuahua, Mexico, [2]Tecnologico de Monterrey Campus Chihuahua, Chihuahua, Mexico, [3]Universidad Autónoma de Nuevo León, Monterrey, Mexico

Background: Human Rotavirus is excreted in the human and animal feces and could contaminate water which is used either for human consumption or other several activities, threatening the population. It has been shown that enteric viruses is not removed from water with conventional treatments, they can survive for a long period time, even months. The evidence for fecal contamination is provided by the high incidence of outbreaks of diarrheal diseases. The aim of this study was to evaluate the recovery of Rotavirus in samples of contaminated water with feces. Material: water samples were artificially contaminated with Rotavirus, were filtered with a capture system of our own design. The viral capture was conducted using Virocap VCM-47® filters whit an absorption-elution method (VIRADEL), next eluted with beef extract, 1% Tween80-0.5M, glycine pH 9 and finally a secondary concentration by organic flocculation. Viral RNA was extracted by the method of Trizol®, and molecular detection of Rotavirus was performed by two steps RT-qPCR. cDNA was synthesized using CON2 primer and it was used as template for SYBR Green qPCR assay. Internal control plasmid DNA was constructed with 876 bp in length of gen 4 of protein VP4 Rotavirus and cloned into the p-GEM vector. This plasmid was used as standard for quantification. Results: Real time protocol was able to detect and quantification of viral particles of Rotavirus in water samples artificially contaminated with 1, 5 and 10 ml of human feces. Conclusion: It was possible to detect Rotavirus in samples of contaminated water and feces.

Poster Session

VIR-PW2066 - Phylodynamics and molecular evolution of re-emerge Rabies virus in Taiwan

Yung Cheng Lin[1,2], Hsin Fu Liu[2], Jih Hui Lin[3]
[1]National Taiwan Ocean University, Keelung, Taiwan, [2]Mackay Memorial Hospital, Taipei, Taiwan, [3]Research and Diagnostic Center, Centers for Disease Control, Taipei,Taiwan

Aims: Rabies is an important zoonotic infectious disease in the world. Taiwan has been the rabies free countries since 1960. However, this situation has been changed in 2013 by the discovery of rabies virus infected ferret badgers. It is interesting to know the branching time of Taiwan lineage and its evolutionary history. The aim of this report is to characterize the molecular phylogeny and phylodynamics of the re-emerge rabies virus in Taiwan. Methods: Phylogeny was reconstructions by the neighbor-joining, maximum likelihood, and bayesian methods based on the full length glycoprotein (G) and matrix protein (M) genes. Selection pressures were estimated as the mean numbers of nonsynonymous and synonymous substitutions per site using the single likelihood ancestor counting, fixed effects likelihood, and internal fixed effects likelihood methods with a significant level of 0.05. The evolutionary rates and population size changes of rabies were determined using Bayesian Markov Chain Monte Carlo method offered in BEAST v1.8.0. The MCMC chains were run for sufficient time to achieve convergence (ESS>200). Results: Phylogenetic trees of the two envelope protein genes showed that isolates from ferret badger in Taiwan were all classified into single cluster and close to the Chinese isolates. No positive selection site was found on both genes. The evolution rates of the 3rd codon were significantly higher than that of the 1st and 2nd codon. Substitution rates of G and M gene was 5.49× 10-4 and 7.71× 10-4 , respective. The times of the most recent common ancestor was estimated around 72.05 and 45.74 years, respective. The phylodynamic pattern of the virus was steady. Conclusions: Our data demonstrated the rabies virus isolates in Taiwan was an independent lineage. This suggested rabies virus has been latent for long time in Taiwan. Extensive surveillance of the virus from different animals is required for epidemic control.

Poster Session

VIR-PW2068 - Deep Sequencing-based analysis of norovirus populations in individuals with acute gastroenteritis

Kazushi Motomura[1,2,3], Hirotaka Ode[3], Masaru Yokoyama[3], Tomoichiro Oka[4], Kazuhiko Katayama[4], Mamoru Noda[5], Tomoyuki Tanaka[6], Naokazu Takeda[1,2], Hironori Sato[3]

[1]Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI), Department of Medical Sciences, Thailand, [2]Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, [3]Pathogen Genomics Center, National Institute of Infectious Diseases, Tokyo, Japan, [4]Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan, [5]National Institute of Health Sciences, Tokyo, Japan, [6]Sakai City Institute of Public Health, Osaka, Japan

Our previous studies using a large set of nearly full-length viral genomes collected in Japan between 2006 and 2009 (Motomura K et al., J. Virol, 2010, 84:8085–8097) suggested that the genome of most of the norovirus (NoV) epidemic variants are composed of a part of the genome derived from recently prevalent NoV variants forming mosaic-like structures. Generation of recombinant viruses should require infection of a single individual by multiple lineages of NoV variants. To address this issue, we conducted 454-pyrosequencing to investigate viral populations in 24 individuals with acute gastroenteritis. We collected 14,000 to 28,000 reads per specimen, and analyzed their phylogenies using the capsid N-terminal/shell coding region (300 nucleotides). In addition to the major NoV variants detected by a conventional direct sequencing method, we detected various lineages composed of minor NoV strains in 9 specimens (38%). They were genetically related to the previously prevalent variants and represented 0.2 to 47.7% of the total populations of individual samples. The minor variants were basically composed of non-GII.4 GII genotypes (GII.3, 6, 9, and 13) and/or past GII.4 epidemic subtypes in Japan (2004/05, 2006b, 2008a, and 2009a). These findings suggest that the genetic recombination due to coinfection of NoV variants classified into distinct lineage is a common event in NoV infections in nature. (This study was collaborated with Norovirus Surveillance Group of Japan. We would like to appreciate that the surveillance group provided clinical specimens to us.)

Poster Session

VIR-PW2070 - Inferring the population structure of Aivian Influenza with Tajima's D and its application to global surveillance

Kiyeon Kim[1], Kimihito Ito[1]

[1]Research Center for Zoonosis Control, Sopporo, Japan

In this research, I propose the use of Tajima's D to detect hidden ongoing outbreaks. I speculate that when the viruses infect natural host animals, they undergo neutral evolution, resulting in Tajima's D value being about zero. When the viruses infect animals other than the natural host, they will have different demography from that of in natural hosts and the Tajima's D will not show zeros. By calculating this statistic from the sequence data, I anticipate we could figure out whether the viruses are in stable state or not. Sequence data of influenza A viruses were downloaded from public databases, including NCBI Influenza Virus Resource, Hokkaido University Influenza Virus Database System and GISAID. Sequences are divided into two categories, which are isolated from natural host and from non-natural host. Then Tajima's D was calculated for each RNA segments. As we have expected, we observed the Tajima's D value around zero in viruses isolated from wild ducks, which is the natural reservoir of influenza viruses. And negative value was obtained from H7N9 influenza viruses isolated in China in March 2013, suggesting that the number of infections in birds or humans were increased during that period. Also, I could find different D value patterns in some RNA gene segment though they are in same host. I believe that this idea could be applied to global surveillance study to detect expanding strain in population and can increase the chance to predict probable outbreak of zoonosis, just by using sequence data of its pathogens.

Poster Session

VIR-PW2072 - Frequency of genotypes of White Spot Syndrome Virus in shrimps from farms of Nayarit state, Mexico using the ORF 75 and ORF 125 as molecular markers

Ma de Jesus Duran-Avelar[1], Norberto Vibanco-Perez[1], Jose Francisco Zambrano-Zaragoza[1], Leobardo Rodriguez-Montoya[3], Alma Castañeda-Duran[1], Ricardo Perez-Enriquez[2]

[1]UA Ciencias Quimico Biologicas y Farmaceuticas Universidad Autonoma de Nayarit Tepic, Nayarit Mexico, [2]Centro de Investigaciones Biologicas del Noroeste, La Paz, Baja California Sur, Mexico, [3]Centro de Investigación en Alimentación y Desarrollo AC, Mazatlan, Sinaloa, Mexico

The virus genome of white spot syndrome (WSSV) is composed of double-stranded DNA has a size of approximately 300 kb and contains 184 open reading frames (ORFs), there are 3 complete sequences of genomes of WSSV isolated from Taiwan (WSSV-TW), China (WSSV-CN) and Thailand (WSSV-TH) that share an identity of 99.32%. Shrimp farming in Mexico takes place mainly in the North West region, Nayarit state is the third largest producer of this crustacean, but production decreases when disease outbreaks occur, including one of the most important is the syndrome caused by white spot virus (WSSV), which can cause mortalities up to 100%. However, sometimes despite the existence of infected organisms this mortality is very low, this suggests the existence of different strains of the virus. In this work the frequencies that were presented in the period 2010-2012 of different WSSV genotypes in Nayarit´s farms were established. Genotyping was performed by sequencing PCR products of ORF 75 and ORF 125. For ORF 75, who has tandem repeats of 45 and 102 bp. 12, 2 and 19 farms were analyzed during 2010, 2011 and 2012 respectively, were found 8 different genotypes , being the most abundant (39 %), one with 7 repeat units (RUs) . Regarding ORF 125, which has tandem repeats of 69 bp with five SNPs by RU (except the first two and last) were found 19 genotypes in 2011-2012, the most frequent was of six RUs, thirteen genotypes were found only one by farm. The great diversity of genotypes based on these ORFs could explain the different behaviors of outbreaks.

Poster Session

VIR-PW2074 - Isolation of Chikungunya Virus from mosquitoes in Ibadan, Nigeria

Johnson Adeniji[1], Adedayo Faneye[1], Kingsley Ayegboyin[1]
[1]Department of Virology, University of Ibadan, Ibadan, Nigeria

Introduction Chikungunya fever is a mosquito- borne viral disease caused by chikungunya virus and spread by Culicine arthropod vectors. The virus, an RNA virus, belongs to the Alphavirus genus of the family Togaviridae. Chikungunya presents with fever, muscle pain, head-ache, fatigue, rash and severe joint pain characteristically. These symptoms are similar to those of dengue fever. The virus is transmitted from human to human by bites from infected females of Aedes aegypti and Aedes albopictus mosquitoes which feed mostly by day. Chikungunya has been reported in over 40 countries, spanning Africa, Asia and Europe as well as the Americas. Recently, there had been pockets of outbreak reported in the Carribean. Objectives This study was aimed at isolating chikungunya virus from mosquitoes in Ibadan Nigeria Methods A total of 489 Day biting mosquitoes were collected between July and November 2013. The mosquitoes were sorted into species and pooled into 19 pools. The mosquito pools were homogenised in Eagles MEM and total RNA was extracted from the solution using RNA extraction kit by Jena Bioscience (Germany). The extracted RNA was transcribed to cDNA by reverse transcription using random hexamers. The cDNA was then amplified by PCR using specific primers targeted at 600bp of chikungunya envelope gene. The amplified products were detected using Agarose gel electrophoresis. Results Of the 19 pools of mosquitoes processed, ten pools were Aedes aegypti while nine pools were Aedes albopiticus. Two of the ten pools of the Aedes aegypti were positive for chickungunya while two of the nine pools of Aedes albopiticus were positive. Conclusion This study showed that chikungunya virus is circulating in Nigeria and that the disease could be endemic in the country.

Poster Session

VIR-PW2076 - The New World and Old World alphavirus nsP3 proteins interact with distinct, virus-specific sets of cellular proteins

Elena Frolova[1], Niall Foy[1], Ivan Akhrymuk[1], Svetlana Atasheva[1], Ilya Frolov[1]
[1]Department of Microbiology, University of Alabama at Birmingham, USA

The Old World (OW) and the New World (NW)alphaviruses represent two groups of geographically isolated viruses, which have evolved independently for almost 3,000 years. These viruses have the same genome organization and encode only four nonstructural proteins, nsP1-4. In addition to being involved in viral RNA replication and formation of infectious virions, these proteins also regulate critical aspects of virus-host cell interactions and modify the intracellular environment to promote efficient virus replication. Thus, most of the nsPs are multifunctional, interact with numerous cellular factors and modify a variety of cellular processes. Functions of nsP1, nsP2 and nsP4 have been intensively investigated and are reasonably well described. However, so far, no specific function has been ascribed to nsP3. This protein is encoded by the genomes of all alphaviruses, but demonstrates a very high level of sequence diversity,determined by their carboxy terminal hypervariable domain (HVD). We have found that the HVD determines formation of virus-specific complexes, which differ in size, morphology and are interaction withcellular proteins.We and others have previously described the set of cellular proteins that interact with nsP3 of several OW alphaviruses. Here, we demonstrate that the host proteins interacting with nsP3 of VEEV, a representative member of the NW alphaviruses, are very different. Alphaviruses with extended mutations in the HVDs rapidly evolve in a cell-specific mode. Interestingly, in spite of a lack of sequence homology between the HVDs of the NW(VEEV) and OW (SINV) alphaviruses, they can functionally replace each other in chimeric alphaviruses..The data suggests that nsP3 of different viruses have a common function, which is achieved by interaction with different sets of host proteins, which we have identified.

Poster Session

VIR-PW2078 - The number and position of N-linked glycosylation on Japanese encephalitis virus envelope protein is important for its replication and virulence

Jian-Jong Liang[1], Min-Wei Chou[1,2], Yi-Ling Lin[1,2]
[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [2]Department of Microbiology and Immunology, National Defense Medical Center, Taipei, Taiwan

The major structural envelope (E) protein of Japanese encephalitis virus (JEV) facilitates cellular binding and entry, and is the primary target of neutralizing antibodies. JEV E protein has one N-linked glycosylation site at residue N154, whereas the related dengue virus E protein might have two N-linked glycosylation sites at residues N67 and N153. To study the importance of N-linked glycosylation on JEV E protein, we generated three recombinant viruses with mutated E protein. JEV mutant E-D67N/N154A, which changed the glycosylation position from residue 154 to 67, and E-N154A, which was not glycosylated, showed reduced viral growth in culture cells and lost their neurovirulence and neuroinvasiveness in challenged mice. Mimicking the E protein of dengue virus, we created a new potential N-linked glycosylation site at reside 67 of JEV E protein and the resulted JEV mutant E-D67N had 2 glycosylation sites on its E protein. E-D67N mutation did not hamper the in vitro viral replication and neurovirulent, however its neuroinvasiveness was greatly lost. Mice immunized with these glycosylation-mutated viruses induced neutralizing antibody and protected mice from lethal JEV challenge. To address the attenuation mechanism, we test whether these glycosylation mutants might interact with cellular lectins, such as DC-SIGN and L-SIGN, differently. The growth of wild-type JEV was similar in cells with or without DC-SIGN overexpression, whereas the extracellular viral production of E-D67N and E-D67N/N154A was lower in cells with DC-SIGN overexpression. Furthermore, the viral release of E-D67N and E-D67N/N154A was also reduced with L-SIGN overexpression. Thus, the patterns of N-linked glycosylation on E protein will affect the interaction of JEV with cellular lectins, and then may contribute to viral replication and pathogenesis.

Poster Session

VIR-PW2080 - Role of HIV-1 envelope glycoproteins variable regions V4 and V5 in the transition to the CD4-bound conformation

Mathieu Coutu[1,2], Andrés Finzi[1,2]
[1]Université de Montréal, Montreal, Canada, [2]CHUM Research Centre, Montreal, Canada

HIV infects cells by fusing it's membrane with the membrane of the target cell. This fusion is performed by the envelope glycoproteins (Env) which are synthesized as a precursor, gp160, which is later cleaved into gp120 and gp41. The transmembrane protein gp41 anchors the envelope complex to the viral particle whereas the gp120 ensures the binding to the cell receptor CD4 and coreceptor CCR5 or CXCR4. These sequential interactions trigger conformational changes on Env that fuel the viral entry process ultimately leading to the insertion of the gp41-derived-fusion peptide into the target cell membrane. The exterior subunit gp120 contains five variable regions (V1 to V5), of which three (V1, V2 and V3) have been shown to restrain the spontaneous sampling of the CD4-bound conformation by gp1201. However, the role of variable regions V4 and V5 in these conformational changes remains unknown. To investigate their effect, mutants of the clade B YU2 primary isolate, comprising a deletion of the V5 or mutating all V4 potential glycosylation sites, have been generated. The effect of mutations on the conformation of the envelope glycoproteins was analyzed by immunoprecipitation with conformation-dependent Abs and surface plasmon resonance. Neither the deglycosylation of the V4 or the removal of V5 affected Env conformational changes as measured by these techniques. Thus, indicating that variable regions V1, V2 and V3 are the major players in preventing Env from spontaneously snapping into the CD4-bound conformation. 1 Do Kwon, Y. et al (2012). Unliganded HIV-1 gp120 core structures assume the CD4-bound conformation with regulation by quaternary interactions and variable loops. Proceedings of the National Academy of Sciences, 109(15), 5663-5668.

Poster Session

VIR-PW2082 - Comprehensive studies on the inactivation of non-enveloped viruses by alcohols

Keiko Ikeda[1], Junko Morishita[2,3], Tamiko Nagao[3,4], Yukiko Suzuki[1], Tomomi Kuwahara[3], A. Hajime Koyama[2]
[1]Sch. Health & Nursing Sci., Wakayama Medical University, Wakayama, Japan, [2]Wakayama Shin-ai Women's Jr College, Wakayama, Japan, [3]Grad. Sch. Medicine, Kagawa University, Kagawa, Japan, [4]Sch. Nursing, Nagoya City University, Nagoya, Japan

Non-enveloped viruses, particularly those transmitted by the enteric route, are relatively resistant to common disinfectants. Considering a convenient use of alcohols for the inactivation of those viruses to prevent viral spread in our daily life, we examined the effects of ethyl alcohol (EtOH), isopropyl alcohol (2-PrOH) or propyl alcohol (1-PrOH) on the infectivity of feline calicivirus (FCV), poliovirus type 1 (PV-1) and Coxsackie B virus type 5 (CB-5) in vitro. Systematic characterization revealed that (1) FCV is inactivated by these alcohol in a pH-dependent manner; alcohols are effective at the neutral pH, but not acidic pH. 1-PrOH is the most effective, but 2-PrOH is marginal for FCV. (2) Although PV-1 was less sensitive to alcohols than FCV, the similar pH-dependence was observed. FCV is transmitted by the droplet infection and PV-1, as a vaccine strain, is temperature-sensitive mutant, CB-5, a wild type enteric virus, was examined. (3) CB-5 showed high sensitivity to EtOH with pH-dependence. (4) Although the inactivation of viruses by alcohols was clearly weak at acidic pH, the virucidal activity at acidic pH of alcohols was dramatically increased in the presence of 0.5 M NaCl, These results showed that the efficacy of alcohols in inactivation of the enveloped viruses is strongly dependent to the virus species, solvents and applied conditions.

Workshop Sessions

BAM-WK113.01 - Bacterial species may exist, metagenomics reveal

Kostas Konstantinidis[1]
[1]*Georgia Institute of Technology, Atlanta, USA*

Whether or not the vast microbial biodiversity is organized in discrete units that correspond to ecologically coherent species remains an unresolved issue of major theoretical as well as practical consequences, in both clinical and environmental settings. Patterns of genomic variation maintained within natural microbial communities can potentially provide new insights into this issue. Examination of such patterns in large metagenomic datasets revealed that bacteria and archaea predominantly form sequence-distinct populations, with the intra-population genomic sequence divergence ranging from ~1% to ~6%. The genotypes of a sequence-discrete population appear to cohere together, at least in part, via means of genetic exchange (homologous recombination) and are typically more uniform among themselves in terms of evolutionary relatedness, functional gene content and gene expression patterns compared to genotypes/strains of many validly "named" species. Therefore, these populations may constitute the important units of microbial communities (species?) and their identification represents a major foundation for beginning high-resolution investigations on how populations are organized, interact, and evolve within communities. An attempt to reconcile these findings with those of previous studies that reported indiscrete species and a genetic continuum within bacterial taxa will be also made during this presentation.

Workshop Sessions

BAM-WK113.02 - Compatible solutes from mesophiles to hyperthermophiles and back

Milton S. da Costa[1]
[1]*University of Coimbra, Coimbra, Portugal*

All microorganisms must adjust, within intrinsic limits, to alterations in the water activity of the environments. The majority of the compatible solutes encountered in hyper/thermophiles are unique to these organisms. These compatible solutes include mannosylglycerate (MG) and di-myo-inositol-phosphate (DIP) derivatives. We have studied the synthesis of (MG) and trehalose in Thermus thermophilus, Rubrobacter xylanophilus and Persephonella marina among other thermophilic and mesophilic organisms. Unlike other species of this genus, the species Thermus thermophilus is capable of growing in media containing 3 to 5% NaCl. The strains of T. thermophilus accumulate primarily trehalose and lower levels of (MG) during osmotic adjustment. Recombinant mutants lacking the genes for the synthesis of trehalose, MG or both, result in a profound effect on the ability of organisms to grow in media containing NaCl. The synthesis of MG by T. thermophilus proceeds via a two step pathway catalyzed by mannosyl-phosphoglycerate synthase and mannosyl-phosphoglycerate phosphatase from GDP-mannose and 3-phosphoglycerate. These enzymes are very similar to those found in other hyper/thermophilic organisms, The homologous enzymes of R. xylanophilus lead, depending of the substrate, to the synthesis of MG or glucosylglycerate (GG). The physiological relevance of MG and GG accumulation in these thermophilic bacteria and the evolution of MG and GG biosynthesis in prokaryotes are discussed. These studies led us to encounter a gene in the species of Mycobacterium that leads to the synthesis of GG that is bound to a polysaccharide but does not serve as a compatible solute. Other compatible solutes have been identified in some deep-branching species of bacteria will be discussed, namely mannosylglycosylglycerate (MGG).

Workshop Sessions

BAM-WK113.03 - Metagenomics of hypersaline environments: phylogenetic and metabolic prokaryotic diversity

Antonio Ventosa[1], Ana B. Fernández[1], María-José León[1], Blanca Vera-Gargallo[1], Cristina Sánchez-Porro[1]
[1]*Department of Microbiology and Parasitology, University of Sevilla, Sevilla, Spain*

Hypersaline environments are represented by aquatic and terrestrial systems in which the high concentration of salts and other environmental factors limit the microbial diversity. In contrast to hypersaline lakes, marine salterns are excellent models for studying the gradient of salinity from seawater to salt saturation. These salterns are composed of a series of ponds in which the seawater is concentrated by evaporation for the commercial production of salt. One of the salterns that has been most extensively studied with respect to the microbial diversity during the last 30 years is located in Santa Pola, Spain. Culture-dependent and more recently molecular approaches have been used in order to determine the microbial populations and the role of these microbes along the salinity gradient. However, most studies were focused on the most concentrated ponds (crystallizers) in which the microbial community is reduced to the square archaeon Haloquadratum walsbyi and Salinibacter ruber, as well as some other halophilic Archaea. We carried out metagenomic studies from the water of four ponds with salinities of 13%, 19%, 33% and 37% salts, from which total prokaryotic DNA was extracted and sequenced by 454 pyrosequencing. The microbial population of the crystallizer pond (37% salts) is mainly represented by H. walsbyi, as well as representatives of Halorubrum, the not yet cultured Nanohaloarchaeon Candidatus Haloredivivus, and Salinibacter ruber as the only abundant extremely halophilic bacterium. However, on the intermediate salinity ponds a more diverse microbial population was observed, represented by Euryarchaeota and members of other seven bacterial higher taxa. In the ponds with 13 to 19% salt a large proportion of the sequences were related to a new gammaproteobacterium (related to the genera Alkalilimnicola and Nitrococcus). We have been able to isolate in pure culture this new bacterium, which present some new interesting features, and described it as Spiribacter salinus.

Workshop Sessions

BAM-WK113.04 - The highly diverse epibiotic microbial community of sulfide tube worms inhabiting deep sea hydrothermal vents is capable of anaerobic respiration on several metal(loid) oxides

Chris Maltman[1], Graham Walter[1], Vladimir Yurkov[1]
[1]University of Manitoba, Winnipeg, Canada

Black smokers have already provided us with many novel bacteria possessing new abilities. However, while much work has been done in regards to the microbial communities of vent chimneys, in plume waters, the surrounding subsurface, or in microbial mats in the vicinity, the epibiotic microorganisms of vent tube worms have received little attention. One of the most amazing capabilities these bacteria possess is dissimilatory anaerobic metal(loid) oxide reduction, which is extremely rare. Since deep sea hydrothermal vents are enriched in tellurium, selenium, and vanadium, many of the microbes from this ecosystem have evolved to deal with their presence. In fact, the first example of anaerobic respiration on a tellurium oxide (tellurate) and orthovanadate was discovered in isolates from a deep sea hydrothermal vent sulfide tube worm. In this study, we screened 107 isolates from worms of the main Endeavor vent field, Juan de Fuca Ridge in the Pacific Ocean for anaerobic metal(loid) oxide respiration on tellurite (TeO32-), tellurate (TeO42-), selenite (SeO32-), selenate (SeO42-), metavanadate (VO3-), and orthovanadate (VO43-). All but strain ER-V-1 were capable of utilizing at least one of the oxides for anaerobic respiration, and showed significant growth in their presence versus absence. Partial 16S rRNA gene sequencing revealed the metal(loid) respiring community associated with sulfide tube worms is comprised of several taxonomical groups, including: Shewanella, Pseudalteromanas, Pseudomonas, Vibrio, Curvibacter, Marinobacter, Okibacterium, and Thalassospira genera. Sequence similarity ranged from as high as 100% to as low as 91%, promising new genera. Our work shows that this extraordinary ability is possessed by many more bacteria than previously thought, and it is carried out by members of various genera. Also, our discovery indicates that highly toxic oxides are of biological importance and can play an integral part in microbial growth/communities.

Workshop Sessions

BAM-WK113.05 - Life in sea ice − proteomic insights into a proteorhodopsin-containing sea-ice dwelling flavobacteria

Shi Feng[1], Shane M Powell[1], Richard Wilson[2], John P Bowman[1]
[1]Tasmanian Inst. Agriculture, University of Tasmania, Hobart, Australia, [2]Central Science Laboratory, University of Tasmania, Hobart, Australia

Although much effort has been put into the investigation of the microbial ecology of sea ice ecosystems physiological studies of sea ice microbes have been rarely reported. The extreme psychrophilic bacterial species Psychroflexus torquis is considered a model sea-ice microorganism, occurring in both the Arctic and the Antarctic, which has adapted to an epiphytic lifestyle in sea-ice algal assemblages. The comprehensive quantitative proteomic study performed here indicated P. torquis responds to changing salinity and illumination conditions by regulating its energy generation, nutrient uptake strategy, and adhesion ability. Furthermore, salinity and illumination intensity were observed to highly interact in affecting the abundance of nutrient uptake transporters, detoxification systems and gliding motility. The data suggests that P. torquis responds to changes in both light energy and salinity to closely modulate its energy production, metabolic processes and other aspects of its lifestyle in sea-ice, a strategy that would be especially advantageous during the polar summer ice algal bloom. This study provides insights into the life strategy of a model sea-ice bacteria species and demonstrates the complex interrelation of physiological processes connected to the light harvesting proton pump proteorhodopsin.

Workshop Sessions

BAM-WK114.01 - Introduction of the Institut Pasteur International Network

Marc Jouan[1]
[1]*Pasteur Institute, Paris, France*

"No abstract available at time of publication"

Workshop Sessions

BAM-WK114.02 - Plasmodium falciparum artemisinin resistance: From phenotype to genotype

Didier Ménard[1], Benoit Witkowski[1]
[1]*Institut Pasteur, Phnom Penh, Cambodia*

The emergence of Plasmodium falciparum resistance to artemisinin derivatives observed in Cambodia threatens the world's malaria control and elimination efforts. Since the first reports in 2008 from Battambang province and 2009 from Pailin province, both in western Cambodia, artemisinin-resistant P. falciparum malaria has been reported elsewhere in western Cambodia, western Thailand, southern Burma and southern Vietnam. The risk of artemisinin-resistant parasites spreading from South East Asia to Africa, as happened previously with chloroquine- and sulphadoxine/pyrimethamine-resistant parasites, is extremely worrisome. Until 2013, artemisinin resistance was only recognized as a relatively slow parasite clearance rate in patients receiving an artemisinin or ACT. Logistically and financially demanding [parasite clearance studies require screening of thousands of febrile individuals over entire transmission seasons to enroll the few patients (<5%) who meet inclusion criteria and agree to several days of hospitalization], as well as inconvenient for patients and their families, this clinical phenotype is not effective to rapidly detect new artemisinin resistance foci and implement containment interventions in areas where ACTs remain the most affordable, effective antimalarials. An urgent need to develop in-vitro/ex-vivo assay readouts that correlate with parasite clearance half-life and to identify a molecular marker of artemisinin-resistant malaria that can be used to map resistance and guide efforts to eliminate it. The presentation will be focused on 2 majors advances achieved in 2013: firstly, the development of two novel in-vitro and ex-vivo ring-stage survival assays (RSAs) capable to detect artemisinin-resistant, slow-clearing P falciparum infections in patients with malaria and secondly, the original approach combining genomics, biology, clinical studies and epidemiology used to identify a molecular marker closely linked to artemisinin resistance in Plasmodium falciparum.

Workshop Sessions

BAM-WK114.03 - Exploratory and confirmatory analysis for biomarker discovery and validation

Magnus Fontes[1]
[1] *Institut Pasteur, Paris, France*

In the preclinical Exploratory Phase in Biomarker discovery today a broad range of statistical and general mathematical methods including different types of data visualizations are often tested, both in unsupervised high throughput set-ups as well as in more targeted approaches. On the other hand in later validation phases for Biomarkers, traditional measures such as True/False Positive Rate and True/False Negative Rate together with the corresponding ROC curves and confidence intervals are often the only statistical measures that are used and presented. Fairly complex dimension and model reduction techniques connected with informative visualizations of the data under consideration can sometimes be very useful not only in an exploratory discovery phase but also as a support in clinical decision making. In this scenario we agrue that it could be useful to complement the traditional statistical measures used for validation with intrinsic statistical stability analyses of the underlying reduction and visualization algorithms.

Workshop Sessions

BAM-WK114.04 - Primary mutations that confer resistance to Raltegravir and Elvitegravir are incompatible with the R263K mutation that is associated with low-level resistance to Dolutegravir

Kaitlin Anstett[1], Thibault Mesplede[1], Peter Quashie[1], Maureen Oliveira[1], Mark A. Wainberg[1]
[1]McGill University AIDS Centre, Jewish General Hospital, Montreal, Canada

Background: No HIV-infected patient, naive to the therapeutic use of integrase strand transfer inhibitors (INSTIs), has yet developed resistance against dolutegravir (DTG). To characterize the resistance profile of DTG, we selected for resistance in tissue culture against this compound. Methods: We grew HIV-1 of different subtypes in both MT-2 cells and in peripheral blood mononuclear cells over protracted periods, with the concentration of DTG being incrementally increased from 0.05 nM, i.e. 4 times less than the EC50. Results: R263K or G118R followed by H51Y were the most frequent integrase resistance mutations to arise in subtypes B and C, respectively. R263K alone conferred an approximate 2-5-fold level of resistance to DTG and a 30% drop in levels of recombinant integrase strand transfer activity and viral replicative capacity. Although H51Y alone did not significantly affect either enzyme activity or DTG resistance, the combination of R263K together with H51Y increased DTG resistance to about 12-fold accompanied by a ≈70% loss ineach of viral replication capacity, the ability of viral DNA to become integrated into host cell DNA, and integrase strand tranfer activity as measured in biochemical assays using purified integrase enzyme. Over > 1 year, no additional possibly compensatory mutations were identified. Conclusions:These results stand in contrast to those obtained with other drugs,whereby secondary mutations increase overall levels of drug resistance and simultaneously increase viral replication and enzyme function, and help to explain why primary resistance to DTG has not yet arisen in clinical studies. Validation of these findings in animal models may support the use of DTG in strategies aimed at purging HIV cellular reservoirs, perhaps over several cycles of DTG treatment, if it can indeed be shown that resistance to DTG is not compensated by other mutations located either within the integrase gene or elsewhere in the HIV genome.

Workshop Sessions

BAM-WK114.05 - Rift Valley fever emergence in Senegal and Mauritania in 2012-2014

Amadou Sall[1]

[1]*Institut Pasteur de Dakaar, Dakaar, Senegal*

"No abstract available at time of publication"

Workshop Sessions

BAM-WK115.01 - The Genomic Avenue to Avian Colisepticemia

Sagi Huja[1], Yaara Oren[1], Dvora Biran[1], Eliora Z. Ron[1]
[1]*Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv, Israel*

Colisepticemia is an invasive infection caused by avian pathogenic *Escherichia coli* (APEC) strains. It is associated with high mortality and morbidity, resulting in significant economic consequences for the poultry industry. To understand the genetic basis of the virulence of avian septicaemia *E. coli*, we sequenced the entire genome of a clinical isolate of serotype O78 (O78-9) and performed systems-wide analyses to characterize the transcriptomes and proteomes of these bacteria and their response to host conditions. We focused on the serum-induced functional genomic changes, as the ability to grow in serum is an essential pre-requisite for septicemia. These studies led to identification of genes and regulators that are involved in serum survival and highlighted the role of metabolic adaptation in colisepticemia and in virulence.

Workshop Sessions

BAM-WK115.02 - Biochemical and functional analysis of modified heptoses from the capsule of Campylobacter jejuni

Matthew McCallum[1], Anthony Wong[1], Dirk Lange[1], Sebastien Houle[2], Steven Shaw[1], Charles Dozois[2], Gary Shaw[1], Carole Creuzenet[1]

[1]Western University, London, Canada, [2]Institut Armand Frappier, Montreal, Canada

The capsule of Campylobacter jejuni is an external polysaccharide layer that is important for colonization and virulence in various infection models. In most C. jejuni strains, the capsule comprises a modified heptose whose biological roles and biosynthetic pathways are unknown. We deciphered the complete biosynthesis pathway for 6-deoxy-D-altro-heptose of strain 81-176 and used a surrogate substrate to decipher the L-gluco-heptose synthesis pathway of strain NCTC 11168. This allowed a direct comparison of novel C3/C5 epimerases and C4 reductases involved in these pathways. Overall, we determined the activity of 7 enzymes, revealing unexpected functions and substrate or product specificities, as well as complex regulatory loops. Knockout mutagenesis studies of four heptose modifying genes in strain NCTC 11168 showed that heptose modification is not necessary for capsule synthesis but affects bacterial resistance to serum and bile salts, and also affects adhesion to and invasion of intestinal epithelial cells. Furthermore, alteration of capsule composition in the mutants leads to decreased phagocytosis by macrophages but does not compromise survival of C. jejuni inside macrophages. We also demonstrate that heptose modifying genes are important for colonization and persistence of C. jejuni within the chicken intestine. Together, these findings suggest that the fine tuning of the composition of the C. jejuni capsule contributes to the survival of the bacterium within intestinal epithelial cells and to efficient colonization, cellular invasion, and persistence within phagocytic cells. This work, combining functional and biochemical data, provides grounds for the elucidation of similar pathways found in other C. jejuni strains, other campylobacters and other pathogens. It provides new molecular tools for the synthesis of carbohydrate antigens useful for vaccination and for the screening of enzymatic inhibitors that may have antibacterial effects and could be used to decrease Campylobacteriosis.

Workshop Sessions

BAM-WK115.03 - Discovery of pheromone-mediated competence for DNA transformation in the zoonotic pathogen Streptococcus suis

Edoardo Zaccaria[1], Peter van Baarlen[1], Astrid van de Greef[2], Donald A. Morrison[3], Hilde Smith[2], Jerry M. Wells[1]
[1]Wageningen University, Wageningen, The Netherlands, [2]Central Veterinary Institute, Lelystad, The Netherlands,
[3]University of Illinois at Chicago, Chicago, USA

Here we show that Streptococcus suis, a major bacterial pathogen of pigs and emerging pathogen in humans responds to a peptide pheromone by developing competence for DNA transformation. This species does not fall within any of the phylogenetic clusters of streptococci previously shown to regulate competence via peptide pheromones suggesting that more species of streptococci may be naturally competent. Induction of competence was dependent on ComX, a sigma factor that controls the streptococcal late competence regulon, extracellular addition of a comX-inducing peptide (XIP), and ComR, a regulator of comX. XIP was identified as an N-terminally truncated variant of ComS. Different comS alleles are present among strains of S. suis. These comS alleles are not functionally equivalent and appear to operate in conjuction with a cognate ComR to regulate comX through a conserved comR-box promoter. We demonstrate that these ‘pherotypes’ can be genetically transferred between strains, suggesting that similar approaches might be used to control competence induction in other lactic acid bacteria that lack ComR/ComS homologues but possess comX and the late competence regulon. The approaches described in this paper to identify and optimize peptide-induced competence may also assist other researchers wishing to identify natural competence in other bacteria. Harnessing natural competence is expected to accelerate genetic research on this and other important streptococcal pathogens. As an example, we show how this new transformation system can be used to generate transposon mutant libraries.

Workshop Sessions

BAM-WK115.04 - Use of a microfluidic system to study host-pathogen interaction in enterohemorrhagic and enteropathogenic Escherichia coli

Yannick Tremblay[1], Philippe Vogeleer[1], Mario Jacques[1], Josée Harel[1]
[1]Groupe de Recherche sur les Maladies Infectieuses du Porc, Université de Montréal, Canada

In laboratory settings, host-pathogens interactions are usually studied using cell lines cultured in flasks or tissue culture plates. These dishes are closed systems and this often leads to the accumulation of cytotoxic bacterial products, such as metabolites and toxins.. Furthermore, there is a lack of shear force in these models and shear force is present at several sites in the host such as the intestinal and urinary tracts. Continuous flow reactors incorporate shear forces and help reduce the accumulation of toxins or other cytotoxic bacterial products. Recently, microfluidic systems, which incorporates shear force and very small volumes (e.g. 20 µl), have been developed to provide cell biology model that resemble in vivo conditions. Therefore, the objective of this study was to determine if the Bioflux 200 microfluidic system could be used to grow a human colorectal cell line, HRT-18, and study host-pathogen interaction in enterohemorrhagic and enteropathogenic Escherichia coli. Type strains of enterohemorrhagic and enteropathogenic E. coli were selected for our investigation. To visualize bacterial attachment to HRT-18 under a microscope, these strains were transformed with a plasmid carrying a fluorescent protein. Initially, different parameters for optimal growth of HRT-18 were tested including initial cell density and the presence or absence of shear force during the formation of the monolayer. HRT-18 was able to form a monolayer within 72 hours in the Bioflux 200 system if the initial cell concentration was 2×10^7. When bacteria were added, the HRT-18 monolayer remained intact. Furthermore, both enterohemorrhagic and enteropathogenic E. coli remained attached to HRT-18, and developed microcolonies in the presence of shear force. In conclusion, conditions have been optimized to allow the study of host-pathogen interactions of enterohemorrhagic and enteropathogenic E. coli in the presence of shear force in a microfluidic system.

Workshop Sessions

BAM-WK115.05 - Antibody responses and fecal bacterial shedding in a subclinical Johne's disease calf model

Kevin J. Stinson[1], Brandon L. Plattner[1]
[1]*University of Guelph, Guelph, Canada*

Johne's disease (JD) is a chronic progressive intestinal infection in ruminants caused by the bacterium Mycobacterium avium subspecies paratuberculosis (MAP). In cattle, initial infection occurs in young calves through the fecal-oral route, and is followed by a lengthy subclinical period lasting months to years. During this subclinical period, live MAP is shed asymptomatically in the feces of infected animals, and therefore represents a potential source of transmission from animal to animal. Early fecal shedding is intermittent, becoming more frequent as the disease progresses. Full clinical signs include diarrhea, chronic wasting and, invariably, death. The associated loss of livestock, either through disease progression or culling of infected animals, is estimated to cost $15M in losses each year in Canada, with an estimated herd prevalence rate as high as 30-60%. Current JD control programs aim to reduce the risk of transmission by identifying and removing infected animals from herds. The most common diagnostic methods include serum antibody ELISA or fecal bacterial culture. Unfortunately, these techniques are poorly sensitive, and so a large portion of subclinical infections go undetected and the disease continues to spread. An urgent need exists to develop novel diagnostic techniques with improved sensitivity. In order to investigate the early MAP-specific antibody response, three-week old calves were infected with MAP via direct ileocecal Peyer's patch injection to induce a state of subclinical JD, as previously described. Though infected calves show limited antibody responses using ELISA by three months post infection, MAP-specific antibodies were detected by western blotting. These antibodies are potential targets for development of highly sensitive diagnostic tests during subclinical JD. Work is ongoing to characterize specific MAP protein epitopes that induce these antibody responses during early intestinal infection. Understanding the mechanisms of early anti-MAP antibody responses is essential to effectively diagnose and control the disease in the field.

Workshop Sessions

BAM-WK115.06 - Role for hemolysin F in the virulence of Escherichia coli

Patricia Martin[1], Kazunori Murase[2], Gaëlle Porcheron[3], Sébastien Houle[3], Emmanuelle Helloin[4], Jean-Philippe Nougayrède[1], Charles Dozois[3], Tetsuya Hayashi[2], Eric Oswald[1]

[1]Physiopathology Center of Toulouse-Purpan, Toulouse, France, [2]Department of Infectious Diseases Faculty of Medicine University of Miyazaki, Miyazaki, Japan, [3]National Institute of Scientific Research, Armand-Frappier Institute, Laval, Canada, [4]National Institute of Agronomic Research, Tours, France

The intestinal microbiota is the reservoir of Extraintestinal Pathogenic Escherichia coli (ExPEC) that can cause colibacillosis in poultry and urinary tract infections, newborn meningitis and sepsis in humans. The pathogenic potential of this pathovar relies on the expression of a versatile array of virulence and fitness-associated factors. The plasmid-borne hemolysin F gene (hlyF) is epidemiologically associated to the most virulent strains of Avian Pathogenic E. coli (APEC) and of Neonatal Meningitis E. coli (NMEC). However, except a putative hemolytic function, the precise role of HlyF in virulence remains unknown. In order to attribute a role for HlyF in virulence, a mutant inactivated for hlyF was constructed and analyzed in a chicken model of colibacillosis. The ΔhlyF mutant displayed reduced persistence in blood and an attenuated virulence compared to the wild type strain. An in silico analysis suggested that the hlyF gene would encode a non-secreted epimerase. The production of outer membrane vesicles (OMVs) was studied by transmission electron microscopy. This showed that the hlyF overexpression resulted in enhanced OMVs production. We evidenced that overexpression of HlyF increased the release of the cytolysin ClyA, which could explain the hemolytic phenotype of certain strains carrying HlyF. The release of other OMV-associated toxins such as the cytolethal distending toxin (CDT) was also augmented upon hlyF overexpression. In addition, OMVs from hlyF-overexpressing bacteria initiated an autophagic response in cultured epithelial HeLa cells, as evidenced by the formation of LC3 puncta, indicating the recruitment of NOD1-dependent innate immune response. In conclusion, we attributed a role for HlyF in the virulence of E. coli. The induction of OMVs by HlyF could contribute to the long-distance delivery of bacterial virulence factors, and may promote inflammation and pathology in infected hosts.

Workshop Sessions

BAM-WK116.01 - Susceptibility to Citrobacter rodentium infection is controlled by R-spondin 2

Olivier Papapietro[1], Sarah Teatero[1], Ajitha Thanabalasuriar[1], Kyoko Yuki[1], Eduardo Diez[1], Eugene Kang[1], Yves Durocher[2], Martin Marcinkiewicz[3], Danielle Malo[1], Samantha Gruenheid[1]
[1]McGill University, Montreal, Canada, [2]Biotechnology Research Institute, Montreal, Canada, [3]Cytochem Inc., Montreal, Canada

Citrobacter rodentium is a mouse pathogen widely used as a model for Enteropathogenic and Enterohemorrhagic Escherichia coli infections in humans. While C. rodentium causes self-limiting colitis in most inbred mouse strains, it induces fatal diarrhea in hyper-susceptible strains. Here we used a forward genetic approach to identify the R-spondin 2 gene (Rspo2) as a major determinant of susceptibility to C. rodentium infection. Rspo2 maps to the minimal genetic interval conferring differential response to C. rodentium infection, and the promoter region upstream Rspo2 is divergent in resistant versus susceptible mice. Robust induction of Rspo2 expression during infection in susceptible mouse strains leads to pathological activation of WNT signaling, subsequent loss of intestinal differentiation, and animal death. Conversely, mouse strains that do not induce Rspo2 following infection undergo milder, self-limiting disease with no mortality. Our data demonstrate a previously unknown role of R-spondins and WNT signaling in susceptibility to infectious diarrhea and identify R-spondin 2 as a key molecular link between enteric infection and control of intestinal homoeostasis.

Workshop Sessions

BAM-WK116.02 - Severe invasive group A streptococcus kills human neutrophils in contact- and Streptolysin O-dependent manner

Manabu Ato[1], Takayuki Matsumura[1], Tadayoshi Ikebe[1], Makoto Ohnishi[1], Haruo Watanabe[1], Kazuo Kobayashi[1]
[1]*National Institute of Infectious Diseases, Tokyo, Japan*

Severe invasive group A streptococcus (GAS) infection is characteristic for a rapid progression of the disease and high mortality of the patients. We and others have shown that high virulent phenotype of these isolates attributes to impair the protective abilities of polymorphonuclear neutrophils (PMNs) by enhanced expressions of multiple virulent factors induced by non-functional mutations of regulatory genes. However, the precise mechanisms by which PMN are selectively impaired remain to be unveiled. Here we show that severe invasive GAS isolates induce PMN necrosis by streptolysin O, shortly after binding to PMN in a Ca++ dependent manner. Either pinocytosis, inflammasome activation, or phagosome maturation is not involved in this process. Rather, activated form of CD11b of the host and a streptococcal protein, which upregulate its expression in severe invasive GAS isolates, are both responsible to PMN-GAS interaction and the subsequent necrosis. These findings provide a novel mechanism of PMN killing by highly virulent gram-positive bacteria, which may lead to understand pathophysiology of invasive bacterial infections.

Workshop Sessions

BAM-WK116.03 - Cell wall dynamics in persistent non-growing Salmonella located inside host cells

Gadea Rico[1], Estel Ramos[1], M. Graciela Pucciarelli[2], Francisco García-del Portillo[1]
[1]*Centro Nacional de Biotecnología-CSIC, Madrid, Spain,* [2]*Universidad Autónoma de Madrid, Madrid, Spain*

Salmonella enterica is a successful intracellular bacterial pathogen that colonizes all eukaryotic host cell types. The ability of this pathogen to persist and/or proliferate inside the infected cell is highly dictated by the host cell type. Thus, in contrast to the high proliferation rate exhibited by *S. enterica* inside epithelial cells, the pathogen adopts a non-growing persistent state upon entry into fibroblasts. The basis of these differences among non-phagocytic cell types are yet unknown. To get insights into the underlying mechanisms responsible for these disparate responses, we analysed changes in cell wall (peptidoglycan) structure and the relative amount of enzymes that synthesize and remodel this macromolecule when the pathogen persists inside fibroblasts. Our results reveal that specific changes in peptidoglycan structure associate to this particular state of coexistence with the infected host cell. We also demonstrate that the relative levels of a few peptidoglycan-related enzymes change dramatically in bacteria adapting to a latent state inside the fibroblast. Some of these changes might be directed to down-regulate signalling cascades based in recognition of peptidoglycan fragments. Taken together, these data point to a delicate balance between the persistent pathogen and the infected host cell that involves, among other processes, defined alterations in peptidoglycan structure and changes in biosynthetic and hydrolytic enzymes.

Workshop Sessions

BAM-WK116.04 - The Group A Streptococcus M1T1 clone evades autophagy for intracellular replication

Timothy Barnett[1], David Liebl[1], Lisa Seymour[1], Christine Gillen[1], Jin Lim[1], Chris LaRock[2], Mark Davies[1], Victor Nizet[2], Rohan Teasdale[1], Mark Walker[1]

[1]The University of Queensland, Brisbane, Australia, [2]University of California San Diego, La Jolla, USA

Autophagy is an important innate immune defence against intracellular bacteria, including Group A Streptococcus (GAS). However, the GAS strains examined to date belong to serotypes infrequently associated with human disease. Here, we show that the globally disseminated serotype M1T1 GAS clone can replicate efficiently in the cytosol of infected cells. Cytosolic M1T1 GAS (strain 5448), but not M6 GAS (strain JRS4), avoids ubiquitylation and recognition by the host autophagy marker LC3 and ubiquitin-LC3 adaptor proteins NDP52, p62 and NBR1. Expression of a streptococcal cysteine protease (SpeB) is critical for this process, as an isogenic M1T1 ΔspeB mutant is attenuated for intracellular replication and is targeted to autophagy. Furthermore, SpeB degrades p62, NDP52 and NBR1 in vitro and within the host cell cytosol. These results suggest a novel proteolytic mechanism utilized by GAS to escape the host autophagy pathway.

Workshop Sessions

BAM-WK116.05 - Active bacterial adaptation through the broad suppression of RNA polymerase II-dependent host gene expression

Ines Ambite[1], Nataliya Lutay[1], Jenny Grönberg Hernandez[1], Gustav Rydström[1], Bryndís Ragnarsdóttir[1], Manoj Puthia[1], Aftab Nadeem[1], Ulrich Dobrindt[2], Björn Wullt[1], Catharina Svanborg[1]
[1]Department of Microbiology, Immunology and Glycobiology, Institute of Laboratory Medicine, Lund University, Lund, Sweden, [2]Institute for Hygiene, University of Münster, Münster, Germany

Mucosal pathogens, elicit inflammation and pathology, reflecting their virulence factor repertoire, while the normal flora furnishes the host mucosa with ecological barriers that prevent pathogen attack while maintaining tissue homeostasis. Urinary tract infections (UTIs) constitute a highly relevant model of host-pathogen interactions and adaptations in which a same specie, Escherichia coli, can induce acute pyelonephritis in some patient, whereas other patients with bacteriuria exhibit an asymptomatic carrier state similar to bacterial commensalism. It is not clear, however, if the lack of destructive inflammation and pathology during asymptomatic carriage reflects the lack of virulence alone or an active inhibition of the disease-associated host response by the bacteria. Here we show that asymptomatic carrier strains actively suppress host gene expression by modifying RNA polymerase II (Pol II)-dependent transcription. In patients with therapy-resistant recurrent urinary tract infection, 63.3% of regulated genes were suppressed 24 hours after inoculation with asymptomatic bacteriuria Escherichia coli 83972 (ABU). Specific repressors and activators of Pol II–dependent transcription were modified. In addition to alteration of Pol-II regulators, Ser2 Pol II phosphorylation was significantly inhibited, which is associated with reduced activity of the polymerase. Pathogen-specific innate immune signaling, including TLR4/IRF3/7 and IFN dependent genes, was suppressed by ABU in different epithelial cell lines and inoculated patients. An increased frequency of strains inhibiting Pol II was epidemiologically verified in ABU compared to uropathogenic isolates ($p<0.001$). These findings suggest that through a new mechanism of transcriptional modulation, certain bacteria may establish a host beneficial, pathology-averse symbiotic state. Doing so, ABU strains promote tissue integrity while inhibiting pathology. Such modulation of innate immune response and gene expression may be essential to sustain asymptomatic bacterial carriage by ensuring that potentially destructive immune activation will not occur.

Workshop Sessions

BAM-WK117.01 - Mechanism of the novel kinase RdoA, a key player in the Envelope Stress Response of Salmonella enterica

Janet Lin[1], Nancy Martin[1]

[1]*Department of Biomedical and Molecular Sciences, Kingston, Canada*

Adaptation and survival in normally hostile conditions plays a critical role in promoting bacterial infection and transmission between hosts, survival on processed foods, and induction of antibiotic resistance. The food borne pathogen Salmonella enterica serovar Typhimurium uses posttranslational modification (PTM) to survive and flourish in the oft-hostile environments surrounding them. S. Typhimurium senses the conditions surrounding the cell and calls for adaptations at the cell surface. A two-phased mechanism called the Envelope Stress Response (ESR) uses phosphoryl-based PTM to control this adaptation process. First, the Cpx signal transduction pathway relays a need for changes at the cell surface to the transcription machinery of the cell, resulting in synthesis of new proteins. A key protein made in response to this initial signal, RdoA, participates in this second ESR phase. RdoA, a novel kinase, is hypothesized to phosphorylate a target protein. This activated protein initiates changes in cellular components that enable bacterial survival. Based on the crystal structure of YihE, RdoA's homologue in E. coli, it was hypothesized that RdoA's carboxyl tail functions akin to the activation loop commonly found in eukaryotic Ser/Thr proteins. Carboxy terminal truncation mutants had lower kinase activity and were structurally unstable compared to wildtype RdoA, indicating that, aside from having a functional role, RdoA's carboxyl tail is necessary for overall structural stability. To elucidate the amino acid residues integral to RdoA's phosphocatalytic mechanism and protein substrate binding, site-directed mutagenesis was performed upon specific residues based on conserved residues found in the catalytic domain of APH(3')IIIa, a small molecule kinase whose structure is similar to that of RdoA. In vitro kinase assays using immunoprecipitated FLAG-tagged RdoA and in vivo complementation assays were performed to test for the effects of these mutations on RdoA's kinase activity. These experiments mapped key amino acids in phosphotransfer, aiding in understanding RdoA activity.

Workshop Sessions

BAM-WK117.02 - Riboswitch discovery in bacteria via 3P-SEQ

Daniel Dar[1], Iris Karunker[1], Rotem Sorek[1]
[1]*Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel*

Riboswitches are cis-acting RNA elements that regulate gene expression upon direct binding to a small molecule ligand. In gram-positive bacteria it is estimated that 2-5% of all genes are regulated by riboswitches, but there is currently no high throughput experimental method that allows riboswitch discovery and characterization in a genome-wide manner. We developed an RNA-seq-based platform that allows unbiased, parallel in-vivo measurements of multiple riboswitch activities in different physiological conditions, and enables high-throughput in vivo ligand identification for regulatory 5' UTRs. The sensitivity and specificity of our approach is demonstrated using the model organism Bacillus subtilis.

Wednesday, 30 July 2014 **15:00 - 15:15 Room 524 B**

Workshop Sessions

BAM-WK117.03 - Riboswitch-mediated regulation of a muralytic enzyme in Streptomyces coelicolor

Renée St-Onge[1], Henry Haiser[1], Mary Yousef[1], Marie Elliot[1]

[1]Department of Biology and Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Canada

Riboswitches are cis-encoded regulatory RNAs predominantly embedded in the 5' untranslated region of an mRNA transcript. They alter the expression of their associated gene(s) upon directly binding a specific metabolite. The ydaO riboswitch, for example, is associated with two transporter-encoding genes in Bacillus subtilis and responds to elevated ATP levels by prematurely terminating transcription of these genes. In streptomycetes, ydaO-like riboswitches are mainly associated with cell wall metabolism genes, including rpfA, which encodes a peptidoglycan-cleaving enzyme required for normal spore development and germination. Interestingly, this rpfA-associated riboswitch lacks an obvious intrinsic terminator as well as key nucleotides required for ATP binding, suggesting it may function in a novel way. Our work focuses on characterizing the riboswitch-mediated regulation of rpfA gene expression in the model organism Streptomyces coelicolor. Immunoblotting analyses of RpfA revealed high levels of the muralytic protein in exponentially growing cultures; however, RpfA was no longer detectable in the culture medium once S. coelicolor transitioned into stationary phase. We found the rpfA riboswitch profoundly affected both full-length transcript abundance and overall levels of the RpfA protein; these effects were due to premature transcription termination within the ydaO-like riboswitch. We also found maximal transcription attenuation required a ten-nucleotide single-stranded region bridging two stem structures; deleting this sequence resulted in increased read-through of the transcriptional unit. Interestingly, deletion of the stem-loop required for ATP binding and ligand-induced gene regulation in B. subtilis had no impact on riboswitch activity in S. coelicolor. Our results suggest a novel mechanism of action for ydaO riboswitches in streptomycetes, and work is on-going to elucidate both the biological impact and mechanistic basis underlying this control.

Workshop Sessions

BAM-WK117.04 - The wider role of RamA in regulating virulence attributes in Klebsiella pneumoniae

Shyamasree De Majumdar[1], Jing Yu[1], Maria Fookes[2], Sean McAteer[3], Enrique Llobet[4], Shaun Spence[1], Rebecca Ingram[1], Jose Bengoechea[1,4], David Gally[3], Thamarai Schneiders[1]

[1]Centre for Infection and Immunity, Health Sciences Building, Belfast, UK, [2]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, UK, [3]Division of Immunity and Infection, The Roslin Institute and R(D)SVS, The University of Edinburgh, Midlothian, UK, [4]Laboratory Microbial Pathogenesis, Fundació d'Investigació Sanitària de les Illes Balears (FISIB) Recinto Hospital Joan March, Bunyola, Spain

Klebsiella pneumoniae is a significant human pathogen that is increasingly multidrug resistant. Despite its success in causing human disease, little is known about intrinsic factors that facilitate microbial survival in host-K. pneumoniae interaction. Intrinsic regulatory proteins such as RamA when overexpressed confer decreased antimicrobial susceptibility, however there is lack of knowledge about its wider role in gene regulation. In this work using a combination of transcriptomic profiling (directional RNAseq) and functional assays, we define the scope of RamA in regulating pleiotrophic phenotypes providing insights into microbial genetic repertoire. RamA-mediated regulation significantly perturbs the transcriptional landscape by directly binding and activating the transcription of 68 genes associated with a myriad of cellular functions. Notably, RamA directly binds and activates lpxC, lpxL-2 and lpxO, genes associated with lipid A biosynthesis which directly results in alterations of the lipid A moiety of LPS in K. pneumoniae. Functional assays demonstrate that these RamA-mediated alterations reduce susceptibility to colistin E, polymyxin and the cationic antimicrobial peptide LL-37. Importantly, we demonstrate that RamA-mediated alterations reduces adhesion and macrophage uptake and significantly more systemic dissemination of K. pneumoniae infection by the ramA overexpressing strain in in vivo intranasal murine model. These data demonstrate that RamA-mediated regulation confers reduced antimicrobial sensitivity and increased protection against the host-immune response to K. pneumoniae which supports microbial survival under selective pressure. Therefore our work defines the role of RamA as a global regulator which impacts on both host-drug and host-microbe interactions in K. pneumoniae which also serves as a paradigm for Salmonella and Enterobacter spp.

Workshop Sessions

BAM-WK117.05 - Unleashing the secondary metabolome of Streptomyces by manipulating chromatin structure

Emma Sherwood[1], Marie Elliot[1]
[1]McMaster University, Hamilton, Canada

Streptomyces bacteria have a complex multi-cellular life-cycle and produce a multitude of valuable medicinal and agricultural compounds that are collectively known as ‘secondary metabolites’. Indeed, the majority of naturally-derived antibiotics used to treat infections are produced by *Streptomyces*. Importantly, genome sequencing has revealed *Streptomyces* are capable of producing far greater numbers of secondary metabolites than had been previously realised. The secondary metabolites produced by the model species *Streptomyces venezuelae* are largely undefined, although it has the genetic capacity to make >30 different molecules. Analysis of the *S. venezuelae* transcriptome has revealed the majority of these secondary metabolic gene clusters are poorly expressed, and this is likely a major factor limiting product identification and characterization. Secondary metabolites are not produced constitutively. Instead their biosynthetic genes are subject to multiple levels of gene regulation; pathway-specific regulators act on one biosynthetic cluster, whereas global regulators affect the expression of multiple clusters. Thus these global regulators are outstanding candidates for use in broadly-stimulating secondary metabolite production. We have recently identified a new class of global regulator: the nucleoid-associated proteins. Nucleoid-associated proteins function to compact chromosomal DNA through bridging, bending and wrapping of DNA, so have the potential to exert enormously pleiotropic regulatory effects through controlling accessibility to the transcriptional machinery. In *Streptomyces*, we have determined that two nucleoid-associated proteins (sIHF and Lsr2) make critical contributions to development and antibiotic production. Deletion of these genes in *S. venezuelae* results in delayed development, a more diffuse nucleoid and a marked alteration in the production of secondary metabolites. We are using a combination of phenotypic, transcriptional and spectrophotometric assays, alongside antibiotic bioassays, to define the full phenotype of these *S. venezuelae* mutants, and will leverage this understanding to manipulate the activity of these proteins in stimulating novel antibiotic production from ‘wild’ streptomycetes.

Workshop Sessions

BAM-WK117.06 - The unique regulation of the DosR regulon in the East-Asian/Beijing lineage of *Mycobacterium tuberculosis*

Pilar Domenech[1], Alexandra Averback[1], Anya Rog[1], Daniele Curtis[1], Nishath Syed[1], Samuel Donato[1], Michael B. Reed[1]
[1]*McGill University, Montreal, Canada*

The dormancy or DosR regulon of *Mycobacterium tuberculosis* (Mtb) is a set of ~ 50 genes that are expressed in conditions that inhibit aerobic respiration. It is controlled by a two-component regulatory system comprising the DNA binding transcription factor DosR (or DevR) and two possible cognate histidine sensor kinases; DosS and DosT. Hypoxia, NO and CO are sensed through a heme-containing sensory domain (GAF domain) that regulates the autokinase activity of DosS and DosT. After autophosphorylation, the phosphate is transferred to DosR resulting in its activation and subsequent expression of the DosR regulon. Mtb DosR mutants are impaired for survival under hypoxic conditions. Although in most Mtb strains this system is inducible, we have previously shown that it is constitutively over-expressed in all “modern” isolates of the East-Asian/Beijing lineage. Interestingly, these strains are also natural mutants in DosT which further limits activation of the DosR system[1]. In our attempts to understand the distinct regulatory control of DosR in the East-Asian background, we previously reported two synonymous SNPs in the sequence of the Rv3134 gene for isolates of this lineage[1]. These SNPs (C507G and C601T) are located upstream of the dosR initiation codon within a known, constitutively active, dosR promoter region. In the present study, we have investigated the impact of these SNPs on dosR expression within the East-Asian strains. To do this we have generated a series of plasmids based on the Rv3134c-*dosR-dosS* operon (+/- the SNPs indicated above) and introduced these into wild-type H37Rv, as well as dosR KO mutants of H37Rv and the HN878-27 East-Asian strain. In addition, we have reconstituted the constitutive *dosR* over-expression phenotype within the non-pathogenic *M. smegmatis* background. Through this combination of approaches we have defined the evolutionary steps that led to the *dosR* over-expression in the East-Asian strains. [1]Fallow et al.,2010 J.Bacteriol. 192:2228-2238

Workshop Sessions

BAM-WK117.07 - The expression of the global post-transcriptional regulator RsmA is capable of self-regulation in the opportunistic pathogen Pseudomonas aeruginosa

Fabrice Jean-Pierre[1], Mariane Seguin[1], Jonathan Perreault[1], Eric Deziel[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada

Post-transcriptional regulation is a vital asset present in bacteria allowing them to rapidly adapt to changing environments. Pseudomonas aeruginosa is an opportunistic pathogen that can colonize a variety of environmental niches and can be the source of diverse infections in immunocompromised individuals. These infections can either be acute or chronic. P. aeruginosa controls the expression of multiple virulence genes using intercellular communication or quorum sensing (QS). The global post-transcriptional regulator RsmA is one element that interacts with QS in this bacterium. This post-transcriptional regulator is a key central factor that is involved in the transition between acute and chronic infection modes by repressing the translation of messenger RNAs (mRNAs) that are necessary for the establishment of chronic infections. Surprisingly, little information is available on the genetic regulation of rsmA. To better understand and further characterize its expression, we have constructed two rsmA-lacZ reporters: one transcriptional and one translational. The expression of rsmA was assessed in different genetic backgrounds. Our lacZ assays revealed that RsmA negatively regulates its own translation. Furthermore, the identification of the rsmA transcriptional start sites by 5'RACE has revealed that RsmA target sites are present on its own mRNA. By performing gel shift assays, we established that RsmA is capable of binding its own mRNA and more interestingly, involves the presence of a binding site located within its coding sequence. Finally, our results show that RsmA can directly auto-regulate itself by inhibiting its own translation.

Workshop Sessions

BAM-WK117.08 - A regulatory feedback loop between RpoS and SpoT supports the survival of Legionella pneumophila in water

Hana Trigui[1], Paulina Dudyk[1], Sebastien P. Faucher[1]
[1]McGill University, Montréal, Canada

Introduction: Legionella pneumophila, the causative agent of Legionnaires' disease, is transmitted to humans by the aerosolization of contaminated water. The nutrient-poor conditions of water systems likely mimic condition found when bacteria reach stationary phase. The objective of this study is to investigate the role of rpoS in the survival of L. pneumophila in water. Results: The rpoS mutant showed a significant survival defect compared to the WT strain in defined water medium (DFM). Thus, RpoS is necessary for survival in water. Then, we analysed the transcriptome of the rpoS mutant during exposure to water using whole genome microarray analysis. We found that RpoS negatively affects the expression of several genes, including genes required for replication, transcription, translation and cell division. Interestingly, decreased expression of these genes was observed when WT cells were exposed to water compared to growth in AYE, indicating that the survival of L. pneumophila in water requires a slowdown in gene expression, probably controlled by the rpoS gene. The rpoS mutant contains more total RNA, is more sensitive to rifampicin and produces more de novo RNA than the WT strain after water exposure. It seems that the rpoS mutant is transcriptionally more active than the WT strain. This could be explained by a mis-regulation of the stringent response in the rpoS mutant, causing an increase in transcription. Indeed, the spoT gene is overexpressed in the rpoS mutant, which would decrease the level of (p)ppGpp caused by its (p)ppGpp hydrolase activity. This would then cause an increase in the transcription level, and a decrease in the survival rate. Conclusion: Our study is the first report highlighting a feed-back loop between RpoS and SpoT. It seems that the lack of rpoS gene causes aberrant regulation of the stringent response, which prevents the induction of a successful response to starvation.

Workshop Sessions

BAM-WK118.01 - Quantitative detection and identification of Arcobacter, Campylobacter and Helicobacter species in a Canadian agricultural watershed

Izhar Khan[1], Michel Cloutier[1], Ed Topp[2], David Lapen[1], André Lévesque[1], Thomas Edge[3], James Tambong[1], Guylaine Talbot[4], Graham Wilkes[1]

[1]Eastern Cereal and Oilseed Research Center, Agriculture and Agri-Food Canada, Ottawa, Canada, [2]Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Canada, [3]Watershed Hydrology and Ecology Research Division, National Water Research Institute (NWRI), Environment Canada, Burlington, Canada, [4]Dairy and Swine Research Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Canada

The occurrence of human pathogens in water due to fecal contamination may cause human health risks, and consequently, prevention is a necessity. Rapid and reliable test results are needed quickly for immediate action. Conventional culture-based methods are laborious and time-consuming and certain fastidious microorganisms are not culturable on laboratory media. Culture-independent quantitative real-time PCR (qPCR) has proven to be rapid and effective tool to detect and quantify pathogens in water within few hours. We employed SYBR Green and TaqMan probe-based qPCR assays in a 4-year study (from 2010-13) for quantitative detection and identification of microaerophilic human pathogens including Arcobacter butzleri, thermophilic Campylobacter spp. (C. jejuni, C. coli and C. lari) and Helicobacter pylori in an agricultural watershed representing mixed-use but primarily dairy dominated livestock operations, located in the South Nation River (SNR) basin Ottawa, Ontario, Canada. Based on a bi-weekly sampling regimen, 356 water samples were collected from eight agricultural sites where one site was selected as a reference site away from significant human and/or agricultural influence. Standard curves for each target species were developed (quantification limit 10-100 cells-1) and used for quantifying the total number of cells present in water samples. Overall, all 5 target species were detected; however, thermophilic Campylobacter spp. was detected more frequently than Arcobacter butzleri; whereas, Helicobacter pylori was detected more infrequently than Campylobacter and Arcobacter species. Interestingly, among Campylobacter species, C. jejuni and C. lari were more commonly detected than C. coli, and the number of cells for each target species was relatively low. Occasionally high cell concentrations were detected in upstream water samples compared to downstream and reference sites. The study results indicate that livestock and wildlife (including birds) and agricultural run-off, as well as human sewage, may contribute to the contamination of these pathogens to the SNR agricultural water.

Workshop Sessions

BAM-WK118.02 - Molecular diversity of environnental Mycobacterium ssp: Ulcer infection in Ivory Coast

Solange Ngazoa Kakou[1], Ngolo Coulibaly David[1], Aka Nguetta[1], Aoussi Serge[1], Dosso Mireille[1]
[1]*Institut Pasteur Abidjan, Abidjan, Cote d'Ivoire*

Buruli Ulcer (BU) is caused by a mycobacterium called M. ulcerans. The events of BU are the skin lesions. The lack of early diagnosis and treatment cause severe disability. Today the emergence to BU in Africa and particularly in Cote d'Ivoire needs faster diagnosis to control and to prevent the M. ulcerans transmission. The lack of circulating strains regarding molecular diversity and prevalence of others environmental mycobacteria in Cote d'Ivoire can contribute to the emergence of the infection in some regions. Tandem repeat (TR) DNA sequences are important sources of polymorphism in the genome of many eukaryotes and prokaryotes. Genomic regions showing polymorphism due to different numbers of TR motifs in different strains or individuals are described as variable number tandem repeat (VNTR) loci. VNTR The main objective of this study was to investigate the genomic diversity of M.ulcerans strains from different regions by using VNTR typing. Materials and methods: 21 schwab samples from ulcer lesions from different regions in Cote d'Ivoire were screening for the study. The samples were first confirmed by PCR IS2404 and KR. After DNA extraction, simplex PCRs in four loci ST1, locus 6, Locus 19 and MIRU1 were applied for each sample to determine the VNTR profile of the strains. Results: 66 % of samples were found positive for MIRU1, 85% positive for ST1, 57 % positive for locus 6 and 66 % for locus 19. The resulting profiles were [3122] Mu Agy99 (C) for 28% samples, and [1122] Mu MK (D) for 9% of the samples. For 61 % of the samples, no VNTR profile can be determinated and suggest the circulating of different strains of Mycobacterium sp. causing Buruli Ulcer. Conclusion: In Côte d'Ivoire the agent of Buruli Ulcer, M. ulcerans was causing from different strains and explain the divergence regarding clinical developing of ulcers and the emergence of Buruli Ulcer in some regions.

Workshop Sessions

BAM-WK118.03 - Association of Vibrio cholerae with phytoplankton and its relationship with the seasonality of cholera in Bangladesh

M. Sirajul Islam[1], M. Shafiqul Islam[1], Zhahi H. Mahmud[1], Gary K. Schoolnik[2], Andrew Collins[3], John D. Clemens[1]
[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, USA, [3]Northumbria University, Newcastle Upon Tyne, UK

Cholera maintains a regular seasonal pattern in Bangladesh. The interepidemic reservoir of V. cholerae is found to be the phytoplankton in the aquatic environment. However, it was not known how the seasonality of cholera is related with the phytoplankton. Therefore the present study was undertaken to find out the relationship of phytoplankton with the seasonality of cholera epidemics. Algal samples were collected from the equatic environment of Matlab, Bangladesh and coast of Indian Ocean in Beira, Mozambique. The association of V. cholerae was monitored in all the collected algal samples using culture and direct fluorescent antibody techniques. Concurrently cholera cases reported to the icddr,b hospital in Matlab were tabulated from the medical record. These data were plotted and statistically analyzed to examine the temporal relationship between algal concentrations in pond water and cholera cases in the same area. V. cholerae O139 was found to be embeded in the mucilaginous sheath of a colonial blue-green alga, Microcystis sp., and a diatom, Melosira sp., collected from the aquatic envirnments of Matlab. V. cholerae O1 was also found in asociation with Melosira sp., collected from the coast of Beira, Mozambique. This is the first time, it has been found that Microcystis sp. and Melosira sp. in Bangladesh harbour V. cholerae O139 and Melosira sp. in Mozambique harbour V. cholerae O1. A statistically significant ($r = 0.67$, $p<0.01$) temporal association was found between algal concentrations in the sentinel pond and cholera cases in the same area. This study therefore provided an insight about the role of various algal species as reservoir of V. cholerae and its relation with seasonality of cholera in Bangladesh.

Workshop Sessions

BAM-WK118.04 - Application of Microbial Source Tracking for identifying sources of fecal contamination in an urban and agricultural-mixed prairie watershed

Dinah Tambalo[1], Tyler Boa[1], Bastien Fremaux[1], Paul Hynds[2], Christopher Yost[1]
[1]Biology Department, University of Regina, Regina,Canada, [2]School of Biomolecular & Biomedical Science, University College Dublin, Belfield, Ireland

Fecal pollution of water can represent a risk to human health when enteric pathogens are present in the contaminating material. Therefore, it is important to have effective tools for identifying sources of pollution to develop effective mitigation and prevent future contamination events. The Qu'Appelle River is in the Qu'Appelle Valley watershed, and is an important source of irrigation water for several vegetable producers in Southern Saskatchewan. This watershed is influenced by a variety of anthropogenic inputs. Weekly/biweekly sampling was conducted during the growing seasons in 2008, 2009, 2012, and 2013. E. coli levels exceeding the recommended limits for irrigation water (100 MPN/100ml) were observed at frequencies ranging from 30% to 61%; while Salmonella and Campylobacter frequencies ranged from 1% to 18% and 3% to 57%, respectively. The results highlight the health risks associated with using contaminated irrigation water and the importance of understanding the nature of the fecal inputs into the watershed. Thus, we evaluated the suitability of real time-quantitative PCR assays developed for the detection of Bacteroidales markers associated with humans, cattles, and ruminant faeces. The markers exhibited high sensitivity (88% to 96%) and specificity (99% to 100%), and shorter persistence compared with E. coli (99% decay in <8 days vs. >15 days) supporting their utility in detecting recent fecal contamination. Marker concentrations matched the observed land use in the study sites. The E. coli and ruminant marker concentrations had a weak correlation ($r_s=0.2544$, $p<0.001$) while there was no correlation between E. coli concentration and Campylobacter detection. The detection of a general Bacteroides marker at >5 log copy number/100 ml and ruminant marker presence increased the probability of Campylobacter detection 2 times and 3 times, respectively. Collectively, the results demonstrate that microbial source tracking can be a useful tool in improving management of water quality.

Workshop Sessions

BAM-WK118.05 - Antimicrobial peptide resistance contributes to host colonization by Crohn's-associated adherent-invasive E. coli

Joseph McPhee[1], Cherrie Small[1], Sarah Reid[1], John Brannon[2], Herve Le Moual[2], Brian Coombes[1]
[1]McMaster University, Hamilton, Canada, [2]McGill University, Montreal, Canada

Crohn's disease is a chronic inflammatory bowel disease characterized by transmural inflammation, fibrosis, and dysbiosis. Antimicrobial peptides secreted by colonocytes and Paneth cells play a key role in innate host defenses in the gut and in shaping gut microbial ecology. Secretion of some antimicrobial peptides is increased in Crohn's disease suggesting that a microbial involvement in Crohn's pathophysiology might require active resistance determinants. Adherent-invasive E. coli (AIEC) are associated with Crohn's disease, however the colonization determinants of AIEC in the inflamed gut are poorly defined. Here we show that resistance to antimicrobial peptides favors host colonization by AIEC. In a clinical isolate of AIEC we identified a plasmid-encoded genomic island called PI-6 that conferred resistance to a wide range of host defense peptides including alpha and beta defensins and cathelicidins. PI-6 encodes three genes, one of which encodes a Mig-14 family protein implicated in resistance to α- and β-defensins and another gene encoding an OmpT family outer membrane protease that we show degrades α-helical cationic peptides. A strain lacking PI-6 was defective for competitive infection of mice and for eliciting intestinal pathology, demonstrating that resistance to host defense peptides confers a selective competitive advantage to AIEC in the gut. These data suggest that, at least in some cases of human colonization, antimicrobial peptides may impart selective pressure on AIEC to acquire or maintain such resistance determinants in the inflamed host environment. Implicit in these findings are new bacterial targets whose inhibition might limit AIEC burden and disease in the gut.

Workshop Sessions

BAM-WK118.06 - Loss of lytic transglycosylases alters beta-lactam resistance and cell morphology in Pseudomonas aeruginosa

Ryan Lamers[1], Ryan Buensuceso[1], Lori Burrows[1]
[1]Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Canada

The opportunistic pathogen Pseudomonas aeruginosa (PA) is a leading cause of hospital-acquired infections; however, treatment is challenging because PA exhibits resistance to most antibiotics. One of the primary mechanisms of resistance is expression of a chromosomally encoded AmpC β-lactamase, which inactivates β-lactam antibiotics. The mechanisms leading to AmpC expression in PA remain incompletely understood; however, they are intricately linked to cell wall metabolism and peptidoglycan recycling. We seek to understand the mechanisms leading to AmpC induction in PA to design strategies to block them, preserving the effectiveness of anti-Pseudomonal β-lactams. A phenotypic screen of deletion mutants identified changes in β-lactam resistance profiles of strains lacking one or more lytic transglycosylases (LTs). Combination mutants were generated to verify the involvement of AmpC and to determine whether the mutations were additive. Strains lacking SltB1 or MltB had high-level β-lactam resistance, while the strain lacking Slt had increased sensitivity. Moreover, SltB1/MltB mutations were additive, as a double mutant had extreme β-lactam resistance (>256 μg/ml cefotaxime vs. 48 μg/ml for the parent). In addition to MltB, single mutants lacking other membrane-bound LTs (MLTs) had increased β-lactam resistance; however, strains lacking multiple MLTs had extreme sensitivity to β-lactams and the bulky antibiotic, vancomycin, which is normally incapable of crossing the outer membrane. These strains were also sensitive to detergent-like bile salts and high osmotic stress, suggesting they had impaired cell walls. Inactivation of ampC in single mutants reverted them to wild type susceptibility, confirming AmpC induction underlies resistance in the single mutants. Loss of only a small subset of peptidoglycan-active enzymes alters β-lactam resistance profiles in PA, suggesting that unique changes in cell wall metabolism and/or architecture triggers AmpC induction. Characterization of these specific triggers related to the loss of particular 'sentinel' activities may reveal avenues for reversal and remediation of β-lactam resistance.

Workshop Sessions

BAM-WK118.07 - Aminoglycoside binding and phosphorylation by APH(2")-Ia - a structural explanation of regiospecificity

Shane Caldwell[1], Albert Berghuis[1]
[1]*McGill University, Montreal, Canada*

The most widespread high-level gentamicin resistance factor is the phosphotransferase (APH) domain of the bifunctional enzyme AAC(6')-Ie/APH(2")-Ia. This domain was previously shown to phosphorylate almost every aminoglycoside antibiotic, although more recent studies have demonstrated that it is predominantly active against 4,6-disubstituted aminoglycosides, while 4,5-disubstituted aminoglycosides bind but are not phosphorylated. We have determined structures of the enzyme domain in complex with aminoglycosides from both groups. These structures illustrate the binding site versatility that allows structurally diverse molecules to bind, and explain why 4,6-disubstiuted, but not 4,5-disubstituted aminoglycosides are phosphorylated by the enzyme. These observations, in combination with a new model for catalytic regulation of the enzyme, highlight the flexibility and adaptability of this enzyme as part of a successful antimicrobial resistance machine.

Workshop Sessions

BAM-WK118.08 - Triclosan selects for AdeIJK Resistance-Nodulation-Division overexpressing Multidrug Resistant mutants of Acinetobacter baumannii ATCC17978

Dinesh Fernando[1], George Zhanel[2], Ayush Kumar[2]
[1]University of Ontario Institute of Technology, Oshawa, Canada, [2]University of Manitoba, Winnipeg, Canada

Triclosan is a commonly used antimicrobial agent used in household cleaning products. The purpose of this study was to investigate if triclosan resistance in Acinetobacter baumannii, an organism notorious for its multidrug resistance, causes cross-resistance to antibiotics. Two triclosan resistant mutants of A. baumannii ATCC17978 were isolated by serial passaging in LB-broth supplemented with triclosan and designated AB042 (MIC = 512 µg/mL) and AB043 (MIC > 512 µg/mL). Antibiotic susceptibility assays displayed decreased susceptibility of AB042 and AB043 to piperacillin/tazobactam, cefepime, ciprofloxacin, moxifloxacin, aztreonam, tigecycline, doxycycline, and trimethoprim/sulfamethoxazole. In order to understand the mechanism of multidrug resistance in AB042 and AB043, we analyzed the expression of five different resistance-nodulation-division (RND) pumps (AdeABC, AdeFGH, AdeIJK, A1S_2818, and A1S_3219), a major facilitator superfamily pump (AbeM), and porins (CarO, OprD). Expression of fabI, the target of triclosan was also examined. We found significant increased expression of fabI in AB043, which partly explains its resistance to triclosan. Expression of the triclosan pump, AbeM, and porins was not altered. However, expression of AdeJ (of AdeIJK complex) was found to be upregulated by 7-fold, which may explain the multidrug resistance phenotype of both mutants. Since the AdeIJK pump has not been shown to efflux triclosan, we expressed the adeIJK operon in a surrogate strain of Pseudomonas aeruginosa that lacks five of its native RND pumps, using a single copy gene expression system. Induction of the adeIJK operon resulted in a moderate increase in resistance to triclosan suggesting that it is a poor substrate of AdeIJK. Therefore, while triclosan can select for adeIJK overexpressing mutants leading to the multidrug resistance phenotype, triclosan resistance is not solely dependent on overexpression of AdeIJK. This is the first study to show triclosan can select for an RND pump (AdeIJK) overexpressing multidrug resistant mutants of A. baumannii.

Workshop Sessions

MEM-WK308.01 - 'Zygomycetes' and the phylogenetic affinity of mycorrhizal fungi (AMF)

B. Franz Lang[1]
[1]*Département de Biochimie, Centre Robert-Cedergren, Université de Montréal*

Arbuscular Mycorrhizal Fungi (AMF) are microbial symbionts that associate with root tissues of virtually all vascular plants. AMF are difficult to investigate at the genomic and molecular level, as they are unable to grow without a host plant, and as they contain hundreds of genetically distinct nuclei. Initially classified as members of the paraphyletic 'zygomycete fungi', taxonomists have more recently transferred them to a distinct phylum 'Glomeromycota' - although in the absence of unequivocal phylogenetic evidence. Even in 2014, with affordable 'new generation sequence technology' widely available, the phylogenetic classification (based on sequence or genome data) of zygomycete-related fungi continues to be a deplorable mess. Because nuclear genomes of AMF are usually too heterogeneous to be assembled into reasonable-size contigs, with DNA samples often containing bacterial and fungal contaminants, we sequence and assemble complete mitochondrial (mt) genomes across zygomycetes. According to our results, mtDNAs across AMF are genetically homogenous, in stark contrast to their nuclear genomes. Our phylogenetic analysis with this data indicates that Glomeromycota are not a separate group of paraphyletic zygomycetes, but form a monophyletic group together with Mortierellales and Harpellales, distinct from a second major lineage of Mucorales.

Workshop Sessions

MEM-WK308.02 - Yeast biodiversity revisited

Teun Boekhout[1], Xin-Zhan Liu[2], Qi-Ming Wang[2], Salvador Capella[3], Bart Theelen[1], Marizeth Groenewald[1], Benjamin Stielow[1], Saad Taj-Aldeen[4], Toni Gabaldon[3], Feng-Yan Bai[2]

[1]CBS Fungal Biodiversity Centre, Utrecht, The Netherlands, [2]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, [3]Centro Regulacion Genomica, Barcelona, Spain, [4]Hamad Medical Corporation, Doha, Qatar

Our knowledge of yeast biodiversity is rapidly advancing. In the 5th edition of The Yeasts, a Taxonomic Study (2011, eds. C.P. Kurtzman, J.W. Fell & T. Boekhout) ca. 1500 species are described, but presently the number of species described is over 2000. The doubling time of species discovery was about 10 years, but likely will be (much) shorter in the future. However, large parts of the biota on earth remain to be sampled with respect to yeast biodiversity. Many of the currently used genera are highly polyphyletic and, consequently, the taxonomy of yeasts needs to be completely revised. The following developments contribute to this new taxonomy: 1. Improved phylogenetic signal in multigene-based phylogenies; 2. The impact of phylogenomics; 3. The introduction of the one species-one name concept; and 3. Ongoing species discovery that add species to new or sparingly sampled lineages. Attempts in order to make the classification of the yeasts more natural, i.e. in line with phylogenetic principles, are ongoing by comparative multigene sequencing and whole genome sequencing of currently described yeast species. Furthermore, phylogenetic information can be used for the selection of lineage specific markers. We will show these developments by presenting some examples from both the asco- and basidiomycetous yeasts, and a discussion of the nomenclatural consequences.

Workshop Sessions

MEM-WK309.01 - Genome-wide approaches to identify and characterize lignocellulolytic enzymes of thermophilic fungi

Adrian Tsang[1]
[1]*Concordia University, Montreal, Canada*

The ability to thrive at high temperatures is rare among eukaryotes. So far, fewer than 30 fungal species are regarded as thermophiles. They have received considerable attention in the search of thermostable enzymes and thermophilic platform organisms for industrial applications, especially in the deconstruction of lignocellulosic biomass. We have sequenced over 20 species of thermophilic fungi, four of which have finished genomes with telomere-to-telomer chromosomes. We have also developed computational tools to improve the identification genes in fungal genomes in general, and genes encoding extracellular proteins in particular because biomass-degrading enzymes are predominantly extracellular proteins. In addition to using informatics tools to identify orthologues of lignocellulolytic enzymes, we have analyzed the transcriptomes and exo-proteomes of the thermophilic fungi when cultured in a variety of agricultural straws to reveal the strategies used by different fungi in the decomposition of lignocellulose as well as identifying novel extracellular proteins that may play a role in biomass decomposition. Over 6000 genes encoding potential lignocellulolytic proteins have been identified. Thermophiles from the Sordariales possess a larger repertoire of lignocellulolytic enzymes than the thermophiles from other orders. The genes predicted to encode lignocellulolytic proteins have been cloned and transformed into Aspergillus niger for the production of recombinant enzymes. Biochemical characterization of the recombinant enzymes show that in addition to producing enzymes that are thermostable, the thermophiles also produce enzymes that have temperature optimum in the 40-50°C range.

Workshop Sessions

MEM-WK309.02 - Multi-Omics analysis of Synechocystis sp. PCC6803 response to stress conditions for biofuel production

Seong-Joo Hong[1], Byung-Kwan Cho[2], Hyung-Kyoon Choi[3], Hookeun Lee[4], Choul-Gyun "CG" Lee[1]
[1]Inha University, Incheon, Korea, [2]KAIST, Daejeon, Korea, [3]Chung-Ang University, Seoul, Korea, [4]Gachon University, Incheon, Korea

Eukaryotic microalgae are attracting a lot of attention due to their potential for sustainable production of biofuel precursors such as lipids, starches, alcohols and hydrogen gas. In addition, they can convert solar energy into biomass using carbon dioxide by photosynthesis. Recently various attempts in biological fields have been made to produce microalgal biofuel. Especially since genome database of Chlamydomonas reinhardtii was published in 2007, high throughput omics data of eukaryotic microalgae have been accumulating at a rapid rate. Among omics data, genomics and transcriptomics researches are developing rapidly by development of NGS technologies. Over 30 whole genome sequencing of eukaryotic microalgae were in progress or completed in the JGI (Joint Genome Institute) and transcriptome of Dunaliella tertiolecta1 and Nannochloropsis gaditana2 was characterized by NGS (next generation sequencing). With accumulation of these enormous data, macroscopic view of cellular network has been required requisitely. To date as the one of research tools to gather and analyze huge information, systems biology is worth of remark. Through the use of systems biology, molecular and cellular phenomena of eukaryotic microalgae could be modeled using integrated and interacting network of genes, transcripts, proteins and biochemical reactions under stressful conditions. Fatty acid contents and carbohydrate contents were observed to be differentially regulated in microalgae under nitrogen depleted condition. To characterize fatty acid accumulation mechanism, genome data was used as a frame work for metabolic reconstruction. Transcriptome, proteome and metabolome analysis of the cells were performed and integrated to reconstruct cellular network in eukaryotic microalgae. To enhance lipid productivity from microalgae, in silico model of eukaryotic microalgae was reconstructed based on OMICS data. It can make us fully understand the lipid accumulation mechanism within eukaryotic microalgae. This in silico analysis integrated multi-omics data could become the driving force for exploiting metabolic and regulatory mechanisms to improve microalgal cells as a biofuel producer.

Workshop Sessions

MEM-WK309.03 - Extracellular and cellular bioremoval of mercury by mercury resistant yeasts, Yarrowia spp.

Ganiyu Oyetibo[1,2], Keisuke Miyauchi[1], Ginro Endo[1]
[1]Faculty of Engineering, Tohoku Gakuin University, Tagajo, Japan., [2]Faculty of Science, University of Lagos, Akoka, Nigeria

Ecotoxicological implications of mercury (Hg) pollution of hydrosphere are global concerns, requiring effective Hg-removal strategies as antidote to the environmental problems. Two viable mercury-resistant yeast strains identified as Yarrowia spp. were studied for distribution of Hg in their cellular components as evidence of bioaccumulation during growyh. Extracellular polymeric substances (EPS) produced by the yeast strains were characterized, and their potential to remove mercuric ions during growth in aqueous system were studied. Of 870 (±23.6) µg Hg2+ bioavailable in liquid medium, 419.0 µg Hg2+ (approx.) adhered onto wet biomasses of the yeast strains. Large portion of the adsorbed Hg was found distributed between cell wall and sphearoplast. Negligible quantities of Hg were present in the mitochondria (0.02-0.02%), and appreciable amount of Hg were observed in nuclei and cell debris (15.2-65.3%). EPS produced by the yeast strains was a complex of protein, carbohydrates and other substances. Secreted EPS in the medium accounted for binding 43.8 (±0.7)% to 58.7 (±1.0)% of initial Hg in medium as soluble precipitates, while 10.13 ±0.4% to 39.2 ±4.3% Hg content was volatilized. Transmission electron microscopy coupled with X-ray energy dispersive energy spectrophotometry confirmed the EPS-Hg complex in dialyzed polymer solution. Comparison of Hg mass balance before and after bioremoval experiment revealed complete Hg removal from the medium by the yeast strains via bioaccumulation, volatilization and biosorption. The two strains of Yarrowia are promising biotechnological tools in knowledge-based information required to design a bioreactor for treatment of mercury-laden industrial wastewater and bioremediation of aquatic environment polluted with mercuric ions.

Workshop Sessions

MEM-WK310.01 - Functional genomics and drug resistance in the parasite Leishmania

Marc Ouellette[1]
[1]*Université Laval, Québec, Canada*

Resistance in Leishmania is often due to gene copy number variations (CNVs) and to point mutations. Next generation sequencing (NGS) of Leishmania cells selected for resistance to antimonials, miltefosine, amphotericin B, and paromomycin has allowed the detection of a plethora of resistance mechanisms. These include chromosome aneuploidy, gene amplification or deletion of specific loci and point mutations. Amplification/deletion happens at the level of repeated sequences that are widespread throughout the Leishmania genome. We obtained evidences that the Leishmania genome is continuously being rearranged at the level of these repeated sequences, which serve as a functional platform for constitutive and stochastic amplification (and deletion) of genomic segments in the population. This process is adaptive as the copy number of the extrachromosomal elements increase upon selection and also reversible since the copy number decreases to baseline levels when selection is removed. As CNVs is an important mechanism of resistance we developed a new functional approach consisting of transfecting cosmid banks of the full Leishmania genome and select step by step with drugs. Cosmids are isolated at each step and subjected to NGS. Enrichment of sequence reads of known resistance genes were observed upon drug selection but most importantly novel resistance genes to all main antileishmanials were also observed. This new technique has proven useful to further our understanding of resistance mechanismes in Leishmania.

Workshop Sessions

MEM-WK310.02 - An antifungal combinations matrix to enhance drug discovery

Gerry Wright[1]
[1]McMaster University, Hamilton, Canada

There is a growing need for new anti fungal therapeutics to overcome resistance and improve efficacy. Our research has shown that combining known antifungal agents with other bioactive compounds with cryptic or negligible anti fungal properties by themselves, can greatly improve efficacy. Using a systematic high throughput screening approach, we have developed a matrix of compounds that synergies with known antifungals across a variety of model and pathogenic fungi. The development of this Antifungal Combinations Matrix will be reported along with selected examples of compound combinations and their mode of action.

Workshop Sessions

MEM-WK310.03 - Targeting fungal stress response circuitry to conquer antifungal drug resistance

Leah E. Cowen[1]
[1]*University of Toronto, Toronto, Canada*

Treatment of invasive fungal infections is notoriously difficult, in part due to the limited number of antifungal drugs and the rapid emergence of drug resistance in pathogen populations. We discovered poignant examples of how targeting fungal stress response circuitry provides a powerful and broadly effective strategy to cripple fungal pathogens and impair their capacity to survive drug-induced stress. Here, I discuss recent advances in understanding the mechanisms by which the molecular chaperone Hsp90 orchestrates signaling through the protein phosphatase calcineurin and the protein kinase C-mediated cell wall integrity pathway, and how these signaling cascades converge on the fungal-specific transcription factor Cas5. I will highlight how dissecting the circuitry that enables fungal pathogens to tolerate drug exposure has revealed new resistance mechanisms and novel therapeutic targets.

Workshop Sessions

MEM-WK310.04 - Elaboration of an enriched cross-species bioactive compound library

Michaela Spitzer[1], Jan Wildenhain[2], Sonam Dolma[3], Gerry Wright[1], Mike Tyers[4]
[1]McMaster University, Hamilton, Canada, [2]University of Edinburgh, Edinburgh, UK, [3]University of Toronto, Toronto, Canada, [4]Université de Montréal, Montreal, Canada

Modest hit rates for complex cell- or organism-based chemical screens often limit the recovery of bioactive compounds. To address this bottleneck, we pre-selected bioactive molecules from a 50,000 compound library based on growth inhibition of S. cerevisiae in a high throughput format and maximization of chemical diversity across the hits. The resulting collection of 1,570 bioactive compounds was then tested against a diverse evolutionary spectrum of species in liquid growth assays (E. coli, S. cerevisiae and S. pombe), whole organism phenotypic screens (C. elegans, D. rerio and A. thaliana) and specific cell-based assays (O. tauri and mammalian cells). Notably, hit rates in the eukaryotic screens ranged from 15 to 40%, far greater than the 2% hit rate observed in the original S. cerevisiae screen against the full 50,000 compound library. The hit rate in the E. coli bacterial screen was only 11%, suggesting that the collection was particularly enriched for bioactivities against eukaryotic species. Despite the initial selection of compounds based on simple growth inhibition in yeast, the bioactives elicited a variety of phenotypes in different species and often caused species-specific effects. Integration of the species-compound network with data on chemical structure also revealed clusters of compounds with interesting patterns of activity across species. To investigate specific target pathways and putative protein targets, we generated genome-wide chemical-genetic profiles for 100 bioactive compounds in S. cerevisiae. Different compound groups specifically interfered with a variety of processes including chitin synthesis, intra-Golgi and ER-Golgi trafficking, microtubule polymerization, sphingolipid biosynthesis and the cell cycle. Access to the bioactive data set is provided via an online portal that enables visualization and exploration of the compound-species matrix. We are currently screening this bioactive compound collection against human pathogens such as Candida, Cryptococcus and Aspergillus to characterize the antifungal potential of the library.

Workshop Sessions

MEM-WK310.05 - Hybrid linkage analyses reveal fluconazole concentration-dependent quantitative trait loci influencing vegetative growth in the human pathogen Cryptococcus neoformans

Aaron Vogan[1], Jordan Khankhet[1], Jiangping Xu[1]
[1]*McMaster University, Hamilton, Canada*

Cryptococcus neoformans is a basidiomyceteous yeast that causes opportunistic meningoencephilitis in humans. It infects up to 1 million people every year and fluconazole is the first line drug for treating C. neoformans infections. There are two divergent varieties in C. neoformans: var. grubii and var. neoformans. However, these two varieties can hybridize in nature and in the laboratory. Some of these hybrids have shown superior virulence and fitness-related traits over the parental lineages. Here, we use the hybrid genetic systems in C. neoformans to identify quantitative trait loci (QTLs) that underlie in vitro growth differences between two parental strains, a fluconazole-resistant var. grubii strain CDC15 and a var. neoformans model laboratory strain JEC20 that is sensitive to fluconazole. A hybrid linkage map was constructed using 55 markers and 230 meiotic progeny. The vegetative growth on both solid agar and in liquid medium was obtained for each of the progeny across a range of fluconazole concentrations. Our analyses identified ~20 regions that contributed to growth differences at at least one concentration of fluconazole. No regions contributed to fitness across all environments, and most were only significant at two or three concentrations. Furthermore, environment-specific interactions between QTLs were observed. Our results are consistent with a diversity of genetic interactions contributing to vegetative fitness and that such interactions are influenced by fluconazole concentrations.

Workshop Sessions

MEM-WK311.01 - **Exploring the physiological diversity of native microalgae for fuel and wastewater reclamation**

Patrick Hallenbeck[1,2], Gustavo Leite[1], Ahmed Abdelaziz[1]
[1]Université de Montréal, Montréal, Canada, [2]United States Air Force Academy, Colorado Springs, USA

Microalgae are being considered as a possible source of biofuel whose production does not compete with food production for resources. In addition, sustainability concerns dictate that wastewater be used as source of both water and nutrients for algal cultivation. Microalgae have an inherently fast growth rate, and can have a high lipid content under different nutrient limiting conditions. Various microalgal cultivation strategies are under study, but just as with farming of terrestrial plant crops, good agricultural practices suggest that local algae will be best adapted to prevailing climatic conditions, more robust, and competitive with other species. Using a 12 well microplate method we have established and characterized a collection of over 100 microalgal strains sourced from local Québec freshwater lakes and rivers. Scatterplots were used to examine the physiological diversity of this collection, examining specific growth rates on synthetic medium (BBM) and wastewater, secondary effluent form a local municipal wastewater treatment plant (WW), at two different temperatures (10±2 ºC and 22±2 ºC). The results allow us to make a number of interesting conclusions. Even though the cultures were initially enriched on BBM at 22 ºC, a large number showed good growth on WW or at 10 ºC. Thus enrichment on BBM at 22 ºC does not seem to bias the cells for growth on this medium versus WW, or at 22 ºC versus 10 ºC. Also, when looked at in this way, no specific location could be associated with any particular subset of properties suggesting that wide ranging diversity is found at each sampling locale. A number of strains were identified that showed good growth at 10 ºC, gave a high lipid content (ranging from 20% to 45% of dry weight), or showed a high capacity for nutrient removal.

Workshop Sessions

MEM-WK311.02 - Interplay between self and non-self recognition mechanisms in Neurospora crassa

Jens Heller[1], David Kowbel[1], Javier Palma-Guerrero[1], N. Louise Glass[1]
[1]Department of Plant and Microbial Biology, The University of California, Berkeley, USA

In filamentous fungi, such as Neurospora crassa, fusion between genetically different mycelial colonies often results in non-self recognition that induces heterokaryon incompatibility (HI), a type of programmed cell death (PCD). HI prevents transmission of potentially infectious cytoplasmic elements, such as mycoviruses and senescence plasmids. Nevertheless, inducing HI due to genetic differences after attraction and fusion seems to be detrimental for the fungus as it is a waste of energy and resources. During asexual spore germination, genetically identical germlings also undergo chemotropic interactions and cell fusion in a highly regulated process that is associated with the early colony establishment. However, the function of currently characterized HI loci and PCD in N. crassa occurs only in the mycelial phase, not during cell fusion of germlings. To determine if there are non-self recognition mechanisms acting during germling fusion, we took advantage of a N. crassa collection of 112 wild strains from a single population. By analyzing chemotropic interactions between germlings in these genetically different strains, we defined three communication groups (CG). Bulked segregant analysis (BSA) identified the genes responsible for the communication group phenotype. However, within a single CG, where genetically different germlings show chemotropic interactions, two additional phenotypes were observed. (1) Cell fusion (cell wall breakdown and membrane merger) was blocked, which segregated as a single gene. (2) Genetically different germlings showed cell fusion, but induced PCD as a post-fusion event associated with vacuolization and cell death of fused germlings. Genetic analyses suggest that two to three loci determine the post-fusion germling cell death phenotype. We will present the results of genetic analyses and BSA to identify the genes that block cell fusion and that induce PCD after non-self fusion of N. crassa germlings in a comparison to genes involved in determining communication group affiliation. Our results suggest that the interactions between germlings are complex and involve both self and non-self interactions.

Workshop Sessions

MEM-WK311.03 - Nucleomorphs: insights into the fate of organelles

Bruce Curtis[1], Geoff McFadden[2], Patrick Keeling[3], Michael Gray[1], John Archibald[1]
[1]Dalhousie University, Halifax, Canada, [2]University of Melbourne, Melbourne, Australia, [3]University of British Columbia, Vancouver, Canada

Mitochondria and chloroplasts are derived from the engulfment and retention of bacteria by eukaryotic organisms. After engulfment, the endosymbionts experienced radical reductions in their functions and cellular components. Their genetic complements were also reduced through gene loss and gene transfer to the host nucleus resulting in small, compact organellar genomes. Green plants, green algae and red algae possess primary chloroplasts derived directly from the engulfed cyanobacterium. In many other photosynthetic algae however the plastids are secondarily derived by the engulfment of a red or green alga. Subsequently, the nuclear genome of the engulfed photosynthetic alga typically vanishes, again through gene loss and endosymbiotic gene transfer. The recent sequencing of the nuclear genomes of the chlorarachniophyte *Bigelowiella natans* and the cryptophyte *Guillardia theta* has shed light on the process of endosymbiotic gene transfer and the persistence of organellar genomes. These two organisms possess nucleomorphs, which are the vestigial remains of the engulfed algal nucleus. In the case of *B. natans* the nucleomorph is derived from a green alga while *G. theta* possesses a vestigial red algal-derived nucleomorph. Since their discovery, the persistence of nucleomorphs in these two unrelated lineages has been perplexing. A detailed examination of the host nuclear genomes for the presence of organellar DNA, including nucleomorph-derived DNA, suggests that in these two lineages endosymbiotic gene transfer has ceased. The nucleomorphs are essentially frozen, no longer able to reduce their genomes through the typical process of transferring essential genes to the host nucleus.

Workshop Sessions

MEM-WK311.04 - Screening of a conditional repressed library of Candida albicans for hyphal formation

Tuana Mesquita[1], Malcolm Whiteway[2]
[1]McGill University, Montreal, Canada, [2]Concordia University, Montreal, Canada

Candida albicans is a commensal organism that can cause life-threatening infections in immunocompromised individuals. Candidemia is not only the main cause of hospital acquired infections in North America, but the mortality rate, depending on the Candida species, can reach up to 81%. Historically people have always used Saccharomyces cerevisiae as a model organism to study C. albicans, however we have reached a point where the reported similarities can no longer explain this organism pathogenicity. One major factor that requires further understanding is that Candida is a polymorphic organism, capable of changing its shape according to the environmental signals. In order to understand the pathogen morphogenesis regulation, we screened a mutant library of C. albicans called G.R.A.C.E. – Gene Replacement and Conditional Expression – for hyphal development in normal growing conditions, and for absence of filamentation in hyphal inducing conditions, in liquid and solid media. A great number of strains were filamentous in normal growing conditions, especially when the library was screened in its shut-off state in solid media. Approximately 14.6% of the shut-off library showed increased filamentation on normal growing conditions. When compared to the liquid screening the number of filamentous strains was reduced significantly. Only 23.4% of those filamentous strains showed filamentation in liquid media containing tetracycline. The cut-off strains were shut off for genes mainly involved in cell cycle regulation and housekeeping processes which might result from genotoxic stress, since they grew consistently slower in liquid media. Only 6.7% of the genes were non-filamentous in hyphal inducing conditions. Surprisingly 15% of the mutants that were consistently non-filamentous in both liquid and solid media are from proteins known to be involved in aerobic respiration and 59% of them are localized in the mitochondria. How aerobic respiration plays a role on hyphal development in Candida is still not well understood.

Workshop Sessions

MEM-WK311.05 - **$^{13}C/^{12}C$ homeostasis of plant cells in symbiotic orchid protocorms revealed by an isotope tracer experiment and isotope microscopy.**

Yukari Kuga[1], Naoya Sakamoto[2], Hisayoshi Yurimoto[2]
[1]Hiroshima University, Higashi Hiroshima, Japan, [2]Hokkaido University, Sapporo, Japan

Orchid seeds are minute, composed of only an embryo and a thin testa. Consequently, the germinating embryo (protocorm) relies on a fungal symbiont for nutrients including carbon. Although the main direction of carbon transport to protocorms is opposite from that in mycorrhizal roots, the colonizing processes and symbiotic structures are almost identical. Furthermore, because of the characteristics of its small size and culturable fungal partners, the symbiotic protocorm of green orchids is an excellent model system to study transfer and allocation of elements in the symbioses. We established a new methodology of visualizing cellular element flow by combining stable isotope tracer experiments, ultrastructural techniques, and high spatial resolution secondary ion mass spectrometry (SIMS). A previous study showed that the interface between the symbionts is involved in nutrient transfer before fungal senescence, but peloton degradation also releases a significant amount of C and nitrogen to host cells. Here we report results of a time course experiment of double labelling of ^{13}C-glucose and $^{15}NH_4{}^{15}NO_3$ to hyphae of *Ceratobasidium* sp. AG-I extending from symbiotic protocorms of *Spiranthes sinensis*. Region of interest analyses of host structures (cytoplasm, nucleus, nucleolus, vacuole, cell wall and amyloplast) showed that $^{12}C^{15}N/^{12}C^{14}N$ increased logarithmically over time and reached *c*. 80 % at 6 days after the labelling. On the other hand, $^{13}C/^{12}C$ remained around 2%, during the entire experimental period. The new methodology revealed new insights into dynamic allocation events of C and N between and within the symbiotic partners.

Wednesday, 30 July 2014 **16:40 - 17:00 Room 512 E**

Workshop Sessions

MEM-WK311.06 - Discovery of gas bubbles inside fermenting yeasts

Chantel Swart[1], Khumisho Dithebe[1], Carolina Pohl[1], Pieter Van Wyk[2], Johan Kock[1]

[1]*UNESCO-MIRCEN: Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa.,* [2]*Centre for Microscopy, University of the Free State, Bloemfontein, South Africa.*

One of the most important biotechnological processes known to man, fermentation, exploits the capabilities of yeasts to produce increased ethanol and carbon dioxide (CO_2) giving rise to products such as leavened bread and alcoholic beverages. Yeasts vigorously release CO_2> into the surrounding medium during fermentation, it is therefore expected that cells would be filled with gas bubbles. Yet no sign of CO_2> bubbles have been reported inside cells despite the fact that the yeast fermentation process is well established. This lack of reports on intracellular gas bubbles is considered a missing link since it is not clear what happens to the CO_2 between production and eventual release from the cells. The main aim of this study therefore became to find this missing link. Here, the brewer's yeast, *Saccharomyces pastorianus* and baker's yeast, *Saccharomyces cerevisiae*, were grown in fermentable and non-fermentable media respectively and analyzed with various microscopic techniques to determine whether gas bubbles are present inside these cells. Imaging techniques used included light microscopy, transmission electron microscopy (TEM), and Auger-architectomics (http://en.wikipedia.org/wiki/Auger_architectomics). Analysis with light microscopy indicated a granular appearance inside the cells, similar to that observed when gas vacuoles were discovered in the Cyanobacteria. Much less granules were however observed in cells grown in non-fermentable media. Further analysis with TEM confirmed the presence of a large number of gas bubbles filling a significant part of the cells when grown in fermentable media. Further visualization with NanoSAM (Auger-architectomics) revealed an interconnecting maze of bubbles. These gas bubbles compress and deform organelles inside the cells although they contained no enveloping membranes as indicated by TEM. The missing link between the production of intracellular CO_2 by glycolysis and eventual CO_2release from the cells has therefore been discovered. The effects of this interaction on metabolism and coding functions of yeasts should be investigated.

Workshop Sessions

MEM-WK312.01 - Mutualistic symbiosis between basal land plants and Endogone: new insights into the evolution of arbuscular mycorrhiza

Katie Field[1], Kate Allinson[1], William Rimington[2,3,4], Martin Bidartondo[2,3], Duncan Cameron[1], Jeffrey Duckett[4], David Beerling[1], Jonathan Leake[1], Silvia Pressel[4]

[1]Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield, UK, [2]Department of Biology, Imperial College London, London, UK, [3]Jodrell Laboratory, Royal Botanic Gardens, Kew, UK, [4]Department of Life Sciences, Natural History Museum, London, UK

The evolution and diversification of land plants has for decades been hypothesised to have involved symbiotic partnerships with arbuscular mycorrhizal fungi of the Glomeromycota. However, recent studies have established that liverworts in the Haplomitriopsida, the most basal extant clade of land plants, associate exclusively with endophytic Endogone fungi that pre-date Glomeromycota. This suggests Endogone played the critical role in initiating land plant-fungal symbioses, but the functional basis of liverwort-Endogone symbiosis has not been determined. We supplied 14CO2 to basal liverworts in the Haplomitriopsida and more advanced thalloid liverworts with Glomeromycotean partners, under early Palaeozoic [CO2]a (1500 ppm) and contemporary [CO2]a (440 ppm), and traced 14C allocation into a rhizoid-excluding compartment colonised by hyphae extending from the plants. Simultaneously, we traced P uptake into the plants from 33P -orthophosphate added to the fungal compartment. Carbon-for-phosphorus plant-fungal exchange established the Endogone-liverwort symbioses are mutualistic, but have lower efficiency than the Glomeromycotean associations of thalloid liverworts, especially under Palaeozoic [CO2]a. This provides the first functional evidence to explain why Glomeromycotean fungal associations evolved and superceded Endogone associations in more advanced plant clades, and raises important questions about the causes and controls on fidelity to Endogone in the basal liverwort clades.

Workshop Sessions

MEM-WK312.02 - Impact of commercial AM fungal inoculant containing Glomus irregulare on structure and diversity of native AM fungal communities under different agro-ecological zones of Saskatchewan prairie

Nazrul Islam[1], Jim Germida[1], Fran Walley[1]
[1]*University of Saskatchewan, Saskatoon, Canada*

Inoculation of soils with beneficial microbes such as arbuscular mycorrhizal fungi (AMF) is considered a tool to promote crop productivity and sustainability of agricultural ecosystems. In recent years, an AM fungal inoculant (bio-fertilizer) was introduced in western Canada for field-scale commercial farming operations. The inoculant contains Glomus irregulare and it is not known what impact the non-native AM fungal propagules may have on indigenous AM fungal communities which are inherently beneficial for nutrient uptake and enhance crop production. The potential ecological consequence of invasions by introduced AMF currently is poorly understood. A three year field incubation study was established in May 2011 to examine the subsequent changes in structure and diversity of resident AM fungal communities in host plant roots in response to the introduction of the AM fungal inoculant strain and to monitor the persistence of the applied inoculant at four sites representing different soil zones (Brown-Swift Current, Dark Brown-Scott & Outlook and Black-Melfort) of Saskatchewan, Canada. Preliminary results from PCR-MiSeq Illumina based meta-genomic sequence analysis of fungal 18S rRNA suggest that at crop harvest in September 2011, compositional structure of AMF in field pea roots was significantly altered as a consequence of inoculant application; specifically, we observed a reduction in species richness and diversity in response to inoculation with the inoculant strain, Glomus irregulare. The relative abundance of both indigenous and commercial AM fungal species in colonized roots as affected by various soil and climatic conditions was highly variable across different soil zones of Saskatchewan. Our findings indicate that application of commercial AM fungal inoculant can, at least in the short term (after one cropping season), alter the number and composition of AMF colonizing pea roots. Further, the persistence of commercial AMF strains largely depends on soil properties (soil type) and local climates where it is introduced.

Workshop Sessions

MEM-WK312.03 - WITHDRAWN - Ectomycorrhizal fungi associated with Himalayan Spruce; a threatened species of the Himalayas

Amna Imran[1], Abdul N. Khalid[1]
[1]Punjab University, Lahore, Pakistan

Himalayan Spruce (Picea smithiana) is native to South Asia, particularly, Pakistan, India, China, Afghanistan and Nepal. The conservation status of this spruce in its native region has been seriously threatened due to habitat loss and deforestation. With the rapidly decreasing numbers of this rare spruce from Himalayan forests, fungal biodiversity is also endangered. Present study focused on the ectomycorrhizal (ECM) fungi associated with the roots of P. smithiana, growing in Himalayan moist temperate forests-a biodiversity hotspot-from Pakistan. In forest ecosystem, belowground ECM fungi play an essential role in nutrient cycling, thus sustaining the ecosystem. Thirteen MOTUs were identified by ITS-barcoding. ITS1/5.8s/ITS2 region was targeted to identify those MOTUs. Apart from these taxa, several other fungi are identified and characterized based on morphotyping. These MOTUs and morphotypes have diverse range of morpho-anatomical features which sets them apart like pale pinkish white to brown colored tips, simple to coralloid to dichotomous ramification patterns, pseudoparenchymatous to plectenchymatous mantle types and simple, aseptate, branched to highly ornamented, clamped, septate emanating hyphae. Phylogenetic relationships, inferred by Maximum Likelihood criterion, are also discussed to verify the divergent affinities of identified MOTUs from one another. Roots of Himalayan spruce are shown to be forming extensive mutualistic associations with Sebacinoid and Tomentelloid fungi, alongwith Clavulina, Geopora, Russula and Pachyphloeus ectomycorrhizas, thus indicating that it is a successful host for ectomycorrhizal fungi. This investigation is the first attempt to explore the below-ground ECM fungi being associated with P.smithiana and these fungi can be of great assistance in conserving this rare spruce.

Workshop Sessions

MEM-WK312.03 - Septate plant endophytes confer tolerance to an extreme environment

Susan Kaminskyj[1], Tim Repas[1]
[1] *University of Saskatchewan, Saskatoon, Canada*

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs with key roles in plant mineral nutrition, particularly for phosphorus. Like AM, certain septate endophyte fungi have important roles in plant nutrition and in conferring growth tolerance to environments impacted by abiotic stresses, including hydrocarbon contamination. Unlike AM fungi, these septate endophytes can be isolated from their host plant and grown in culture. Endophyte spores readily colonize crop and other herbaceous plants, where the fungus confers the same type of tolerance as for their isolation environment. One promising application of fungal endophyte technology is in revegetation of land impacted by strip mining in the Athabasca tar sands. During processing the bitumen-sand mixture is treated with hot water, lye and other chemicals. Tailing sands (TS) are a high volume byproduct of bitumen extraction, that comprises ~80 % of the total mined. TS are stripped of plant mineral nutrients during bitumen extraction, and are hydrophobic due to residual hydrocarbon. TS are stored in tailings management areas prior to remediation. Currently there are >750 km2 of TS, but only 1 km2 has been remediated with standard technologies. This includes filling and contouring the mined area, covering with a 1 metre thick layer of plant mulch, fertilizing and planting. A weedy pioneer plant that was growing on TS was shown to contain several endophyte fungi, including a strain of Trichoderma harzianum, named TSTh20-1. Plants including tomatoes and a selection of native grasses and legumes that were colonized with TSTh20-1 were able to germinate and grow vigorously on TS when watered with rainwater. A biocompatible pretreatment was sufficient (in plant growth room conditions) to overcome TS hydrophobicity. Field tests (when approved) will allow us to attempt economical revegetation of TS, as a first stage in primary restoration of a healthy soil microflora.

Workshop Sessions

MEM-WK312.04 - The NsdD GATA factor and its downstream regulators control sexual and asexual development in Aspergillus nidulans

Dong-Soon Oh[1], Mohammed Abdoelgabbar[1], Seul-A Gu[2], Jong-Hwa Kim[1], Dong-Min Han[2], Kap-Hoon Han[1]
[1]Department of Pharmaceutical Engineering, Woosuk University, Wanju, Korea, [2]Division of Life Sciences, Wonkwang University, Iksan, Korea

In a homothallic filamentous fungus Aspergillus nidulans, sexual and asexual developmental processes are affected by the genetic controls and various environmental factors. Previously, we identified the nsdD gene, encoding a GATA-type transcription factor that positively regulates sexual development. Here, we identified the fkhA gene, which encodes a putative forkhead transcription factor which is possibly regulated by nsdD and involved in sexual development. The fkhA deletion resulted in the complete loss of fruiting body formation under all conditions favoring sexual development, indicating that the fkhA gene is required for normal sexual development in A. nidulans. Overexpression of fkhA showed enhanced production of fruiting bodies under induction condition not only in the normal condition but also in the inhibiting condition of sexual development. Furthermore, we isolated the ndrA gene by transcriptome analysis, which encodes a Helix-Loop-Helix DNA binding protein that can be regulated by nsdD. Knock-out mutant of ndrA produces a lot of fruiting bodies but no conidia while overexpression of ndrA produces many conidia without fruiting bodies, indicating that the ndrA gene is a negative regulator of sexual development as well as a positive regulator of asexual development. Taken together, two genes, fkhA and ndrA, are positively regulate sexual and asexual development, respectively, under the genetic regulation of NsdD.

Workshop Sessions

MEM-WK312.05 - Cloning and characterization of resistance related regulation genes from an AM fungus Rhizophagus intraradices

Bin Zhao[1], Zhongfeng Sun[1]
[1]Huazhong Agricultural University, Wuhan, China

Arbuscular mycorrhiza known as a reciprocal symbiont of vascular plants and mycorrhizal fungi widespread existence in nature. The maintenance of this mutualistic symbiosis is through vascular plants provide carbon source as nutrition material to mycorrhizal fungi, mycorrhiza fungi transfers plant Pi through its hyphae of complex network as reverse. Moreover mycorrhizal fungi can also increase the resistance of plants. The found of Mycorrhiza fungi phosphorus transport(PT) genes provide the basis for understanding the mechanism Pi transport. But resistance enhancement of mycorrhizal fungi involves complex regulatory network, and the research of this aspect is still poorly understood by now. Here, we report the HOG1, PKA and YAK gene of Rhizophagus intraradices. As an important ingredient of the MAP way, GiHOG1 can adjust the activity of resistance related transcription factors such as msn2, hot1 to inspire the downstream cascade. GiPKA and Gi14-3-3 are believed can improve fungi resistance by active target proteins through the phosphorylation and interaction in fungi. GiYAK1 encodes a negative factor of resistance, which can influnce many resistance related proteins. Through HIGS, we can study the role of all these resistance related protein in the mediation of fungal resistance as well as the role they played in the process of early symbiotic respectively. Taken together, through the series of related genes we can get a deep understanding the resistance mechanism of mycorrhizal fungi, and provide some basis in the molecular level for the subsequent research on the mechanism of mycorrhizal fungi improving the resistance of plants.

Workshop Sessions

MEM-WK313.01 - The commensal yeast *Malassezia*–host-microbe interactions

Annika Scheynius[1]
[1] *Translational Immunology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden*

Malassezia yeasts are present on the skin of warm-blooded animals and dominate the skin human mycobiome. There are currently 14 recognized species, eleven of which have been found on humans. *Malassezia* are dependent on host lipids and secrete lipases and phospholipases that likely release host fatty acids. Colonization starts right after birth, increases during puberty with the highest densities on sebum-rich areas of the skin such as the trunk and head. *Malassezia* are associated with skin diseases such as pityriasis versicolor, seborrheic dermatitis, dandruff, folliculitis and atopic eczema (AE). Approximately 50% of adult patients with AE have allergen-specific IgE- and T-cell reactivity and/or positive atopy patch test reactions to *Malassezia* leading to the hypothesis that in AE *Malassezia* act as allergens rather than infectious agents. Currently, thirteen allergens from *Malassezia* species are reported by the official allergen nomenclature list (www.allergen.org) and at least 12 genes encoding different classes of the proteins have been confirmed to be present in both the *M. sympodialis* and *M. globosa genomes*. Interestingly, several of the identified allergens are proteins of unknown function, whereas others are homologous to host proteins, such as thioredoxin and manganese superoxide dismutase, suggesting the possibility of cross-reactive immune responses. In fact, new subgroups of AE patients have been discovered who, in addition to IgE reactivity to *Malassezia* allergens, have an autoimmune IgE-mediated reactivity against self-antigens. Such cross-reactivity might contribute to the pathogenesis of AE by perpetuating skin inflammation in patients with AE sensitized to *Malassezia*. Another mechanism by which *Malassezia* can interact with the host is the release of extracellular exosome-like nanovesicles carrying allergens. These nanovesicles, designated MalaEx, can induce inflammatory cytokine responses with a significantly higher IL-4 production in AE patients compared to healthy controls. These findings suggest that MalaEx might be potential therapeutic targets.

Workshop Sessions

MEM-WK313.02 - Caspase 1 mediates protection against Aspergillus fumigatus chronic airway infection through an IL-1 independent mechanism

Benjamin Ralph[1], Melanie Lehoux[1], Brendan Snarr[1], Maya Saleh[1], Donald Sheppard[1]
[1]McGill University, Montreal, Canada

Backgound: Aspergillus fumigatus is a ubiquitous mold which commonly colonizes the airways of patients with chronic lung disease such as cystic fibrosis. The acquisition of A. fumigatus colonization is associated with increased levels of pulmonary inflammation and declining airway function, even in the absence of allergic disease. We therefore hypothesized that the caspase 1 inflammasome may play an important role in governing the inflammatory response to A. fumigatus colonization. Methods: C57BL/6 mice were infected intratracheally with agar beads containing A. fumigatus conidia. Fungal colonization was quantified by pulmonary galactomanan content and the host immune response was assessed by measuring cytokine levels, leukocyte recruitment, and histopathology. Results: Fungal colonization was associated with an increase in the pulmonary levels of pro-inflammatory cytokine IL-1β levels. Consistent with a role for the IL-1 β in mediating the response to A. fumigatus airway colonization, both IL-1 receptor deficient mice and mice deficient in caspase 1, the canonical protease responsible for pro-IL-1β maturation, were more susceptible to fungal colonization and displayed a higher fungal burden in the first week of infection when compared to wild-type mice. Surprisingly, caspase 1 deficient mice were found to have an intact Il-1β response, suggesting that the increased susceptibility of these mice to A. fumigatus was IL-1β independent. To test this hypothesis, caspase-1 deficient mice were treated with IL-1Ra, a competitive inhibitor of the IL-1 receptor; and IL-1 receptor deficient mice were treated with YVAD to inhibit caspase-1 activity. Following infection, chemical inhibition of either 1L-1 receptor signalling in caspase 1 deficient mice or caspase 1 inhibition in IL-1 receptor deficient mice resulted in an increase in pulmonary fungal burden. Conclusion: Collectively these results suggest that the IL-1 axis and caspase 1 inflammasome mediate protection against A. fumigatus airway colonization through independent mechanisms.

Workshop Sessions

MEM-WK313.03 - High content investigation of neutrophil immune response to Candida albicans

Nicholas Geoffrion[1], Malcolm Whiteway[2]
[1]McGill University, Montreal, Canada, [2]Concordia University, Montreal, Canada

As one of the main components of the innate immune system, neutrophils play a key role in eliminating blood borne pathogens. For C. albicans to become invasive, it must first disseminate through the bloodstream and bypass neutrophils, which remain largely untouched in patients suffering from HIV, but can be seriously diminished in patients undergoing certain medical procedures (e.g. transplants, chemotherapy). The HL60 promyelocytic cell line can easily be differentiated to a neutrophil-like state, providing a convenient, in vitro means of investigating the cellular basis by which C. albicans overcomes neutrophils; taking advantage of the HL60 cell line as a control, interactions that are neutrophil specific can be investigated. Using the HL60 cell line to challenge a collection of non redundant knockouts yields potential candidate genes responsible for C. albicans' ability to overcome the hosts innate immune response. Further investigation of knockouts for these genes via microscopy and killing curves seeks to cement their relevance and elucidate their role in immune evasion beyond the classical paradigm of reduced virulence being associated with loss of either hyphal or yeast morphology.

Workshop Sessions

MEM-WK313.04 - **Immunology and persistence of the microsporidia.**

Imtiaz Khan[1], Magali Moretto[1], Louis Weiss[2]
[1]*Department of Microbiology, Immunology and Tropical Medicine. George Washington University, Washington, USA,*
[2]*Departments of Medicine and Pathology, Albert Einstein College of Medicine, Bronx, USA*

The microsporidia are a diverse phylum of obligate intracellular parasites that are related to the Fungi, possibly as a sister taxon. These pathogens can cause life-threatening infections in immune compromised hosts, such at those with HIV infection or organ transplantation. Microsporidia can also cause infections in immune competent individuals including travelers, contact lens wearers and the elderly. Interestingly, in several instances, microsporidia have been shown to persist in their hosts causing at opposite ends of the spectrum either an intractable chronic diarrhea and wasting in patients with advanced-stage AIDS or asymptomatic shedding of spores in healthy populations. This presentation will review data on the immune response to microsporidia. While it is clear that CD8+ T cells are essential in clearing this infection the role of CD4+ T cells is not as well defined. As microsporidia are acquired via the oral route gut associated immunity plays an important role in host protection. Interestingly a very early and rapid intra epithelial lymphocyte response (IEL) was observed in infected hosts. Amongst these IELs (a heterogeneous population) the CD8 alpha beta subset exhibited a major effector response. The protection mediated by these cells was primarily mediated by perforin dependent cytotoxic activity. The mechanism involved in the priming and maintenance of this population is critical for understanding long-term protection against the pathogen and developing immunotherapeutic agents. A better appreciation of the host response to microsporidia should provide insights into the survival strategies of these pathogens that enable them to persist in hosts of varied immune status.

Workshop Sessions

MEM-WK313.05 - Characterization of B Cells subpopulation responses during Leishmania donovani infection

Sasha Silva-Barrios[1], Albert Descoteaux[1], Simona Stäger[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada

Leishmania donovani is one of the protozoan parasites that cause Visceral Leishmaniasis (VL), a zoonotic disease in which clinical manifestations are not specific and are frequently obvious at the chronic stages. Hypergammaglobulinemia is one of the relevant characteristics of VL and a consequence of polyclonal B cell activation induced by the parasite. Interestingly, B-cell deficient mice are highly resistant to VL. B cells are best known for their role in the production of antibodies to facilitate the elimination of pathogens and antigens. However, several publications have now demonstrated that these cells can also regulate adaptive T cell responses by various antibody independent mechanisms, such as cytokine production. Indeed, B cells can both enhance or suppress CD4+ T cell responses, depending on the disease model. We have recently shown that B cells and marginal zone B cells (MZB) suppress protective T cell responses during experimental VL. This suppression is mediated in part by IL-10 production by MZB. Here, we characterize the interaction between L. donovani and various splenic B cell subsets, assessing cytokine production, activation markers, and possible activation pathways. We show that IL-10 production by MZB is MyD88-dependent; in contrast, upregulation of the costimulatory molecule CD86 by the parasite does not require the MyD88 or the B cell receptor. Other pathways of activation will be discussed.

Workshop Sessions

MEM-WK313.06 - L. major promastigotes escape LC3-associated phagocytosis through GP63-mediated proteolytic cleavage of VAMP8

Christine Matte[1], Neda Moradin[1], Pierre-André Casgrain[1], Albert Descoteaux[1]
[1]Institut National de la Recherche Scientifique - Institut Armand-Frappier, Montreal, Canada

Protozoan parasites of the genus Leishmania cause a wide spectrum of diseases in humans, ranging from self-healing skin ulcers to life-threatening visceral infection. These pathogens primarily infect macrophages and are renowned for their ability to sabotage host antimicrobial responses. Previous studies from our laboratory have shown that soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), key mediators of membrane fusion, are targeted by Leishmania virulence factors to promote the establishment of an intracellular infection. Notably, we have recently shown that the zinc-dependent metalloprotease GP63 of L. major promastigotes cleaves vesicle-associated membrane protein 8 (VAMP8) and thereby prevents NADPH oxidase assembly at the phagosomal membrane. In this study, we aimed to investigate the effect of L. major on LC3-associated phagocytosis (LAP), a newly-identified process triggered by the recognition of pathogens by Toll-like receptors (TLR) or Fcγ receptors (FcγR) and subsequent reactive oxygen species production by NADPH oxidase. Infection of bone marrow-derived macrophages (BMDM) with L. major promastigotes resulted in LC3-I conversion to its lipidated form, LC3-II. No effect on Beclin-1 protein expression or intracellular localization was observed, suggesting that LC3 conversion did not result from autophagy induction. Interestingly, LC3 recruitment to phagosomes containing GP63-expressing L. major promastigotes was significantly impaired in comparison with GP63-deficient parasites, most likely as a result of GP63-mediated VAMP8 cleavage. Indeed, infection of BMDM from VAMP8 knock-out mice showed that, in the absence of functional VAMP8, LC3 recruitment to phagosomes containing GP63-deficient parasites was inhibited. Further analyses are currently under way to evaluate the role of NADPH oxidase in these events and to determine the importance of LAP in the control of Leishmania infections. Altogether, this study highlights a novel mechanism by which L. major interferes with macrophage responses and provides a better understanding of Leishmania pathogenesis.

Workshop Sessions

VIR-WK222.01 - Role of the Polyomavirus micro RNA

Michael Imperiale[1], Nicole Broekema[1]
[1]*University of Michigan, Ann Arbor, USA*

Viral microRNAs (miRNAs) play an important role during infection by post-transcriptionally regulating both host and viral gene expression. However, the function of many viral miRNAs remains poorly understood. In this study, we investigated the role of the BK polyomavirus (BKPyV) miRNA in regulating virus replication. The function of the polyomavirus miRNA was investigated in an archetype BKPyV, which is the transmissible form of the virus and thought to establish a persistent infection in the host urinary tract. In agreement with previous studies, we show that the BKPyV miRNA targets large T antigen (TAg) mRNA. Importantly, we show that the miRNA plays a significant role in limiting archetype BKPyV replication in a natural host cell model of infection. This regulation control is accomplished through the balance of regulatory elements located within the non-coding control region controlling early gene expression and miRNA expression prior to genome replication. We therefore provide evidence for a novel function of the polyomavirus miRNA that may have important implications for the mechanism of viral persistence.

Workshop Sessions

VIR-WK222.02 - DDX3 and pVIII interaction with tripartite leader (TPL) of BAdV-3 enhances translation of late viral mRNAs and production of progeny virions

Lisanework Ayalew[1,2], Amit Gaba[1,2], Suresh Tikoo[1,2,3]
[1]*Vaccine and Infectious Disease Organization-International Vaccine Center (VIDO-InterVac), Saskatoon, Canada,* [2]*Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada,* [3]*School of Public Health, University of Saskatchewan, Saskatoon, Canada*

Adenovirus infection modulates cellular protein synthesis at late times post infection. This has been attributed to the activity of one or more late adenovirus proteins. One of the late bovine adenovirus (BAdV)-3 proteins, pVIII interacts with a cellular protein DDX3 in virus infected cells and affects cellular protein synthesis by reducing the level of DDX3, and other eukaryotic initiation factors in the cap binding complex. Moreover, the reduction in the cellular protein synthesis is evidenced by less functional 80S ribosomes and polysomes in pVIII expressing transfected cells or BAdV3 infected cells (late times post infection). Interestingly, knockdown of DDX3 resulted in significant reduction in virus yield and expression of BAdV-3 late proteins suggesting that DDX3-pVIII interactions appear to be involved in selective BAdV-3 late mRNA translation. Furthermore, evidence of binding of DDX3 and pVIII to BAdV-3 tripartite leader (TPL), and enhanced translation of a reporter gene containing TPL at 5' UTR in the presence of pVIII and DDX3 proteins in DDX3 negative cells support our conclusion that pVIII and DDX3 are involved in the selective translation of late viral mRNAs that contain TPL.

Workshop Sessions

VIR-WK222.03 - Human Enteric Adenoviruses 40 and 41 in 293 and Caco-2 cells

Casandra Mangroo[1], Martha Brown[1,2]

[1]Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Canada, [2]Department of Molecular Genetics, University of Toronto, Toronto, Canada

Fastidious human enteric adenoviruses, serotypes 40 and 41 (HAdV-40/41; species F), are important agents of paediatric enteritis. Unlike other adenoviruses, they grow to high titre in the small intestine but typically do not cause disease elsewhere in the body. In cell culture, infectivity is poor, even in 293 cells, whose endogenous HAdV-5 E1 sequences complement the low level E1 expression of HAdV-40/41. Recent work in this laboratory identified a major, though incomplete, block in virion uptake in 293 and A549 (lung epithelial) cells. A comparable block in intestinal epithelial (Caco-2) cells, grown under conditions optimized for susceptibility to HAdV-40/41, was unexpected. Caco-2 cells are less susceptible to infection as cells become differentiated with time, and are most susceptible in sub-confluent undifferentiated cultures, in particular, when the edges are exposed. Recent experiments using HAdV-41-EGFP and 293 cells have addressed the hypothesis that virions are stable during passage through the stomach, but require modification by intestinal conditions for successful entry/genome delivery. Consistent with the hypothesis, virion infectivity is stable upon exposure to synthetic gastric fluid, as determined by flow cytometric analysis of infected cultures. Modest increases in infectivity were found under certain experimental conditions. Subsequent experiments will address structural modifications induced by gastric and intestinal conditions and steps in the entry pathway facilitated by these modifications. These studies should help determine the molecular basis for the restricted tropism of enteric adenoviruses.

Workshop Sessions

VIR-WK222.04 - Genomic and bioinformatics analysis of simian adenovirus 19 confirms the need to establish a new adenovirus species

Iva Podgorski[1], Laura Pantó[1], Mária Benkő[1], Balázs Harrach[1]
[1] *Institute for Veterinary Medical Research, Hungarian Academy of Sciences, Budapest, Hungary*

Besides being an ideal model for study of viral evolution, adenoviruses (AdVs) also keep gaining popularity as gene delivery vectors and vaccines. However, preexisting antibodies in the human population limit the medical use of human AdVs (HAdVs), phylogenetically grouped into seven species (HAdV-A to HAdV-G) within the genus *Mastadenovirus*. In order to identify appropriate alternatives, we seek and analyze non-human primate AdVs. Ape AdVs (the closest relatives to HAdVs) and several monkey AdVs phylogenetically belong to HAdV species. Species *Simian adenovirus A* (SAdV-A) is the only species officially approved for monkey AdVs exclusively. This study examined the genetic content and phylogenetic relationships of SAdV-19 from yellow baboon (*Papio cynocephalus*). Full genome sequencing revealed a genome of 34,063 bp, with 38 putative genes characteristic of the genus *Mastadenovirus*. Two fiber genes, coding for the cellular attachment protein, of different lengths were found. For the first time in a SAdV, two additional exons belonging to the so-called U exon were also identified. Genomic study of several novel baboon AdV (BaAdV) strains has been recently published by others, with BaAdV-2 and BaAdV-3 proposed to form novel species SAdV-C. Phylogenetic calculations based on the major capsid protein, the hexon, implied that SAdV-19, BaAdV-2 and BaAdV-3 represent three different (sero)types within the proposed species SAdV-C. Phylogeny inference based on the viral DNA-dependent DNA polymerase protein supported this species classification. However, significant divergence was found between the shorter fiber proteins (fiber1) of SAdV-19 and BaAdV-2 or BaAdV-3, respectively. The closest relative of fiber1 of SAdV-19 was that of SAdV-1, sharing ~47% amino acid sequence identity. This finding may reflect that a usually rare, inter-species homologous recombination event took place between the two viruses (or their close ancestors) in the past. *Support: EU FP7 ADVance grant*.

Workshop Sessions

VIR-WK222.05 - Downregulation of cell surface major histocompatibility complex class I expression is mediated by a small transmembrane protein, ORF1C, of fowl adenovirus type 9

Bryan D Griffin[1], Li Deng[1], Yanlong Pei[1], Eva Nagy[1]
[1]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada*

Adenoviruses (AdVs) are members of the family Adenoviridae and are nonenveloped, medium-sized, double-stranded DNA viruses that infect a wide range of vertebrate hosts. Fowl adenoviruses (FAdVs) that infect birds are in the genus Aviadenovirus. Some FAdVs are associated with inclusion body hepatitis (IBH), characterized by hepatic necrosis and intranuclear inclusion bodies. While mammalian adenoviruses are well-studied, protein functions of the most studied FAdV, FAdV-1, have been demonstrated for only three of the predicted 19 genus-specific genes, ORF1, ORF22, and GAM-1. Although FAdVs do not have an E3 region, the region responsible for major histocompatibility complex class I (MHC-I) downregulation in human adenoviruses, we hypothesized that one or more of the FAdV genus-specific genes down-regulate surface expression of BF2, the MHC-I molecule of the chicken. As determined by flow cytometric analysis with anti-chicken MHC-I, the normally high MHC-I cell surface expression of chicken hepatoma cells (CH-SAH) was reduced to approximately 15% of initial levels upon infection with FAdV-9 at a multiplicity of infection of 5. The left-end transcription unit deletion virus (FAdV-9Δ4), in contrast, reduced cell surface MHC-I to approximately 50%. Western blotting of protein lysates of uninfected, parental, and knockout virus-infected CH-SAH with anti-chicken MHC-I antibody revealed that total MHC-I was reduced equally. We, therefore, hypothesize that MHC-I is sequestered intracellularly via direct binding with an FAdV-9 protein(s) or through disruption of intracellular transport. To examine the potential roles of the predicted open reading frames (ORFs) within the leftmost transcription unit, we systematically mutated (FAdV-9-ORF1-stop, FAdV-9-ORF1C-stop) or knocked out (FAdV-9ΔORF2) each individual ORF and examined infected cells for loss of the ability to downregulate cell surface MHC-I. Cells infected with FAdV-9-ORF1C-stop showed a 50% reduction in cell surface MHC-I downregulation versus wtFAdV-9. To our knowledge, this is the first study to identify an immunomodulatory role for an FAdV protein.

Workshop Sessions

VIR-WK222.06 - Characterization and functional studies of fowl adenovirus 9 dUTPase

Li Deng[1], Xiaobing Qin[2], Bryan Griffin[1], Betty-Anne McBey[1], Peter Krell[3], Ray Lu[3], Shayan Sharif[1], Éva Nagy[1]
[1]Department of Pathobiology, University of Guelph, Guelph, Canada, [2]College of Animal Science and Veterinary Medicine, Qingdao Agricultural University, Qingdao, China, [3]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Canada

Fowl adenoviruses (FAdVs) are potential vaccine vector alternatives to human adenoviruses due to their larger genomes and having a larger transgene capacity. In particular, fowl adenovirus 9 (FAdV-9, strain A-2A), a non-pathogenic virus, has shown great potential as a vaccine vector for poultry based on findings from our laboratory. However, in contrast to human adenoviruses, the molecular biology of fowl adenoviruses is only poorly understood. Previous work in our laboratory has demonstrated that a region at the left end of the genome, bearing 6 open reading frames (ORFs0, 1, 1A, 1B, 1C and 2), is non-essential for virus replication in vitro but contributes to less efficient virus replication in vivo. However, the role of the individual ORFs remains unknown. In the present study, we characterized ORF1, a deoxyuridine triphosphatase (dUTPase) homologue, and explored its functions. Firstly, we confirmed through a PCR-based enzyme assay that ORF1 is a functional dUTPase that is able to hydrolyze dUTP. Additionally, three mutant viruses (FAdV-9ORF1null, ResFAdV-9ORF1null and FAdV-9-ORF1:HA) were generated through site-directed mutagenesis, homologous recombination with a FAdmid in BJ5183 E. coli cells and transfection in CH-SAH cells. Results from RT-PCR and Western blotting showed that ORF1 is transcribed from 2 hours post-infection (hpi) and its protein is translated from 6 hpi and both continue to the late phase of the virus infection. An immunofluorescence assay (using anti HA antibody) with FAdV-9-ORF1:HA indicated that the ORF1 protein is localized in both the cytoplasm and nucleus. Moreover, we compared wtFAdV-9 and FAdV-9ORF1null in terms of virus replication and expression of type I interferons. The results showed that ORF1 is able to up-regulate the expression of type I interferons both in vitro and in vivo.

Workshop Sessions

VIR-WK222.07 - Crystal structure of adenovirus E3-19K protein bound to HLA-A2 reveals mechanism for immunomodulation

Lenong Li[1], Yasameen Muzahim[1], Marlene Bouvier[1]
[1]*University of Illinois at Chicago, Chicago, USA*

The presentation of viral antigens by MHC class I molecules to T-cells is critical for the elimination of host infected cells. Notably, viruses have evolved multiple strategies to interfere with MHC I antigen presentation. For example, the adenovirus (Ad) E3-19K protein binds to and retains MHC I molecules within the endoplasmic reticulum (ER) of infected cells, suppressing the activity of anti-Ad T-cells. E3-19K is a type I transmembrane glycoprotein that comprises a N-terminus ER-lumenal domain, a transmembrane domain, and a C-terminus cytosolic tail. The ER-lumenal domain of E3-19K associates with the ER-lumenal domain of MHC I, while the dilysine motif in its cytosolic tail provides the signal for localization of the E3-19K/MHC I complex within the ER. Here, we will describe a novel "rescue refolding strategy" that allowed formation of the Ad serotype 2 (Ad2) E3-19K/HLA-A2 complex, from which we grew crystals diffracting to 1.95 Å resolution. The x-ray crystal structure of Ad2 E3-19K/HLA-A2 shows that Ad2 E3-19K binds to the N-terminus of the HLA-A2 groove, contacting the α1-, α2-, and α3-domains and β2m. Ad2 E3-19K has a unique structure comprised of a large N-terminal domain formed by two partially overlapping β-sheets arranged in a V-shape, a short α-helix, and a tail segment. Our structure reveals determinants in both E3-19K and HLA-A2 that are important for complex formation; conservation of some of these determinants in E3-19K of different Ad species and MHC I of different HLA loci suggests a universal binding mode for all E3-19K/MHC I complexes. Importantly, our structure offers explanations for the mechanism by which E3-19K modulates antiviral cellular immunity.

Workshop Sessions

VIR-WK223.01 - Needs and opportunities in antiviral drug development

Mike Bray[1]
[1] *NIH, Bethesda, USA*

Recent progress in developing a range of direct-acting drugs for hepatitis C and the success of experimental therapies for some severe diseases has provided encouragement to the field of antiviral drug development, but treatments are still lacking for many acute viral infections. My talk will highlight a number of areas in which research efforts are needed, or where opportunities exist for making rapid progress.

Workshop Sessions

VIR-WK223.02 - Resistance-associated mutations from clinical varicella-zoster virus sequences as the cause of drug resistance proven by targeted recombinants of a cloned clinical wild type isolate

Anne-Kathrin Brunnemann[1], Kathrin Bohn[2], Roland Zell[2], Martin Walther[2], Helmut Fickenscher[1], Andreas Sauerbrei[2], Andi Krumbholz[1]

[1]Institute for Infection Medicine, Christian Albrecht University Kiel, Germany, [2]Institute of Virology and Antiviral Therapy, Reference Laboratory for HSV and VZV, Friedrich-Schiller University Jena, Germany

Particularly, in immunosuppressed patients, the resistance development of varicella-zoster virus (VZV) against aciclovir (ACV) and other antivirals is observed with increasing frequency. Resistance-associated mutations usually affect the thymidine-kinase gene (TK, ORF36) and less frequently the DNA polymerase gene (Pol, ORF28). However, VZV can only be isolated in rare cases. Thus, it often remains unclear if a specific resistance-associated mutation is actually causative for the therapeutic failure. Initially, eight ORF36 sequences from ACV-resistant VZV strains with novel amino acid substitutions were amplified and the derived TK was expressed in E. coli. The enzymatic TK activity was abolished in all but one of these cases. Subsequently, the respective nucleotide substitutions were inserted by site-directed mutagenesis into the wild type ORF36 of a transfer construct. The sequence variants were transferred in E. coli into the VZV wild type isolate HJO, which was cloned as a bacterial artificial chromosome (BAC). By two-step en passant mutagenesis, the ORF36 of HJO was replaced by the mutated variants. Additionally, VZV recombinants with Pol mutations were generated. For better plaque identification, the gene for the monomeric red-fluorescent protein was inserted into a non-coding intergenic region. After transfection of the BAC mutant into VZV-permissive cells, the reconstituted virus strains were tested by plaque assay using various antivirals. While all TK-mutated strains showed phenotypic resistance against ACV, penciclovir, or brivudine, the Pol mutants showed resistance against cidofovir, only. These results were confirmed by qPCR and quantification of the fluorescence. In conclusion, a recombinant system was generated and validated to introduce any TK or Pol mutations into a wild type VZV background in order to prove the causative role of the specific mutation for resistance to antiviral drugs. The study was supported by an intramural grant of the CAU Kiel awarded to AK/HF. AKB and KB contributed equally.

Workshop Sessions

VIR-WK223.03 - Genotype-dependent inhibition of Influenza A M2 ion channel activity by non-cytotoxic, acylguanidine-based small molecules

Ian Tietjen[1], Scott Miller[1], Daniel Kwan[1], Brent Johnson[2], Hannah Boycott[1], Jodene Eldstrom[1], Doug Chou[1], David Busath[3], David Fedida[1]

[1]Department of Anesthesiology, Pharmacology, and Therapeutics, University of British Columbia, Vancouver, Canada, [2]Department of Microbiology and Molecular Biology, Brigham Young University, Provo, USA, [3]Department of Physiology and Developmental Biology, Brigham Young University, Provo, USA

The rapid emergence of drug-resistant Influenza A viruses highlights an urgent need for new antiviral development. Amantadine and rimantadine are potent inhibitors of M2, an ion channel encoded by Influenza A, but increased viral resistance has rendered these drugs ineffective. Here we describe a series of novel acylguanidine-based small molecules that are potent inhibitors of M2 as measured by whole cell patch clamp electrophysiology. The lead molecule, SM111, inhibits wild-type M2 with an IC50 of 0.2μM, making it more potent than amantadine or hexamethylene amiloride (HMA) in this assay (respective IC50s = 0.6 and 1.3μM). SM111 is also less toxic than HMA (respective CC50s = 130 and 16μM; respective Selectivity indices (SIs) = 650 and 12.3) with weaker blockade of hERG cardiac ion channel (respective IC50s = 1.5 and 3.2μM; respective SIs = 16.0 and 1.2). SM111 also competes with amantadine for blockade of M2. A structure-activity-relationship study further identifies an SM111 derivative called SM122 that blocks adamantane-resistant M2 with an S31N mutation (IC50=70+/-10μM). Interestingly, the activity of SM122 is dependent on distinct M2 polymorphisms that do not influence adamantane resistance. Finally, we show that these molecules block wild-type and/or adamantane-resistant Influenza A virus propagation in vitro (e.g., EC50 for SM122 against adamantane-resistant virus = 2.4μM). Our study emphasizes the use of non-adamantane chemical scaffolds to develop potent influenza A ion channel inhibitors and illustrates the potential of additional M2 polymorphisms to underlie viral drug resistance.

Workshop Sessions

VIR-WK223.04 - WITHDRAWN - Clinical relevance of antiviral compounds that disable the paramyxovirus by premature activation of fusion protein

Ilaria De Vito[1], Laura Palermo[1], Matteo Porotto[1], Anne Moscona[1]
[1]Department Pediatrics & Microbiology and Immunology, Weill Medical College of Cornell University, New York, USA

Paramyxoviruses, enveloped RNA viruses that include human parainfluenza virus type 3 (HPIV3), cause the majority of childhood cases of croup, bronchiolitis, and pneumonia worldwide. HPIV3 enter host cells by fusion of the viral and target cell membranes. Fusion proceeds via a multistep reaction orchestrated by the two glycoproteins that comprise the HPIV3 fusion machinery: the receptor binding protein (hemagglutinin-neuraminidase; HN) and the fusion protein (F). The first step is recognition and binding of cell surface sialic acid receptors by HN. HN-receptor interaction leads to activation of the F protein, via a series of conformational changes in F that render it fusion-competent. To successfully promote fusion, the triggering of F must occur while HN is in contact with its receptor and the F protein is proximal to the target cell membrane. Previously, we have identified a new antiviral small molecule, CSC11, a HPIV3 receptor mimic whose interaction with HN results in F-activation prior to receptor binding. The fusion machinery is prematurely activated and disabled, preventing fusion of the viral membrane with target cells and precluding viral entry. While CSC11 provided a proof of concept for a new antiviral strategy, it was effective only at mM concentrations. Here we made directed modifications to the CSC11 molecule and generated compounds effective in the nM concentration range. These compounds were effective at inhibiting HPIV3 clinical strains in differentiated human airway epithelial cell cultures (HAE), suggesting that they merit in vivo study.

Workshop Sessions

VIR-WK223.05 - Anti-HCV activity of novel pyranone carboxamide derivatives and their structure-activity relationship

Masanori Baba[1], Masaaki Toyama[1], Wataru Ito[1], Mika Okamoto[1], Ananda Kumar Konreddy[2], Chandralata Bal[2], Ashoke Sharon[2], Koichi Watashi[3], Takaji Wakita[3]

[1]Kagoshima University, Kagoshima, Japan, [2]Birla Institute of Technology, Ranchi, India, [3]National Institute of Infectious Diseases, Tokyo, Japan

The current standard of treatment for HCV infection includes one of the NS3 protease inhibitors combined with pegylated interferon-α (PegIFN) and the nucleoside analog ribavirin. This regimen has improved sustained viral response rate up to 75−80%. However, the use of pegIFN/ribavirin is associated with considerable side effects. Therefore, it would be ideal, if effective treatment for HCV infection could be achieved with pegIFN/ribavirin-free regimens. A number of novel HCV inhibitors, termed direct acting antivirals (DAAs), targeting not only NS3 protease but also NS5B RNA polymerase and NS5A protein have been identified and currently being under clinical development. We have recently synthesized a novel series of pyranone carboxamide derivatives and examined for their antiviral activity in cell cultures. Although these compounds were not inhibitory to HIV-1 and HBV replication, some of them displayed selective inhibition of HCV replication. The most potent compound of the series was 4-(hydroxyamino)-2-oxo-6-phenyl-N-(p-tolyl)-2H-pyran-3-carboxamide. Its 50% effective concentration and 50% cytotoxic concentration were 0.18 ± 0.09 μM and > 20 μM, respectively, in subgenome HCV (genotype 1b) replicon cells. The compound also showed potent anti-HCV activity in full-genome HCV replicon cells. Interestingly, it did not have any inhibitory effects on NS3 protease or NS5B RNA polymerase activity in cell-free enzyme inhibition assays. When their structure-activity relationship was analyzed, the minimum structural requirement for anti-HCV activity was 4-(hydroxyamino)-N-methyl-2-oxo-6-phenyl-2H-pyran-3-carboxamide. These results suggest that the pyranone carboxamide derivatives have potential as anti-HCV agents with a novel mechanism of action, including the inhibition of NS5A or host cellular factors. Further experiments, such as induction of drug-resistant replicons, are in progress to elucidate the target molecule.

Workshop Sessions

VIR-WK223.06 - Targeting lipid droplet formation for the discovery of indirect-acting antivirals: A tale of two flaviviridae members, hepatitis C virus and dengue virus

Anastasia Hyrina[1], Andrea Olmstead[1], Steven McArthur[1], François Jean[1]
[1]*Dept. of Microbiology and Immunology, University of British Columbia, Vancouver, Canada*

In this study, we hypothesize that targeting cellular enzymes acting as master regulators of lipid homeostasis could represent a powerful approach to developing a novel class of broad-spectrum antivirals against infection associated with *Flaviviridae* viruses whose lifecycle depend on the interaction with lipid droplets (LDs), the main lipid store in eukaryotic cells. Here, we report that strategic manipulation of cellular SKI-1/S1P enzymatic activity by the active-site-directed small-molecule inhibitor PF-429242 provides a means of effectively inhibiting dengue (DENV-2) infection of human hepatoma (Huh-7.5.1) cells. SKI-1/S1P is a master regulator of the lipid homeostasis/sterol regulatory element binding protein (SREBP) pathway critical for LD formation. We demonstrate that inhibition of SKI-1/S1P using PF-429242 results in a dose-dependent inhibition of DENV infection, pre- and post-establishment of viral infection in Huh-7.5.1 cells (EC50 = 0.76 microM). Using plaque assays, we observed a ~3-log decrease in DENV-2 titer following pretreatment of Huh-7.5.1 cells with 20 microM of PF-429242 prior to infection with DENV-2. The antiviral effect of the SKI-1/S1P-directed inhibitor is associated with a robust block of SKI-1/S1P dependent proteolytic cleavage of SREBPs in hepatoma cells, a critical step in the hijacking of the host cholesterol pathways by DENV. Further, we demonstrated that SKI-1/S1P inhibition in hepatoma cells is correlated with a dramatic reduction in the abundance of LDs and the LD marker: adipose differentiation-related protein (ADRP)/perilipin 2. The results of our studies support our research hypothesis that targeting cellular SKI-1/S1P may represent a powerful approach to developing a novel class of broad-spectrum antivirals against human *Flaviviridae* viruses such as hepatitis C virus and DENV, whose lifecycle depend on the interaction with LDs. Our results also reveal the potential therapeutic opportunities associated with the use of lipid droplet-modulating agents for controlling infection of viruses that hijack the host cell lipid metabolism to support their lifecycle.

Workshop Sessions

VIR-WK224.01 - Arenavirus host cell invasion: molecular mechanisms and target for anti-viral intervention

Stefan Kunz[1]
[1]*Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland*

Several arenaviruses cause severe viral hemorrhagic fevers in humans and represent serious public health problems. The Old World Lassa arenavirus (LASV) causes several hundred thousand infections per year in Africa resulting in significant mortality and morbidity. In the Americas, the South American hemorrhagic fever viruses have emerged as etiological agents of severe diseases with high fatality rates. The first and most fundamental steps of every virus infection are the interaction of a virus with its cellular receptors and subsequent entry into the host cell. During cell invasion, the virus heavily depends on the molecular machinery of the host cell. Cellular factors implicated in viral entry represent thus key determinants for epidemiology, cellular tropism and disease potential of a virus. The past years have seen remarkable progress in our understanding of the molecular mechanisms underlying arenavirus host cell invasion, which will be covered here. Structural studies of receptor recognition by New World arenaviruses provided exciting molecular insights into receptor use and the zoonotic potential of these emerging pathogens. Investigation of LASV interactions with its receptor revealed unprecedented complexity of functional glycosylation of the receptor with implications for human diseases. The current lack of licensed vaccines against arenaviruses and the limited therapeutic options at hand make the development of novel anti-viral therapeutics an urgent need. Several screening approaches, including RNA silencing and cutting-edge proteomics, revealed novel and interesting cellular factors that play crucial roles in arenavirus cell entry and represent promising targets for therapeutic anti-viral intervention.

Workshop Sessions

VIR-WK224.02 - Boid Inclusion Body Disease–associated ArenaViruses (BIBDAV), a novel taxonomic entity in the family Arenaviridae

Tarja Sironen[1], Udo Hetzel[1,2], Jussi Hepojoki[1], Anja Kipar[1,2], Heikki Henttonen[3], Olli Vapalahti[1]
[1]University of Helsinki, Helsinki, Finland, [2]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zürich, Switzerland, [3]Finnish Forest Research Institute, Vantaa, Finland

Arenaviruses, which belong to the family Arenaviridae, have been known as rodent-borne viruses, with the exception of Tacaribe virus isolated from bats. Arenaviruses are currently divided into two serogroups: New World arenaviruses (NWA) and Old World arenaviruses (OWA). Recently, we and others have recovered arenaviruses from boid snakes with boid inclusion body disease (BIBD). BIBD is a progressive, usually fatal disease of constrictor snakes, characterized by cytoplasmic inclusion bodies in a wide range of cell types. It appears to affect captive snakes, and can lead to the loss of entire boid collections. Arenaviruses have been identified as the causative agent of the BIBD, and they can be isolated from diseased animals using cultured boid cells. We have sequenced a full genome of an individual isolate, which we tentatively designated University of Helsinki virus (UHV). Sequencing confirmed UHV to be a novel arenavirus species, distinct from other known arenaviruses including those identified in snakes with BIBD elsewhere. RT-PCR was used to study samples of diseased snakes, which revealed the presence of genetically diverse arenaviruses in a large cohort of snakes with BIBD. Here we analyse and discuss the phylogenetic relationship of these arenaviruses, and their relatedness to rodent-borne arenaviruses. In conclusion, we propose that the newly identified lineage of arenaviruses associated with BIBD as a novel taxonomic entity in the family Arenaviridae.

Workshop Sessions

VIR-WK224.03 - Roles of viral specific RNAs in the assembly of LCMV

Masaharu Iwasaki[1], Nhi Ngo[1], Beatrice Cubitt[1], Juan C. de la Torre[1]
[1]*The Scripps Research Institute, La Jolla, USA*

Several arenaviruses, chiefly Lassa virus (LASV), cause hemorrhagic fever disease in humans and pose a great public health concern in their endemic regions. Moreover, evidence indicates that the worldwide-distributed prototypic arenavirus lymphocytic choliomeningitis virus (LCMV) is a neglected human pathogen of clinical significance. Arenaviruses are enveloped viruses with a bi-segmented, negative-strand RNA genome and a life cycle restricted to the cell cytoplasm. Each genome RNA segment, S and L, uses an ambisense coding strategy to direct the expression of two viral polypeptides in opposite orientation, separated by a non-coding intergenic region (IGR). We have documented that the IGR serves as a bona fide transcription termination signal, but functional viral mRNA species are produced in the absence of the IGR. However, the absence of IGR prevents formation of infectious virus like particles, indicating its involvement in virus assembly. In this study, we have further investigated the roles played by viral specific cis-acting RNA sequences in the arenavirus life cycle. We found that efficient interaction between the nucleoprotein (NP) and polymerase (L protein) of LCMV requires the presence of virus-specific RNA sequences that not include the IGR. Moreover, a recombinant LCMV containing the LASV, instead of LCMV, S-IGR exhibited growth properties similar to LCMV WT, suggesting that structure rather than sequence specificity within the IGR is required for the production of arenavirus infectious progeny. We will discuss also the role of S-and L-IGR interaction in the production of arenavirus infectious progeny.

Workshop Sessions

VIR-WK224.04 - Chronic liver infection in mice Infected with Lymphocytic choriomeningitis Virus (lcmv)

Pascal Lapierre[1], Valérie Janelle[1], Marie-Pierre Langlois[1], Esther Tarrab[1], Tania Charpentier[1], Alain Lamarre[1]
[1]*Laboratory of Immunovirology, INRS-Institut Armand-Frappier, Laval, Canada*

To study the factors responsible for the establishment of a chronic liver infection in an immune competent host, TTR-NP transgenic mice, that express the LCMV nucleoprotein (NP) specifically in hepatocytes, were infected with LCMV-Arm or -WE strains, which normally induce acute infections. Infected TTR-NP mice showed high serum ALT levels and 20% mortality compared to infected B6 mice. Expansion of both NP396 and GP33 specific CD8 T cells occurred in TTR-NP mice, however, intracellular cytokine staining showed a polyfunctional T cell response restricted mostly against LCMV-GP. PD-1 expression by CD4+ and CD8+ T cells was increased in TTR-NP mice 55 days after infection indicative of T cell exhaustion. While LCMV-Arm or –WE was cleared by day 8 post-infection in B6 mice, TTR-NP mice remained chronically infected up to 189 days post-infection with the highest virus titers found in the liver. In vivo cytotoxicity assays revealed that TTR-NP mice have an increased CTL response to the GP33 epitope and an almost absent response against the NP396 epitope. Adoptive transfer experiments of T cells in RAG/TTR-NP mice revealed that liver-restricted expression of NP led to increased peripheral conversion/expansion of CD4+ regulatory T cells. In vivo depletion of Tregs in infected TTR-NP mice restored T cell functionality against NP, lowered PD-1 levels and led to viral clearance. In conclusion, expression of NP in the liver of TTR-NP mice induced a strong peripheral tolerance mediated by CD4+ regulatory T cells leading to reduced T cell cytokine secretion and poor cytotoxic activity against NP. This reduced immune response against one viral protein was sufficient to induce T cell exhaustion and chronic LCMV infection by limiting the antiviral T cell responses in both magnitude and breadth. Similar mechanisms might be at play in chronic HCV infections during which an expansion of Tregs has also been observed.

Workshop Sessions

VIR-WK225.01 - Evaluation of immune evasion by Crimean-Congo Hemorrhagic virus encoded deubiquitinase and deISGylase

Éric Bergeron[1], Jessica Spengler[1], Michelle Deaton[2], Ayan Chakrabarti[1], Scott Pegan[2], Christina Spiropoulou[1]
[1]Centers for Disease Control and Prevention, Atlanta, USA, [2]University of Denver, Denver, USA

Interferon production and signaling are regulated by the addition and removal of Ubiquitin (Ub) and ubiquitin-like interferon-stimulated gene-15 (ISG15) to proteins orchestrating the innate immune response. Deconjugation of Ub and ISG15 by deubiquitinases (DUB) and deISGylases negatively regulate immune signaling after virus infection. Interestingly, the large RNA dependent RNA polymerase (L-RdRp) of Crimean-Congo Hemorrhagic fever virus (CCHFV) contains an ovarian tumor (OTU) domain with strong deubiquitinating and deISGylating activity. Previous studies demonstrated that the L-RdRp viral OTU domain (vOTU) alone was capable of blocking the induction of type I interferon and tumor necrosis factor-α signaling. These activities of the vOTU are proposed to be critical for countering cellular antiviral responses. However, evidence for this in the context of CCHFV infection is lacking. Our goal was to address vOTU function in the context of infection by combining biochemical and reverse genetics approaches to obtain CCHFV mutants with altered vOTU activities. Using three-dimensional structure assisted mutagenesis, a vOTU that was deficient in cleaving ubiquitin and ISG15 substrates in vitro was generated by mutating 3 residues (DUB-/ISG-). We also inactivated the vOTU catalytic site by mutating the cysteine 40 to alanine. When introduced into the L-RdRp both mutants retained RNA replication activity similar to WT. In contrast, only DUB-/ISG- yielded a viable CCHFV. The CCHFV (DUB-/ISG-) growth in A549 cells was severely attenuated. The mechanism(s) explaining the attenuation of CCHFV (DUB-/ISG-) are currently being investigated. In conclusion, these data suggest that CCHFV the vOTU catalytic activity is essential for virus replication and that the CCHFV genome can be engineered to address vOTU DUB and deISGylase roles in innate immune evasion.

Workshop Sessions

VIR-WK225.02 - Creation of Rift Valley Fever Viruses with four-segmented genomes reveals flexibility in Bunyavirus Genome packaging

Paul Wichgers Schreur[1], Nadia Oreshkova[1,2], Rob Moormann[1,2], Jeroen Kortekaas[1]
[1]Central Veterinary Instituteof Wageningen UR, Lelystad, The Netherlands, [2]Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Bunyavirus genomes comprise a small (S), medium (M) and a large (L) RNA segment of negative polarity. Although the untranslated regions (UTRs) have been shown to comprise signals required for transcription, replication and encapsidation, the mechanisms that drive the packaging of at least one S, M and L segment into a single virion to generate infectious virus are largely unknown. One of the most important members of the Bunyaviridae family that causes devastating disease in ruminants and occasionally humans is the Rift Valley fever virus (RVFV). Here we studied the level of flexibility of RVFV genome packaging by splitting the glycoprotein precursor gene, encoding the (NSm)GnGc polyprotein, into two individual genes encoding either (NSm)Gn or Gc. Using reverse genetics, six viruses with a segmented glycoprotein precursor gene were rescued, varying from a virus comprising 2 S-type segments in the absence of an M-type segment to a virus consisting of 4 segments (RVFV-4S) of which 3 are M-type. Despite that all virus variants were able to grow in mammalian cell culture, they were unable to spread efficiently in mosquito cells. Moreover, in vivo studies demonstrated that RVFV-4S is unable to cause disseminated infection and disease in mice, even in the presence of the main virulence factor NSs, but induced a rapid protective immune response against a lethal challenge with wild-type virus. Collectively, splitting bunyavirus glycoprotein precursor genes offers new opportunities to study bunyavirus genome packaging and open doors for the development of next-generation live-attenuated bunyavirus vaccines.

Workshop Sessions

VIR-WK225.03 - A 3D human lung model for characterization of long-term Andes virus infection

Karin B. Sundström[1], Anh Thu Nguyen Hoang[2], Shawon Gupta[2], Clas Ahlm[3], Mattias Svensson[2], Jonas Klingström[2]
[1]Uppsala University, Uppsala, Sweden, [2]Karolinska Institutet, Stockholm, Sweden, [3]Umeå University, Umeå, Sweden

Hantaviruses are zoonotic viruses that cause diseases in humans by inhalation of virus containing excreta. The lungs are believed to be the initial site of infection, from where the virus spreads to the rest of the body. Flu-like symptoms appear after an incubation period of two, sometimes up to six, weeks. In some individuals the virus infection progresses to full-blown hemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome (HCPS), depending on species of infecting virus. Lung symptoms are part of the HCPS pathogenesis and recent evidence suggests a stronger pleural involvement in HFRS than earlier believed. Due to the late onset of disease symptoms, initial and early hantavirus infection cannot easily be studied in patients. So far, all in vitro studies on hantaviruses have been performed with cells in monolayer. However, to better understand the effect hantavirus have on their surroundings, 3-dimensional models are needed. Here we used a 3-dimensional in vitro model of human lung mucosa to study infection of the HCPS-causing Andes virus for longer than five weeks. Ten to eighteen days post infection, we observed a high dose of progeny virus released in a burst period of four to five days, followed by a low level of progeny virus production. Increased levels of certain cytokines and chemokines in the supernatants, e.g IP10, Eotaxin-1 and VEGF, followed this burst period. In contrast, the chemotactic cytokine RANTES were over time detected in lower amounts relative to mock infected models. The sudden burst of progeny virus observed, suggest that this model might mirror the clinical time point for onset of symptoms in patients. By further study complex in vitro models we will have better tools to understand early pathogenesis of hantavirus infection, and how hantavirus progeny virus production is regulated.

Workshop Sessions

VIR-WK225.04 - Genome-wide RNA interference screening for host factors required in Rift Valley Fever virus infection

Brooke Harmon[1], Benjamin Schudel[1], Anson Hatch[1], Oscar Negrete[1]
[1]Sandia National Laboratories, Livermore, USA

Rift Valley Fever virus (RVFV) is a zoonotic pathogen capable of causing serious morbidity and mortality in both humans and livestock. The lack of efficient countermeasure strategies, the potential for dispersion into new regions, and the pathogenesis in humans and livestock make RVFV a serious public health concern. To identify host proteins that are required for RVFV infection, we conducted a genome-wide RNA interference (RNAi) screen using a human siRNA library. In the screen, HeLa cells were reverse transfected with siRNAs in a 384 well format then infected with the recombinant vaccine strain RVFV MP12 encoding GFP in place of the nonstructural protein NSs. Genome-wide RNAi screening identified 252 cellular genes whose knockdown reduced viral infection as indicated by reduced GFP fluorescence. These 252 genes were classified into canonical pathways and the Wnt/β-catenin signaling pathway was among the most represented. Further analysis indicated that RVFV-GFP infection increased Wnt signaling in a β-catenin reporter assay, inhibitors of Wnt signaling decreased RVFV-GFP infection, and RVFV-GFP infection was enhanced when Wnt signaling was pre-activated. Similar results were found using authentic (non-recombinant) RVFV-MP12. We will report the results of the screen and follow-up experiments to confirm the role of this specific host pathway in RVFV infection.

Workshop Sessions

VIR-WK225.05 - Abundant host specific accumulation of head-to-tail dimers and/or defective dimers of the small genomic segment in members of the genus Tospovirus, family Bunyaviridae

Marina Ciuffo[1], Paolo Margaria[1], Laura Miozzi[1], Hanu Pappu[2], Renato Resende[3], Massimo Turina[1]
[1]Plant Virology Institute-CNR, Torino, Italy, [2]Dept. Plant Pathology-WSU, Pullman, USA, [3]Dept. Biologia Celular- UnB, Brasilia, Brazil

Polygonum ringspot virus (PolRSV) is a Tospovirus species recently characterized from a common weed (Polygonum convolvulus) from a number of sites in Italy. Dictyothrips betae is the natural thrips vector. During its molecular characterization, we carried out Northern blots with S derived probes of total RNA from infected Nicotiana benthamiana leaf extracts and of RNA from purified virus: direct comparison of such blots showed that the most abundant viral RNA in N. benthamiana extracts was a molecule of ca 4.8 kb, which was not encapsidated in the virion. Such molecule was cloned and was shown to be a head-to-tail incomplete dimer of the S genomic segment RNA. We developed a sensitive real time PCR assay to detect the defective dimer in a number of hosts and in its thrips vector. The defective dimer accumulated only in N. benthamiana and to a lesser extent in N. clevelandii. Dimer accumulation was not isolate specific, although each isolate had a different deletion at the head-to-tail junction. Dimer accumulation was strictly dependent on temperature: we could not detect the dimer by northern blot in N. benthamiana maintained at 21° C, whereas at 32° C its accumulation was very abundant. We repeated the experiment with Tomato spotted wilt virus, and surprisingly an abundant accumulation of a nearly full-length dimer was observed in N. benthamiana at 32° C. Such dimer was absent in the same conditions in a TSWV silencing suppressor deficient isolate. In order to detect a possible host specific silencing component in generating the dimer, we are currently comparing the siRNA and miRNA profile of N. benthamiana and Solanum lycopersicum infected by PolRSV, since accumulation of the dimer is not detected in tomato. We will speculate on the nature, origin and biological significance of the accumulation of such dimer species in tospoviruses.

Workshop Sessions

VIR-WK225.06 - Epidemiology investigation of SFTS virus infection in China

Mifang Liang[1], Shiwen Wang[1], Jiandong Li[1], Guoyu Liu[1], Quanfu Zhang[1], Dexin Li[1]
[1]*National Institute for ention Viral Disease Control and Prevention, Beijing, China*

SFTS Virus (SFTSV), a new member of Phlebovirus, family Bunyaviridae, is a high pathogenic causative agent of an emerging infectious disease severe fever with thrombocytopenia syndrome (SFTS) identified in China in 2010. SFTSV infection in human can lead to serious disease with a case fatality rate around 10-30%, and human-to-human transmission of SFTSV due to close contact with infected blood has been described. Up to now, SFTS cases have been reported in 17 provinces of China and the confirmed case number reach over 2000. To investigate the maintenance and transmission of the virus, a total of 3039 animals were sampled and 3,145 ticks were collected from animals (71.6%), and grazing fields (28.4%) in an endemic region of Shandong province, China. SFTSV-specific antibodies were detected in 328 of 472 sheep (69.5%), 509 of 842 cattle (60.4%), 136 of 359 dogs (37.9%), 26 of 839 pigs (3.1%), and 250 of 527 chickens (47.4%). SFTSV RNA was detected in all sampled animal species with a detection rate ranging from 1.7% to 5.3%. Of the ticks, the majority was H. longicornis (96.9%), which included both adults (59.4%), and the immature ticks (40.6%). All collected ticks were tested by real –time PCR, of them, 4% of H. longicornis and 4.8% of the adult R. microplus were detected as SFTSV RNA positive. Viral RNA was identified from all developmental stage of H. longicornis including adult, nymphal, larval, and eggs. Viable viruses were isolated from sheep, cattle, dogs, and adult ticks. Sequences analysis revealed that tick and animal-derived isolates shared high homology of nucleic acid sequences with human isolates. These findings extend the understanding of SFTSV natural maintenance and transmission, which suggest that the ticks may act as both vectors and reservoir of the virus, while domestic animals are likely playing a role of amplifying hosts.

Workshop Sessions

VIR-WK225.07 - Gene vaccine encoding Hantavirus Gn, targeted to MIIC by trafficking molecule LAMP, significantly enhances specific immune responses and harvests satisfying immune protection

Dongbo Jiang[1], Yuanjie Sun[1], Linfeng Cheng[1], Fanglin Zhang[1], Kun Yang[1]
[1]Fourth Military Medical University, Xi'an, China

Lysosome-associated membrane protein (LAMP) can target and bind to endosome/lysosome, one of the most important components of the MHC class II-processing compartment (MIIC) in the exogenous antigen-processing pathway. LAMP trafficking greatly enhance the immune response because endogenous antigen can take advantage of LAMP being directly carried into MIIC, activating CD4+ helper T cells for effective immue response and long-term immune memory. Hantavirus glycoprotein N-terminal, named Gn, could induce neutralizing antibody production with a low serum titer as natural infection. To analyze the influence of LAMP on Hantaan virus (HTNV) Gn vaccine potency and develop a novel effective vaccine against HTNV, we constructed three eukaryotic vectors as naked DNA vaccine named pVAX-Gn, pVAX-LAMP and pVAX-LAMP/Gn, respectively. Balb/c mice were immunized with those plasmids, the specific humoral and cellular responses elicited against HTNV Gn were measured by ELISA, cytotoxicity assays and ELISPOT assay (IFN-γ). To measure the protective efficacy, virus challenging in vivo and neutralizing antibody valence were conducted by viral load detection (qRT-PCR and sandwich ELISA) and the cell microculture neutralization test. We found that HTNV Gn showed a strong immunogenicity to elicit both humoral and cellular responses with LAMP as a chimera. Compared with the current prophylactic inactive vaccine, pVAX-LAMP/Gn showed a stronger cellular response. Being worth raising, a significant long term memory reponse was observed only from the group of pVAX-LAMP/Gn. Histopathological analysis by HE staining demonstrated that pVAX-LAMP/Gn was not harmful. Results of protection assay in vivo indicated that the immune response established was HTNV specific and protective. These findings not only demonstrated that the LAMP as a trafficking molecule can introduce Gn to MHCII presenting pathway and significantly enhanced HTNV specific immune response, but also suggested that the pVAX-LAMP/Gn as a DNA vaccine had potential application on clinic for HTNV infection immunoprophylaxis.

Wednesday, 30 July 2014 **16:01 - 16:20 Room 511 D**

Workshop Sessions

VIR-WK226.01 - Molecular identification of the genetically defined vegetative incompatibility loci involved in restricting transmission of virulence-attenuating mycoviruses in the chestnut blight fungus Cryphonectria parasitic

Donald Nuss[1]

[1]*Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, USA*

Mycovirus infections of plant-pathogenic fungi can result in attenuation of fungal virulence (hypovirulence) on plant hosts, providing potential applications for disease control. Because mycoviruses generally replicate without an extracellular phase, their transmission depends on intracellular mechanisms such as exchange of cytoplasmic contents during hyphal fusion (anastomosis) or incorporation into asexual spores. As first shown for hypoviruses causing hypovirulence of the chestnut blight fungus *Cryphonectria parasitica*, fungal non-self recognition vegetative incompatibility (vic) systems act as a population level defense system that can limit hypovirulence efficacy by restricting mycovirus transmission. The restriction imposed by the vic system is a consequence of the incompatible reaction that ensues after fusion of hyphae of fungal strains that are heteroallelic at one or more of the vic genetic loci. The fusion between incompatible strains results in localized programmed cell death that constrains cytoplasmic exchange, limiting virus transmission. We now report the use of a polymorphism based comparative genomics approach for molecular identification of all six genetically defined *C. parasitica* vic loci. Functional analysis confirmed the role of vic in restriction of mycovirus transmission and revealed evidence for both polymorphic and idiomorphic allelic components as well as allelic and nonallelic interactions. The molecular identification of the full complement of genetically defined *C. parasitica* vic loci provides new prospects for manipulation of vic alleles to enhance hypovirus transmission and biological control.

Workshop Sessions

VIR-WK226.02 - A novel dsRNA mycovirus associated with reduced pathogenicity of Aspergillus fumigatus in a mouse infection model

Azusa Takahashi-Nakaguchi[1], Misa Yahara[1], Erika Shishido[1], Hiromitsu Moriyama[2], Tohru Gonoi[1]
[1]MMRC, Chiba University, Chiba, Japan, [2]Tokyo Univ. Agr. Tech., Tokyo, Japan

The filamentous fungal pathogen Aspergillus fumigatus is the most common causal agents of invasive fungal infection in humans, and is associated with an alarmingly high mortality rate. Emergences of drug resistant fungal and toxic side effects of antifungal drugs are two major problems encountered in treatment of fungal infections. Therefore, development of new therapeutic strategies is urgently required, which may include discovery of new chemotherapic drugs based on identification of novel fungal targets and finding novel therapeutic methods for alleviating the pathogenic effects. One such hypothetical therapeutic strategy may involve the use of mycoviruses which are able to selectively infect pathogenic fungi. In this study, we tried to find dsRNA mycoviruses which have effect in reducing virulence of A. fumigatus in a mouse infection model. By screening 44 isolates of A. fumigatus, we found four isolates which contained distinct groups of dsRNA segments. Using a genome sequencer (illumina, MiSeq), we read dsRNA sequences from the four isolates, and found four distinct dsRNA mycoviruses, two of which were members of the genus Chrysovirus and the genus Partitivirus, while the rest two were novel mycovirus strains. Isogenic lines of virus-infected and virus-free A. fumigatus strains were established using one of the novel mycovirus strains. In vitro experiments showed mycovirus infection had apparent influence on phenotypes of A. fumigatus in culture, such as numbers of conidium formation, mycelia morphology, adherence of fungal resting conidia to pulmonary epithelial cells, tolerance for macrophage phagocytosis. When immunosuppressed mice were infected with the virus-free or virus-infected A. fumigatus strains, respectively, the virus-infected strain showed reduced mortality of mice in comparison to the virus-free strains. We conclude a novel mycovirus have ability to reduce pathogenicity in mouse infection model, and can be potential resources for developing new therapies strategies in treatment of Aspergillosis.

Workshop Sessions

VIR-WK226.03 - Transfection of the megabirnavirus RnMBV1 to a model filamentous fungus, Cryphonectria parasitica: biological significance and expression strategies

Lakha Salaipeth[1], Sotaro Chiba[1], Ana Eusebio-Cope[1], Satoko Kanematsu[2], Nobuhiro Suzuki[1]
[1]IPSR, Okayama University, Kurashiki, Japan, [2]IFTS, NARO, Morioka, Japan

Rosellinia necatrix megabirnavirus 1 (RnMBV1) with a bipartite dsRNA genome (dsRNA1 and dsRNA2) confers hypovirulence to its natural host, the white root rot fungus, thus being regarded as a potential virocontrol (biocontrol) agent. Each segment has two large open reading frames (ORFs): ORF1 and partially overlapping ORF2 on dsRNA1 encodes the major capsid protein (CP) and RNA-dependent RNA polymerase (RdRp), while ORF3 and ORF4 on dsRNA2 encode polypeptides with unknown functions. Here we report the biological and molecular characterization of this virus in the chestnut blight fungus Cryphonectria parasitica, a filamentous fungus that has been used as a model for mycovirus research. Transfection with purified RnMBV1 particles into an RNA silencing-defective strain (Δdcl-2) of C. parasitica and subsequent anastomosis with the wild-type strain (EP155) resulted in stable persistent infection in both host strains. However, its accumulation levels in the two strains were different, being ~20-fold higher in Δdcl-2 than in EP155. Intriguingly, while RnMBV1 reduced both virulence and growth rate in Δdcl-2, it attenuated virulence without significantly affecting other traits in EP155. Western analysis using antiserum against recombinant proteins encoded by either ORF1 or ORF2 demonstrated the presence of a 250-kDa protein in purified virion preparations, suggesting that RdRp is expressed as a CP-fusion product via -1 frameshift. Antiserum against the ORF3-encoded protein allowed the detection of 150-kDa, 30-kDa and 23-kDa polypeptides specifically in RnMBV1-infected mycelia. Some properties of an RnMBV1 mutant with genome rearrangements, which occurred after transfection of Δdcl-2 and EP155, were also presented. This study provides an additional example of C. parasitica serving as a versatile, heterologous fungus for exploring virus/host interactions and virus gene-expression strategies.

Workshop Sessions

VIR-WK226.04 - Molecular characterization a negative-stranded RNA virus that infects fungal plant pathogen Sclerotinia sclerotiorum

Lijiang Liu[1], Jiatao Xie[1], Jiasen Cheng[1], Yanping Fu[1], Guoqing Li[1], Daohong Jiang[1]
[1]*HZAU, Wuhan, P R China*

Mycoviruses, or fungal viruses, are viruses that infect and replicate in fungal cells, and hypovirulence-associated mycoviruses can be explored to control fungal plant diseases possibly. Mycoviruses are common in nature and often with dsRNA and (+) ssRNA genomes, or rarely with ssDNA genomes. Previously, Kondo et al (2012) identified genes with sequence similarity to those of (-) ssRNA viruses in two fungal plant pathogens and suggested that (-) ssRNA viruses are likely to exist in fungi. However, so far whether (-) ssRNA viruses occur in fungi is not known. Here, we isolated and characterized a (-) ssRNA virus (SsNSRV-1) from a hypovirulent strain of a fungal plant pathogen Sclerotinia sclerotiorum. The complete genome of SsNSRV-1 is 10002 nt with six putative ORFs (ORF Ⅰ-Ⅵ), ORF Ⅱ encodes a nucleoprotein and ORFⅤ encodes the largest protein which contains a conserved mononegaviral RdRp domain, while the functions of proteins encoded by other ORFs are not known. The enveloped virions of SsNSRV-1 are 25-40 nm in diameter and ~1000 nm in length, while the nucleocapsids are long, flexible and helical, with 22 nm in diameter and 200-2000 nm in length. Phylogenetic analysis based on RdRP domain of SsNSRV-1 and other selected viruses shows that SsNSRV-1 is a new member of the order Mononegavirales and is most closely related to viruses of Nyavirus and Bornaviridae which majorly infect mammal and human. SsNSRV-1 was successfully transfected a virus-free strain of S. sclerotiorum, and could convert hypovirulence to its host. Moreover, SsNSRV-1 is widely distributed since it could be detected in different regions of China. Our finding demonstrates that (-)ssRNA virus occurs naturally in fungi and expands our knowledge of ecology and evolution of (-)ssRNA viruses, and will provide a potential viral agent to control Sclerotinia disease.

Workshop Sessions

VIR-WK227.01 - The interferon system: the first line of defense against viruses

Ganes Sen[1], Volker Fensterl[1], Saurabh Chattopadhyay[1]
[1]*Department of Molecular Genetics, Cleveland Clinic, Cleveland, USA*

The interferon (IFN) system comprises the primary innate immune defense against viral infection. The host has evolved to use many signaling pathways triggered by a variety of viral products, including double-stranded RNA, that lead to the induction of antiviral genes which inhibit virus replication. Viruses, in turn, employ a variety of strategies to evade the IFN system, thus maintaining cell-virus homeostasis. It has become apparent that different IFN-induced genes target different families of viruses and their antiviral functions can be cell type-specific as well. Moreover, the IFN system uses a gene-induction independent antiviral action of the interferon-regulatory factor, IRF-3. This pathway does not require IFN induction but it causes early apoptosis of the infected cell by virus-activated IRF-3, thus limiting the viral spread. Our observations suggest that the IFN system employs multi-pronged antiviral mechanisms which are regulated at multiple levels.

Workshop Sessions

VIR-WK227.02 - Early detection and sequence analysis of measles virus copyback defective-interfering RNAs: mechanism of generation, and biased hypermutation

Christian K. Pfaller[1,2], Xiao Ma[1], Charles E. Samuel[2,3], Roberto Cattaneo[1]
[1]Molecular Medicine; Virology and Gene Therapy track, Mayo Clinic, Rochester, USA, [2]Molecular, Cellular and Developmental Biology, UC Santa Barbara, USA, [3]Neuroscience Research Institute, UC Santa Barbara, USA

Defective-interfering viral genomes (DI-RNAs) can form during infections of negative strand viruses and temporarily outgrow full-length genomes, modulating infection severity. We have observed that C-protein-deficient measles virus (MV-C^{KO}) efficiently generates double-stranded RNA at viral replication sites; dsRNA detection precedes autophosphorylation of protein kinase R (PKR) and induction of cellular stress and innate immune responses; we also documented generation of “copyback” DI-RNAs (J. Virol. 88, 456-468, 2014). We now document that multiple copyback DI-RNAs are generated as early as passage 1 after independent rescue events for both vaccine (vac2) and wild type (IC323) MV strains. This class of RNAs is generated even more frequently in C^{KO} derivatives of these viruses. We characterized the breakpoints and re-initation sites of several dozen individual DI-RNAs and conclude that: (1) The abundance of DI-RNAs is reciprocal to their size. Most DI-RNAs are about 500 nucleotides in length, but some are up to 4000 nucleotides. (2) The length of the potentially dsRNA portion is 100-170 nucleotides: breakpoints are widely distributed, whereas re-initiation sites cluster in a narrow window 130 (+/-35) nucleotides from the genomic 5’ end. (3) A sequence with homology to the MV genomic and antigenomic promoters is located within this window. It may favor transcription re-initiation. (4) All DI-RNAs characterized so far are of hexameric length, which is consistent with their selective replication. (5) However, DI-RNAs may not be properly encapsidated, possibly leading to the formation of dsRNA that can activate PKR and other cellular responses. (7) Some DI-RNAs incurred up to 35% A-to-G mutations (biased hypermutation events). This is consistent with modification by adenosine deaminase acting on RNA 1 (ADAR1), which may destabilize the dsRNA structures. Finally, we have passaged multiple infections several times and are currently reconstructing the evolution of the most interesting DI-RNA species.

Workshop Sessions

VIR-WK227.03 - Structural basis for the recognition of 5´-triphosphate viral RNA by human IFIT proteins

Yazan Abbas[1], Andreas Pichlmair[2], Maria Górna[3], Giulio Superti-Furga[3], Bhushan Nagar[1]
[1]McGill University and Groupe de Recherche Axé sur la Structure des Protéines, Montreal, Canada, [2]Max Planck Institute of Biochemistry, Munich, Germany, [3]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

IFIT proteins are interferon-inducible antiviral effectors that can recognize viral RNA in the cytoplasm. Specifically, IFIT1 recognizes markers of viral infection such as 5´-triphosphate RNA (PPP-RNA) or capped mRNA lacking 2´-O methylation at the ultimate and penultimate 5´-nucleotides. IFIT5, on the other hand, recognizes only PPP-RNA. To understand the molecular basis by which IFIT proteins can discriminate self from non-self viral RNA bearing a 5´-PPP, we determined the crystal structure of human IFIT5, an N-terminal fragment of human IFIT1, and IFIT5 in complex with various PPP-RNAs. The structures reveal that IFIT proteins form a narrow, positively charged cavity that can accommodate only single-stranded RNA, and engages the ends of PPP-RNA in a sequence non-specific manner. Mutational analysis and gel shift assays support the crystal structures and implicate IFIT5 and IFIT1 in a similar mechanism of PPP-RNA recognition. IFIT5 and IFIT1 mutants that are defective in binding PPP-RNA have impaired anti-viral activity against viruses that generate PPP-RNA during their life-cycle. These results shed light on the mechanism of viral RNA recognition by IFIT proteins and will hopefully pave the way for novel immuno-modulating therapeutics.

Workshop Sessions

VIR-WK227.04 - Sustained activation of IRF-3 during infection by Paramyxoviruses requires MDA5

Nathalie Grandvaux[1,2], Xiaochun Guan[1,2], Yoboua Fabrice[1,2], Nicolas Zucchini[1,2], Karin Fink[1,2], Doyon Priscilla[2], Lydie Martin[1,2], Marc Servant[2], Stéfany Chartier[1]
[1]CRCHUM, Montreal, Canada, [2]Université de Montréal, Montreal, Canada

Amongst pathogen recognition receptors, RIG-I and MDA5 are the main cytosolic sensors of ssRNA viruses, including Paramyxoviruses. Sensing of nucleic acids in the cytosol is required to initiate a quick and robust innate antiviral response. Recent research has been mainly focused on the characterization of specific RIG-I- and MDA5-ligands. Although it is now clear that each receptor have different ligand binding properties, the consensus view is that RIG-I and MDA5 trigger common downstream signal(s) to activate IRF-3 and NF-κB and downstream antiviral and proinflammatory cytokines expression. Here, we performed a thorough analysis of the temporal involvement of RIG-I and MDA5 in the regulation of IRF-3 during RSV infection. Based on specific RNAi-mediated knockdown of RIG-I and MDA5 in A549 cells, we confirmed that RIG-I is critical for the initiation of IRF-3 activation and downstream antiviral gene expression. On the other hand, our experiments yielded evidence that MDA5 is not essential for the initiation of IRF-3 activation but rather prevents active IRF-3 degradation, thereby sustaining IRF-3 activation and downstream gene expression. Importantly, ectopic expression of MDA5 prolonged 5'ppp-dsRNA-induced (RIG-I-induced) IRF-3 activation. Altogether, these results support a novel model in which activation of RIG-I is essential for initial IRF-3 activation, while induction of MDA5 expression prevents early degradation of IRF-3, thereby sustaining IRF-3-dependent antiviral gene expression. MDA5 plays a similar role during SeV infection suggesting that this model is not restricted to RSV amongst Paramyxoviruses.

Workshop Sessions

VIR-WK227.05 - Antagonism of the Phosphatase PP1 by the paramyxovirus V protein is required for innate immune escape of MDA5

Meredith Davis[1], May Wang[1], Linda Rennick[2], Florian Full[1], Sebastian Gableske[1], Annelies Mesman[4], Sonja Gringhuis[4], Teunis Geijtenbeek[4], W. Paul Duprex[2,3], Michaela Gack[1]

[1]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, USA, [2]Department of Microbiology, Boston University School of Medicine, Boston, USA, [3]School of Medicine, Queen's University of Belfast, Belfast, UK, [4]Department of Experimental Immunology, University of Amsterdam, Amsterdam, the Netherlands

The cytosolic innate immune sensor MDA5 detects various RNA viruses, including picornaviruses and paramyxovirus family members such as measles virus. Upon sensing of viral RNA, MDA5 activates signaling cascades that result in the expression of type-I interferons (IFNs) to block virus replication. A recent series of studies identified sophisticated regulatory mechanisms that modulate MDA5's antiviral signaling. MDA5 is constitutively phosphorylated in uninfected cells, preventing premature antiviral signaling. Upon viral infection, MDA5 is rapidly activated through dephosphorylation by the protein phosphatases PP1α and PP1γ. Most viruses have evolved means to block detection by the innate immune system. Here we present a novel evasion mechanism of paramyxoviruses to escape the MDA5-induced innate immune response. The V proteins of measles virus and the related Nipah virus interact with PP1α/γ, thereby preventing MDA5 dephosphorylation and innate immune signaling. We further identified a conventional PP1-binding motif in the C-terminal region of the measles V protein that mediates PP1 interaction. Mutation of the PP1-binding motif in the measles V protein abrogated PP1 interaction and consequently MDA5 inhibition. To determine the physiological relevance of the V-PP1 interaction for MDA5 antagonism, we generated a recombinant measles virus carrying a PP1-binding deficient V protein. The V mutant recombinant virus was unable to inhibit MDA5 signaling, evidenced by efficient IRF3 activation, robust IFN-β gene expression and a strong attenuation in replication compared to the parental virus. In summary, our study identifies a novel immune evasion strategy of paramyxoviruses and opens up new avenues for the development of novel therapeutics for paramyxovirus-associated diseases.

Workshop Sessions

VIR-WK227.06 - TRIM5α cellular partners for the restriction of incoming Retroviruses and the activation of innate immune responses

Paulina Pawlica[1], Nasser Masroori[1], Valerie Le Sage[2], Andrew J Mouland[2], Lionel Berthoux[1]
[1]UQTR, Trois-Rivières, Canada, [2]McGill University, Montreal, Canada

The cellular anti-retroviral factor TRIM5α, a member of the tripartite motif family of proteins, is present in the cytoplasm and can intercept incoming retroviral cores in a species-specific, virus-specific fashion. In addition, TRIM5α promotes the induction of an antiviral state involving the activation of transcription factors NF-κB and AP-1. The possible intervention of other cellular factors in the TRIM5α anti-retroviral functions is still poorly understood. My laboratory is committed to characterize the role of these additional cellular factors in TRIM5α functions. I will show recent experiments from two of our projects on this topic: 1. TRIM5α cytoplasmic bodies move along microtubules, but there was little evidence for a functional role of microtubules or microtubule-associated molecular motors in TRIM5α-mediated retroviral restriction. Using (i) pharmacological inhibitors of microtubules assembly or disassembly, (ii) a drug targeting dynein molecular motor, and (iii) dynein heavy chain siRNAs, we now demonstrate that the integrity of the microtubule network is important for TRIM5α-mediated retroviral restriction. In addition, dynein heavy chain depletion alters the size and subcellular distribution of TRIM5α cytoplasmic bodies and decreases retroviral restriction. 2. The PML (promyelocytic leukemia) protein also belongs to the TRIM family of proteins, and TRIM5α localizes at PML nuclear bodies when its nuclear export is inhibited by treatment with leptomycin B. We used PML knockout murine embryo fibroblasts (MEFs) to investigate the possible role of PML in TRIM5α-mediated restriction. We find that PML is largely unnecessary for efficient restriction of HIV-1 by Rhesus TRIM5α in MEFs. Interestingly however, TRIM5α overexpression in MEFs also seems to induce retroviral restriction by an indirect mechanism independent of TRIM5α-capsid interactions, and that particular restriction is modulated by PML.

Workshop Sessions

VIR-WK227.07 - Role of TRAF7 in the regulation of Type I IFN antiviral response during Influenza Virus infection

Yiliu Liu[1], Rongtuan Lin[2]
[1]*Dept. of Experimental Medicine, McGill University, Montreal, Canada,* [2]*Lady Davis Institute - Jewish General Hospital, McGill University, Montreal, Canada*

Influenza viruses are highly contagious human pathogens that causes up to 500 million seasonal respiratory infections. The innate immune response to influenza virus infection plays an important role in limiting virus multiplication and pathogenesis. Type I interferons (IFN) have a major role in the first line of defense against viral infection. Influenza viral RNA triggers the cytoplasmic viral sensor retinoic acid-inducible gene I (RIG-I), one of the major Pattern Recognition Receptors, and ultimately leads to the production of type I IFN, as well as pro-inflammatory cytokines. TBK1 is a critical kinase implicated in TLR and RIG-I dependent IFN transcription. The tumor necrosis factor receptor-associated factor (TRAF) protein family members are cytoplasmic regulatory molecules that function as signal transducers for receptors in both innate and adaptive immune responses. TRAF7 is the last protein identified in the TRAF protein family and possesses an E3 ligase activity. Given that the role of TRAF7 in the regulation of type I IFN response -mediated by either TLR or RIG-I like receptors- has never been investigated; and that TRAF7 has been identified as a negative regulator of NF-kB pathway, we hypothesize that TRAF7 could be involved in type I IFN signaling. Our results show that: 1) TRAF7 inhibits IFN response, downstream of TBK1 and upstream of IRF3. 2) TRAF7 interacts with TBK1 and promotes degradation of TBK1. 3) TRAF7 is phosphorylated by TBK1. 4) TRAF7 ubiquitinates TBK1. Altogether, our data demonstrated that TRAF7 negatively regulates type I IFN antiviral response by ubiquitination of TBK1, thus creates a suitable environment for virus, such as influenza, to establish an efficient viral infection and replication.

Workshop Sessions

VIR-WK228.01 - Reovirus FAST proteins usurp host exosome biogenesis to enhance cell-cell fusion and virus dissemination

Jolene Read[1], Roy Duncan[1]
[1]*Dalhousie University, Halifax, Canada*

The fusion-associated small transmembrane (FAST) proteins, encoded by the fusogenic orthoreoviruses, are autonomous membrane fusion proteins whose only known function is to induce cell-cell fusion. Syncytium formation promotes virus dissemination and is a virulence determinant of these viruses. Interestingly, the FAST proteins contain many hallmarks of proteins targeted to exosomes. A yeast two-hybrid screen identified components of the exosome pathway as genetic interaction partners of the p14 FAST protein. The composition and morphology of exosomes from various transfected or virus-infected cell lines expressing FAST proteins were analyzed using immunoblotting, biochemical assays, and electron microscopy. Results indicate the intracellular endodomain of the p14 FAST protein serves as an exosome-sorting signal to traffic p14 to exosome-like vesicles as an integral membrane protein with an Nout/Cin topology. Expression of p14 increased exosome release from cells by three-fold compared to mock-transfected cells. Quantitative mass spec analysis of purified exosomes from mock- and p14-transfected cells indicated overlapping, but distinct, protein profiles, and inhibiting exosome release from p14-expressing cells reduced cell-cell fusion by >80%. Furthermore, addition of purified p14 exosomes to naïve cells induced syncytium formation. The fusogenic reoviruses are the first example of a nonenveloped virus that exploits exosome pathways to to enhance cell-cell fusion as a means to promote virus dissemination.

Workshop Sessions

VIR-WK228.02 - Reovirus replication organelles recruit membranes derived from the ERGIC

Paula Zamora[1,2], Isabel Fernandez de Castro[3], Bernardo Mainou[2,4], Cristina Risco[3], Terence Dermody[1,2,4]
[1]Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, USA, [2]Lamb Center for Pediatric Research, Vanderbilt University School of Medicine, Nashville, USA, [3]CNB-CSIC, Campus UAM, Madrid, Spain, [4]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, USA

Many viruses reorganize cellular membranes to create an environment to facilitate their replication and form neoorganelles called inclusions, factories, or viroplasms. Reoviruses are nonenveloped double-stranded RNA viruses that display broad cell, tissue, and host tropism. Replication and assembly of these viruses take place within inclusions, which contain dsRNA, viral and cellular proteins, and both complete and incomplete viral particles. Reovirus inclusions have been described as membrane-free structures that do not associate with cellular organelles. We used transmission electron microscopy and three-dimensional image reconstruction of reovirus-infected cells to demonstrate that reovirus inclusions form within a membranous network. To determine the source of inclusion-associated membranes, we stained reovirus-infected cells for specific organelle markers and imaged these cells using confocal microscopy. Our results show reorganization of the endoplasmic reticulum Golgi intermediate compartment (ERGIC). In uninfected cells, ERGIC markers ERGIC-53 and KDEL receptor segregate to the perinuclear region. In reovirus-infected cells, ERGIC markers localize within inclusions. Endoplasmic reticulum (ER) marker protein disulfide isomerase redistributed in infected cells but was not observed within the inclusion matrix. Golgi apparatus markers giantin and wheat germ agglutinin were not modified during infection. These results suggest that the ERGIC is a source of membranes associated with reovirus inclusions and that the ERGIC functions in reovirus replication and assembly.

Workshop Sessions

VIR-WK228.03 - Rotavirus activates lymphocytes from non-obese diabetic mice by triggering toll-like receptor 7 signaling and type 1 interferon production in plasmacytoid dendritic cells

Jessica Pane[1], Nicole Webster[1], Barbara Coulson[1]
[1]*Department of Microbiology and Immunology, The University of Melbourne, Parkville, Australia*

Rotavirus infection is proposed to promote the progression of genetically-predisposed children to type 1 diabetes, a chronic autoimmune disease marked by infiltration of activated lymphocytes into pancreatic islets. Non-obese diabetic (NOD) mice provide a model for the human disease. Infection of adult NOD mice with rhesus monkey rotavirus accelerates diabetes onset independently of pancreatic infection. Instead, RRV infection of NOD mice is associated with spread of virus to the pancreatic and mesenteric lymph nodes, association with and maturation of dendritic cells and activation of B and T cells at these sites. Here we tested the hypothesis that rotavirus induces bystander activation of lymphocytes from NOD mice by provoking dendritic cell activation and proinflammatory cytokine secretion. NOD mouse splenocytes were stimulated with rotavirus and assessed for activation by flow cytometry. This stimulation activated B cells independently of virus strain and replicative ability. Instead, B cell activation depended on virus dose and was prevented by blockade of virus decapsidation, inhibition of endosomal acidification, depletion of dendritic cells and interference with signaling through toll like receptor 7 and the type 1 interferon receptor. Interestingly, the level of B cell activation by rotavirus was significantly greater in cells from NOD mice compared to non-autoimmune mice. B cells were activated when cultured with either plasmacytoid or conventional dendritic cells in the presence of rotavirus. Rotavirus-exposed plasmacytoid dendritic cells also contributed to T cell activation, including the activation of islet-autoreactive CD8+ T cells. Our findings suggest that bystander activation mediated by toll like receptor 7 signaling and type 1 interferon contributes to the lymphocyte activation observed following RRV infection of NOD mice, and may play a role in diabetes acceleration by rotavirus.

Workshop Sessions

VIR-WK228.04 - Molecular tracking of enteric viral indicators in a swine production network

Virginie Lachapelle[1], Julie Brassard[1,2], Ann Letellier[1], Philippe Fravalo[1], Yvan L'Homme[1,3]
[1]University of Montreal, St-Hyacinthe, Canada, [2]Agriculture and Agri-Food Canada, St-Hyacinthe, Canada, [3]Canadian Food Inspection Agency, St-Hyacinthe, Canada

In the swine industry, a complex network of exchanges exist between farms and slaughterhouses associated with transportation of pigs, but also frequent farm visits from animal health professionals which can multiply the risk for introduction and dissemination of microbial contamination. However, limited information is available concerning the distribution of enteric contamination within and between swine herds and slaughterhouses, especially regarding viral agents. The objective of this project was to evaluate the potential of specific enteric viruses as indicators of fecal contamination associated with the movement of vectors (vehicles of livestock transporters, veterinarians and animal nutritionists) between ten finisher swine farms belonging to the same network and a slaughterhouse over a one year period. On each farm, composite fecal samples and environmental swabbings (e.g. landing stages, front doors, fans and portable solid panels) were collected twice. Environmental samples (surfaces of truck tire tracks) on the slaughterhouse site during unloading of two separate animal shipments from selected farms were also collected. Finally, samples from each stakeholder's vehicle were also collected twice. For each sample, two enteric viral families (group A rotavirus and astrovirus) were targeted by RT-PCR and genetically characterized in order to assign a molecular fingerprint to each farm and stakeholder. Results demonstrate the presence of unique and sometimes novel viral strains on most farms, particularly for rotavirus, which allowed epidemiological monitoring between specific farms and the slaughterhouse through stakeholders such as the livestock transporters. Extensive genetic viral diversity detected at the slaughterhouse site suggests its potential implication in the propagation of viral contamination throughout the network. Results also point out the possible role of environmental fomites in the contamination process of pigs within farm sites. The implementation of new biosecurity measures or the strengthening of key measures already in place within the network is advisable in light of our results.

Workshop Sessions

VIR-WK228.05 - Inhibition of the innate immune response by rotavirus

Michelle Arnold[1]
[1] *LSU Health Sciences Center, Shreveport, USA*

The first line of defense against a viral infection is the host innate immune response, which uses complex signaling cascades to induce the production of interferon (IFN). The secretion of IFN creates an antiviral state that limits the replication and spread of the invading pathogen. As with most viruses, rotavirus encodes an antagonist of the host innate immune response. The nonstructural protein NSP1 inhibits the production of IFN by inducing the degradation of IFN regulatory factors IRF3, IRF5, IRF7, and IRF9, but the targets vary depending on the viral isolate from which the NSP1 is derived. When activated by upstream signals, IRF proteins dimerize and translocate to the nucleus where they induce transcription of IFN mRNA. NSP1 has been shown to specifically target the dimerization domain of the IRFs for degradation, which can be prevented by treatment with proteasome inhibitors. These observations, along with the presence of a RING domain, suggest that NSP1 acts as a virally encoded E3 ubiquitin ligase to target proteins for proteasomal degradation. E3 ubiquitin ligases containing a RING domain serve as a bridge between the target protein and an E2 ubiquitin-conjugating enzyme, which is responsible for transfer of the ubiquitin moiety. At least one cellular E2 ubiquitin-conjugating enzyme has been found to associate with NSP1, providing further evidence that NSP1 acts as a virally encoded E3 ubiquitin ligase. Despite the introduction of vaccines, rotavirus continues to result in the death of more than 400,000 young children globally each year, in part because the vaccines are less efficacious in impoverished nations. One approach to improving vaccine efficacy is to interrupt the activity of NSP1. Because the host innate immune system plays a key role in the development of a strong adaptive response, interfering with viral immune antagonists may improve vaccine success.

Workshop Sessions

VIR-WK228.06 - The Nogo receptor NgR1 mediates infection by mammalian reovirus

Jennifer Konopka-Anstadt[1], Bernardo Mainou[1], Danica Sutherland[2], Stephen Strittmatter[3], Terence Dermody[1,2]
[1]Department of Pediatrics, Lamb Center for Pediatric Research, Vanderbilt University School of Medicine, Nashville, USA,
[2]Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, USA,
[3]Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven USA

Neurotropic viruses must spread from an initial site of replication in the periphery to the central nervous system (CNS). The coordinated engagement of specific cellular receptors is often a major determinant of exquisitely specific patterns of viral spread and tropism in the host. By virtue of the multiple tissue types encountered in the infected host, reovirus is an ideal system to investigate stepwise mechanisms of spread and tropism used by neurotropic viruses. Reovirus binds to cell-surface glycans and proteinaceous receptors via an adhesion-strengthening mechanism to productively infect cells. Binding to junctional adhesion molecule-A (JAM-A), an immunoglobulin superfamily protein expressed on hematopoietic cells and in tight junctions, is required for reovirus bloodstream spread in mice. However, JAM-A is dispensable for reovirus replication in the CNS. We identified Nogo receptor NgR1 as a reovirus entry mediator using an RNA interference screen. NgR1 is a leucine-rich-repeat protein expressed on CNS neurons. Remarkably, sites of NgR1 expression in the murine brain parallel those targeted by reovirus. Expression of NgR1 confers reovirus binding and infection of otherwise non-susceptible cells. Blocking cell-surface NgR1 on transfected cells or primary cortical neurons abrogates reovirus infection. Incubating reovirus virions with soluble NgR1 or removing NgR1 from the cell surface neutralizes infectivity. Reovirus virions bind directly to soluble JAM-A and NgR1. However, infectious disassembly intermediates bind only to JAM-A. These results suggest that reovirus uses different capsid components to bind structurally distinct cell-surface molecules, engaging independent receptors to facilitate discrete steps in pathogenesis.

Workshop Sessions

VIR-WK228.07 - Rotavirus inhibits MHC class I expression in intestinal epithelial cells by suppressing type I interferon-dependent transcription of NLRC5

Gavan Holloway[1], Fiona Fleming[1], Barbara Coulson[1]
[1]*The University of Melbourne, Australia*

Detection of viral infection by host cells leads to secretion of type I interferon (IFN). The action of type I IFN results in expression of antiviral genes, and genes involved in the adaptive immune response. Rotavirus, an important human pathogen, blocks IFN signalling through inhibition of the nuclear translocation of STAT1 and STAT2. The class I major histocompatibility complex (MHCI) is required for viral antigen presentation and subsequent killing of infected cells by cytotoxic T lymphocytes. MHCI promoters contain a binding element for interferon response factor 1 (IRF1), and a region that binds NOD-like receptor family CARD domain-containing 5 (NLRC5). NLRC5 is regulated by an IFNγ-activated sequence (GAS) that binds STAT1 homodimers, and an IRF1-binding element. IRF1 contains a GAS element in its promoter. Therefore, activation of STAT1 by IFNγ or type I IFN can induce IRF1 and NLRC5 expression, which in turn promote MHCI expression. To determine if rotavirus inhibition of STAT1 signalling affects MHCI expression, we assessed MHCI levels in HT-29 intestinal cells following rotavirus infection. Total and cell surface MHCI levels were upregulated in uninfected bystander cells but not infected cells. Increased MHCI expression in bystander cells depended on signalling through the type I IFN receptor. MHCI and NLRC5 mRNA expression was elevated in bystander, but not infected cells, supporting a transcriptional block as a mechanism for the reduced MHCI expression. In addition, the total MHCI protein level in infected cells was reduced compared to mock-infected cells, supporting the existence of an additional non-transcriptional mechanism that suppresses MHCI expression in infected cells. These findings suggest that inhibition of MHCI expression in rotavirus-infected cells is at least partially due to the ability of rotavirus to block type I IFN-induced STAT1 nuclear translocation. Suppression of MHCI may limit cytotoxic T cell-mediated killing of rotavirus-infected enterocytes.

Workshop Sessions

VIR-WK228.08 - Challenges in the development of recombinant rotaviruses expressing foreign proteins

Aitor Navarro[1], John T. Patton[1]
[1]*Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, USA*

Rotaviruses (RVs), members of the Reoviridae family, are an important cause of acute gastroenteritis in infants and young children. Several single-gene reverse genetics systems have been established for RV, and these have been used for generating reassortant viruses, introducing foreign sequences into the viral genome, and altering the structure and function of viral molecules (Komoto et al., 2006, Troupin et al., 2010). We developed a RV single-gene reverse genetics system that allows replacement of the segment 8 (s8) RNA of the temperature-sensitive mutant virus, tsE, with a recombinant s8 RNA that encodes a fully functional NSP2 (Trask et al., 2010). We have used this system to engineer recombinant (r)RVs with s8 RNAs that contain sequence duplications (25 to 200 bp in size) and heterologous sequences (e.g., FLAG tag, PP7 and MS2 RNA recognition sites). However, attempts to recover rRVs that harbor mutations in the 5'-3' terminal panhandle and/or stem-loop structures of viral RNAs or that express foreign proteins under the control of Cricket Paralysis Virus (CrPV) IRES (e.g., HA-Ubiquitin, EGFP) have been unsuccessful. To further explore using RVs as expression vectors for foreign proteins, T7 transcription vectors for s8 RNA were constructed in which a translational stop-go 2A element was used to link a downstream Gaussia luciferase ORF sequence to an upstream NSP2 ORF sequence. Recovery of rRVs containing the modified s8 RNA was attempted using a cell line that expresses wildtype NSP2 and an shRNA that targets tsE s8 RNA. Based on RT-PCR assays, rRVs containing the modified s8 RNA may be generated by the single-gene reverse genetics system but may be lost during serial plaque isolation. Modifications are being made to the system in hopes of using it to engineer next-generation RV vaccines that induce protection against not only RV, but also other enteric pathogens such as norovirus.

Workshop Sessions

VIR-WK228.09 - Whole-genome characterization of animal Rotavirus C

Tohru Suzuki[1]
[1] *NIAH, Tsukuba, Japan*

Rotaviruses, one of major pathogens of gastroenteritis in humans and animals, are currently divided into eight species (A-H) on the basis of antigenic and genetic analyses. Rotavirus C (RVC) was first detected in a piglet with diarrhea in 1980s, and has subsequently been identified in humans, cows, ferrets and dogs. Human RVCs has been detected not only predominantly in children under 3 years old, but also in all age groups in many countries. In addition, a high prevalence of antibodies was detected in the United States and Japan in cattle and pigs. However, it is difficult to cultivate RVCs serially in cell culture, the molecular characterization of RVCs remains still unknown, despite their potential economical and epidemiological impact. In the present study, we attempt to determine full-length nucleotide sequences of all RNA segments from multiple bovine and porcine RVC strains obtained in Japan. Moreover, we conduct comparative sequence and phylogenetic analyses for individual genes using all RVC strains including our data. Comparative sequence analyses reveal that animal RVCs indicate low sequence identities to human RVCs, and animal RVCs do possess unique genetic diversity in a certain gene. Phylogenetic analyses demonstrate that RVCs can be classified into different genotype according to host species, and especially animal RVCs can differentiate into multiple genotypes. The data presented here suggest that multiple animal RVC strains with distinctive genotypes are broadly distributing and circulating throughout Japan. Our findings would provide an important progress into understanding of molecular characterization and evolutionary dynamics of animal RVCs.

Workshop Sessions

VIR-WK229.01 - Generalized adaptation to host innate immunity broadens RNA virus tropism

Brian Wasik[1], Andres Munoz[1], Kathryn Miller-Jensen[1], Paul Turner[1]
[1]Yale University, New Haven, USA

Understanding how virus populations evolve in changing host environments is critical for elucidating patterns and predictions of virus emergence. Theory predicts that historical exposure to single vs. multiple hosts should select for host-use specialization vs. generalization in viruses, and that generalist viruses should be relatively advantaged to successfully emerge on novel hosts. However, the molecular mechanisms underlying viral generalism remain unclear. Experimental evolution of viruses in simple vs. complex host environments provides a powerful approach to address these questions. Our previous work showed that experimental evolution of vesicular stomatitis virus (VSV) in single vs. alternating hosts led to specialist and generalist lineages, which diverged molecularly, particularly in the VSV matrix (M) gene. Here we tested whether evolved host-use generalism in VSV was explained by the M protein's role in evasion of cellular innate immunity, especially ability for VSV generalists to better circumvent the innate pathway regardless of cell type. Using measures of viral growth kinetics, apoptosis induction, and host innate gene expression levels, we observed that specialist and generalist viruses diverged in innate immune evasion, but that at least two alternative molecular mechanisms evolved among replicate lineages of VSV generalists. Our results shed light on the potential role of innate immune evasion as a primary mechanism in generalist evolution in an RNA virus. Furthermore, our results suggest that the virus is evolutionarily versatile in its adaptive response to innate immunity.

Workshop Sessions

VIR-WK229.02 - Emergence and transmission of arbovirus evolutionary intermediates with epidemic potential

Kenneth Stapleford[1], Lark Coffey[1], Sreyrath Lay[2], Ofer Isakov[3], Antonio Borderia[1], Herve Blanc[1], Stephanie Beaucourt[1], Anna-Bella Failloux[1], Philippe Buchy[2], Marco Vignuzzi[1]
[1]Institut Pasteur, Paris, France, [2]Institut Pasteur, Phnom Penh, Cambodia, [3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Isreal

Adaptive mutations are essential for the emergence of Chikungunya virus (CHIKV) epidemics, yet the mechanisms involved are poorly understood. We hypothesized that by investigating CHIKV viral subpopulations in vivo we could address the role of viral adaptation and evolution in the lab. We first infected individual Aedes albopictus mosquitoes with the pre-epidemic CHIKV strain and identified the emergence of the epidemic mutation A226V in vivo. Furthermore, we identified two novel E1 mutations in both a clone-derived and wildtype isolate of CHIKV. These mutations increased both transmission in mosquitoes as well as pathogenesis in mice, and viruses containing these mutations were transmitted and caused disease in an insect-to-mammal transmission study. Finally, in vitro studies showed that these mutations increase stability and fusogenic activity of these virions providing a possible mechanism for their selection. Taken together, in this study we used deep-sequencing technologies to identify the emergence of a previous epidemic strain of CHIKV and novel changes in the E1 glycoprotein which increased transmission in mosquitoes and pathogenesis in mice. These studies provide valuable insight into CHIKV adaptation and a powerful platform on which to study both the temporal and spatial aspects of viral evolution in vivo, as well as viral and host components that influence these processes.

Workshop Sessions

VIR-WK229.03 - HIV-1 subtype A divergence and Gag epitope evolution

Syed Hani Abidi[1], Marcia Kalish[2], Farhat Abbas[1], Sarah Rowland-Jones[3], Syed Ali[1]
[1]Aga Khan University, Karachi, Pakistan, [2]Vanderbilt University, Nashville, US, [3]University of Oxford, Oxford, UK

Objective: The aim of this study was to examine course of time-dependent evolution of HIV-1 subtype A on a global level, especially with respect to the dynamics of immunogenic HIV Gag epitopes. Methods: We used a total of 1893 HIV-1 subtype A gag sequences representing a timeline from mid-1980s to late 2000s, as well as 19 different countries of Africa, Europe and Asia. Out of 1893 sequences, 94 sequences were from our Kenyan, Pakistani and Afghan cohorts. The phylogenetic relationship of subtype A gag and its epidemic dynamics was analysed through Maximum Likelihood tree and Bayesian Skyline plot, whereas genomic variability was measured in terms of GtoA substitutions and Shannon entropy. Additionally, time-dependent evolution of HIV subtype A Gag epitopes was examined. Finally, to confirm observations on globally reported HIV subtype A sequences, we analysed the Gag epitope data from our Kenyan, Pakistani, and Afghan cohorts, where both cohort-specific gene and epitope variability and HLA restriction profile of Gag epitopes were examined. Results: HIV subtype A epidemic stabilized around late-80s and eventually declined from 2003 onward. During the course of evolution, a gradual increase in genomic variability was observed that peaked in 2005-2010. We observed that the number of point mutations and novel epitopes in Gag also peaked concurrently during 2005-2010. Conclusion: It appears that as the HIV subtype A epidemics travel around the world, changing population immunogenetic pressures have steered the immune-evolution of this subtype in new directions. This trend is apparent in the genomic and epitope diversity of HIV-1 subtype A Gag sequences.

Workshop Sessions

VIR-WK229.04 - Characteristic patterns of gene fragmentation in orthopoxvirus evolution.

Eneida L. Hatcher[1], Robert C. Hendrickson[1], Elliot J. Lefkowitz[1]
[1]*University of Alabama at Birmingham, Birmingham, USA*

Although the most pathogenic member of the virus family Poxviridae, variola virus, has been eradicated in nature, other closely related viruses may have the potential to develop more highly pathogenic phenotypes. In particular, the genus Orthopoxvirus contains several members that are capable of causing disease in humans as well as in agriculturally-important animals. As poxviruses evolve, certain genes experience changes in selection pressure to maintain function, as evidenced by accumulation of mutations. These mutations may be progressive, starting with the development of single nucleotide polymorphisms, followed by small insertion and deletions, and then gene truncation and gene fragmentation, eventually leading to loss of expression (translational and subsequently transcriptional). Examination of these fragmentation patterns leading to gene loss inform us about the roles individual genes may play in virus biology, and how selection pressures impacting virus replication may affect gene and virus evolution. Our previous work has demonstrated that gene loss is one of the major observable effects of orthopoxvirus evolution. Investigating the fragmentation patterns of orthopoxvirus genes will allow us to better understand the mechanisms guiding virus evolution in greater detail. We systematically predicted the complete functional gene sets for representative orthopoxvirus strains, as well as assessed the presence, quantity, and causes of gene fragmentation. Fragmented genes were examined in detail to identify the indels, single nucleotide polymorphisms, and large deletions that lead to truncation or loss of the open reading frame through the introduction of early stop mutations (ESMs). Our work demonstrates that short deletions are a major cause of gene truncation, and that the length of genomes is inversely associated with the number of ESMs they contain. By clarifying the operative mechanisms and products of virus evolution, we will be able to better understand poxvirus biology, virus-host interactions, and the future evolutionary potential of these virus pathogens.

Workshop Sessions

VIR-WK230.01 - Cellular and molecular mechanism of alphavirus pathogenesis: implications for disease interventions

Lisa Ng[1]
[1]*Singapore Immunology Network, Biopolis, Singapore*

The last two decades has seen a dramatic increase in the emergence and/or re-emergence of a number of serologically distinct alphaviruses. Factors such as the absence of herd immunity and a lack of vector control have been instrumental in the re-emergence of several virus infections. Questions remain about the role of possible microevolution on viral virulence and severity of the associated disease. Importantly, the exact nature of the protective immune defense and the pathogenic mechanisms of debilitating arthralgia and arthritis upon virus infection are still poorly known. Studies have found that the interplay between the levels of viral load, interferon-stimulated genes (ISGs) and the induction of neutralizing antibodies, could mediate efficient viral clearance and protect against severe diseases. Understanding some of these cellular and molecular mechanisms will provide insight into future control and therapeutic strategies.

Workshop Sessions

VIR-WK230.02 - Calcium Is critical for Rubella Virus fusion, membrane interaction, and infection

Mathieu Dubé[1], Felix A. Rey[2], Margaret Kielian[1]
[1]Department of Cell Biology, Albert Einstein College of Medicine, New York, USA., [2]Unité de Virologie Structurale, Institut Pasteur and CNRS UMR 3569, Paris, France

Rubella virus is a (+)ssRNA enveloped virus causing benign infection in children or adults, but severe fetal malformations. Rubella virus enters cells by receptor-mediated endocytosis and undergoes membrane fusion in endosomes through the fusogenic activity of its class II E1 fusion protein. A crystal structure of E1 recently revealed a homotrimeric hairpin-like post-fusion conformation that includes Ca2+ coordinated between the two membrane-interacting fusion loops, a unique feature among the currently characterized viral fusion proteins. However, the role of this cation during Rubella virus entry remained unknown. Here we demonstrated a specific and dose-dependent requirement for calcium during Rubella virus fusion. The role of calcium in Rubella virus receptor binding, lipid interaction, lipid mixing and low pH-triggered E1 conformational changes was further assessed by taking advantage of receptor-binding, liposome co-floatation, DiD-labelled Rubella virus dequenching and trypsin-resistance assays, respectively. Strikingly, only the lipid interaction and lipid mixing steps required calcium. Alanine substitution of the polar Ca2+-coordinating residues E1 N88 and D136 did not affect virus assembly but completely abrogated virus infectivity and membrane interaction, thus excluding that Ca2+ only acts by compensating N88 and D136 polarity. Calcium-dependence appeared to confer a critical selective advantage to Rubella virus since no calcium-independent revertant was recovered from cells electroporated with virus RNA encoding Ca2+-binding deficient E1 mutants. Overall, our data identified calcium as a major entry cofactor essential for the membrane interaction of Rubella virus E1, and described for the first time among known enveloped viruses a calcium-dependent fusogen.

Workshop Sessions

VIR-WK230.03 - Pathogenesis of epidemic Chikungunya Virus in the Common Marmoset (Callithrix jacchus)

Chang-Kweng Lim[1], Yasushi Ami[2], Meng Ling Moi[1], Mutsuyo Takayama-Ito[1], Kenji Shirai[1,3], Akira Kotaki[1], Eri Nakayama[1], Masayuki Saijo[1], Ryuji Suzuki[3], Tomohiko Takasaki[1]

[1]Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan, [2]Division of Experimental Animal Research, National Institute of Infectious Diseases, Tokyo, Japan, [3]Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, Sagamihara, Japan

Chikungunya virus (CHIKV) virus has re-emerged as an important mosquito-borne pathogen causing epidemics in several parts of the world. The CHIKV belongs to the Alphavirus genus in the family Togaviridae. A large-scale epidemic of CHIK fever started in Kenya in 2004 and spread to Indian Ocean islands, India, Sri Lanka, Thailand, Malaysia, Indonesia, and the Philippines. Feral neurological syndromes were associated with CHIKV infection for the first time in these outbreaks. During Jan. 2007-Aug. 2013, 43 imported CHIK cases were detected in Japan from South and Southeast Asia. One of the main vectors responsible for transmission between humans is Aedes albopictus, which is widely distributed in urban areas of Europe, the USA and East Asia including Japan. This fact raises concern that the virus could be introduced and become established in these areas. As primates possess physiological characteristics more reflective of humans, we assessed a nonhuman primate model of CHIKV infection in adult, immunocompetent common marmosets (Callithrix jacchus). In this study 10 common marmosets were inoculated subcutaneously with recent 2006 epidemic CHIKV strain. All marmosets developed viremias that persisted for 4-5 days. Neutralizing antibodies were detected after day 7 with the fading viraemia. At 4 dpi, focal necrosis and mononuclear cells infiltration were observed in the liver. The viral antigens were immunohistochemically detected in the hepatocytes. At 7 dpi, the proliferation of lymphocytes around the central artery and macrophage accumulation in red pulp were observed. In the cytoplasm of macrophages, viral antigens were detected. No viral antigens were detected on control animal. These results demonstrate that CHIKV effectively infects common marmosets. This is the first common marmoset model of CHIKV infection to allow for analyses of the molecular mechanisms of the CHIKV lifecycle directly in a non-human primate.

Workshop Sessions

VIR-WK230.04 - The mechanism of early sensing of alphavirus replication and innate immune response development

Ilya Frolov[1], Svetlana Atasheva[1], Ivan Akhrymuk[1], Elena Frolova[1]
[1]*University of Alabama at Birmingham, Birmingham, USA*

Members of the Alphavirus genus in the Togaviridae family cause a variety of diseases with the symptoms varying between mild polyarthritis to severe meningoencephalitis with lethal outcome. The characteristic feature of alphaviruses is their exceptionally rapid replication in cell culture and development of viremia in vivo. The efficient spread of infection in vitro was found to be strongly determined by the ability of alphaviruses to interfere with the development of the innate immune response, to inhibit cellular transcription and to isolate their dsRNA intermediates into cellular membrane spherules, thus, protecting them from interacting with PRRs. However, in spite of these mechanisms, the inhibitory effect of alphaviruses on the development of the cellular antiviral response is incomplete, and animals do respond to alphavirus infection with cytokine release. Our data provide a plausible explanation for the mechanism of development of the innate immune response to alphavirus replication, and explain the differences between the data generated in vivo and in vitro. Two cellular PRRs, RIG-I and MDA5, were found to be the only cellular sensors involved in this process. They play critical but redundant roles in the induction of the early anti-alphaviral response and sense virus replication before the development of transcriptional shutoff. The rates and magnitude of type I IFN induction are determined by the intracellular concentrations of these PRRs. We also demonstrate that the low doses of type I IFN released from the primarily infected cells play a critical role in development of infection. The uninfected cells, primed for a short time by exposure to very low doses of IFN-ß, respond to the next rounds of virus replication with dramatically more efficient IFN release and activation of ISGs, compared to unprimed cells. Thus, the very early events in alphavirus infection are the critical determinants of the overall infection outcome.

Workshop Sessions

VIR-WK230.05 - The Americas are more than ever under the threat of chikungunya virus spread.

Anubis Vega Rua[1], Karima Zouache[1], Anna-Bella Failloux[1], Ricardo Lourenco-de-Oliveira[2]
[1]Institut Pasteur, Department of Virology, Laboratory Arboviruses and Insect Vectors, Paris, France, [2]Instituto Oswaldo Cruz, FioCruz, Río de Janeiro, Brasil

Chikungunya virus (CHIKV) mainly transmitted by the mosquitoes Aedes aegypti and Aedes albopictus, causes a major public health problem. Intensification of intercontinental travels tends to facilitate the expansion and invasion of mosquito vectors contributing to the (re)-emergence of arthropod-borne diseases when environmental conditions are conducive to viral transmission. Intriguingly, no local transmission of CHIKV has been reported in the Americas until very recently despite the presence of competent vectors with dozens annually-reported imported cases in the last eight years. Here, we analyze the risk of chikungunya emergence in the Americas by assessing the ability of 35 American Ae. aegypti and Ae. albopictus populations to disseminate and to transmit three different CHIKV genotypes. We also compared the number of viral particles of different CHIKV strains in mosquito saliva at two different days after infection. Primarily, viral dissemination rates were high for all mosquito populations irrespectively to the tested CHIKV isolate. In contrast, differences in transmission efficiency (TE) were underscored in populations of both species through the Americas, suggesting the role of salivary glands in selecting CHIKV for highly efficient transmission. Nonetheless, both mosquito species were capable to transmit all three CHIKV genotypes, and TE reached alarming rates as high as 83.3% and 96.7% in Ae. aegypti and Ae. albopictus populations, respectively. Ae. albopictus better transmitted the epidemic mutant strain CHIKV_0621 of the East-Central-South African (ECSA) genotype than Ae. aegypti, whereas this latter species was more capable of transmitting the original ECSA CHIKV_115 strain and also, the Asian genotype CHIKV_NC. Therefore, a high risk of establishing and spreading of CHIKV transmission all over the tropical, subtropical and even temperate regions of the Americas is more than ever a reality.

Workshop Sessions

VIR-WK230.06 - A multiagent DNA vaccine delivered by electroporation elicits protective immunity against aerosolized eastern and western equine encephalitis viruses in nonhuman primates

Lesley Dupuy[1], Callie Bounds[1], Michelle Richards[1], Drew Hannaman[2], Connie Schmaljohn[1]
[1]USAMRIID, Fort Detrick, USA, [2]Ichor Medical Systems, San Diego, USA

Next-generation vaccines that can safely and effectively protect against human infections caused by the biological threat agents Venezuelan, eastern, and western equine encephalitis virus (VEEV, EEEV, and WEEV) are needed. Previously, we demonstrated that DNA constructs expressing codon-optimized envelope glycoprotein genes of VEEV, EEEV, or WEEV delivered individually or as a multiagent combination by intramuscular (IM) electroporation (EP) elicited high levels of virus-neutralizing antibodies in multiple animal species and provided protective immunity against lethal homologous aerosol viral challenge in mice. Recently, we have completed studies to assess the immunogenicity and protective efficacy of the individual and combined DNA vaccines delivered by IM EP in nonhuman primate (NHP) models of aerosol viral challenge. After EEEV exposure, all NHPs that received empty vector DNA developed severe neurological signs indicative of acute encephalitis and were euthanized on days 5-7 postchallenge. Although neurological signs of disease were observed in some NHPs vaccinated with the individual EEEV plasmid, these were of lesser severity than those observed in the negative control animals and all survived. In contract, no significant clinical signs of disease were observed in NHPs that received the combined DNA vaccines. Similarly, NHPs that received the combined vaccines also did not display significant clinical signs of disease after WEEV exposure. We are currently investigating the apparent synergistic protective effect achieved with the combined vaccines in further detail. In addition, we are completing a study with the individual VEEV and combined vaccines delivered by IM or intradermal EP in the NHP model of aerosol VEEV challenge. Taken together, the results of our studies demonstrate that EP delivery of a multiagent formulation of VEEV, EEEV, and WEEV DNA vaccines represents a potent means of protecting against aerosolized encephalitic alphavirus infections and support its continued development into a mature vaccine candidate suitable for future clinical testing.

Workshop Sessions

VIR-WK230.07 - DNA-Launched Live Attenuated Vaccines for Alphaviruses

Irina Tretyakova[1], Eryu Wang[2], Igor Lukashevich[3], Scott Weaver[2], Peter Pushko[1]
[1]Medigen, Inc., Frederick, USA, [2]UTMB, Galveston, USA, [3]University of Louisville, Louisville, USA

Alphaviruses such as Venezuelan equine encephalitis virus (VEEV) and chikungunya virus (CHIKV) cause outbreaks of life-threatening diseases and represent emerging epidemic and pandemic threats. Vaccine development against alphaviruses has proved challenging. Currently there are no approved vaccines or specific therapies for VEEV or CHIKV. To develop an experimental VEEV vaccine, we applied novel iDNA infectious clone technology that combines the advantages of DNA and live-attenuated vaccines. We generated an iDNA plasmid that encodes the full-length infectious RNA genome of live-attenuated VEEV strain TC-83 virus downstream from a eukaryotic promoter. The iDNA plasmid was designed to initiate replication of live-attenuated VEEV from the plasmid in vitro and in vivo. Experimental iDNA vaccine was evaluated in Vero cells and in BALB/c mice. Transfection with as little as 10 ng of iDNA was sufficient to initiate replication of vaccine virus in vitro. Furthermore, vaccination of BALB/c mice with a single 10 mg dose of iDNA plasmid resulted in seroconversion, elicitation of neutralizing antibodies, and protection from experimental challenge with wild-type, virulent VEEV. To confirm the broad applicability of the iDNA approach, we also prepared an iDNA plasmid that encoded the full-length infectious RNA genome of live-attenuated CHIKV strain 181/25. Our data suggest that live-attenuated alphavirus vaccines can be delivered in vitro and in vivo using DNA vaccination. We conclude that iDNA immunization represents a promising vaccination strategy for VEEV and CHIKV alphaviruses.

Workshop Sessions

VIR-WK231.01 - Crystal structures of intermediates and post-fusion conformations of Chandipura virus glycoprotein highlight the pathway of the fusion associated transition of vesiculovirus glycoprotein

Eduard Baquero[1], Aurélie Albertini[1], Malika Ouldali[1], Linda Buonocore[2], John K Rose[2], Jean Lepault[1], Stéphane Bressanelli[1], Yves Gaudin[1]

[1]CNRS, Laboratoire de Virologie Moléculaire et Structurale, Gif sur Yvette, France, [2]Yale University School of Medicine, New Haven, USA

Vesicular stomatitis virus (VSV) G is the prototype of the third class of viral fusion glycoproteins. At low pH, G undergoes a huge conformational change during which it exposes two hydrophobic fusion loops which interact with the target membrane as a first step of the fusion process. Crystal structures have provided atomic, static pictures of the pre- and post-fusion conformations of VSV G which are both trimeric. Although experimental data suggest that the conformational change proceeds through monomeric intermediates, the transition pathway between the pre- and post-fusion states remains elusive. We have recently determined the post-fusion structure of the glycoprotein of Chandipura virus (CHAV), another vesiculovirus responsible for deadly encephalopathies. CHAV G evidences a high structural identity with VSV G. However, we also identified several differences which reflect the evolutionary constraints acting on the glycoprotein. Unexpectedly, in a second crystal form, two distinct conformations corresponding to early and late refolding states of CHAV G form a fusion loop-exposing flat heterotetramer with twofold symmetry. Consistent with these data, electron microscopy and tomography show different intermediates at the viral surface depending on experimental conditions. This work reveals the chronological order of the structural changes in the protein for the spikes located outside the contact zone with the target membrane. Particularly, our data confirm that the conformational change involves monomeric intermediates and that it likely proceeds to an elongated hairpin monomer before subsequent collapse into the post-fusion trimer. Furthermore, our data and previously published mutagenesis analysis indicate that after dissociation of the pre-fusion trimer into monomers, vesiculovirus fusion glycoprotein could re-associate not only into trimers but also into a dimeric (and even tetrameric) assembly which is optimally organized to forming the initial bridge between the target and viral membranes.

Workshop Sessions

VIR-WK231.02 - Soluble receptor binding domains (RBD) derived from retroviral envelope glycoproteins as ligands for nutrient transporters and new metabolic markers

Svilena Ivanova[1,2,3], Donatella Giovannini[1,2], Jawida Touhami[1,2,3], Julie Laval[1,2], Vincent Petit[3], Jean-Luc Battini[1,2], Marc Sitbon[1,2]

[1]Institut de Génétique Moléculaire de Montpellier (IGMM), CNRS-UMR5535, Montpellier, France, [2]Université de Montpellier, Montpellier, France, [3]Metafora biosystems, Evry, France

Retroviral infections rely on the binding of viral envelope glycoproteins (Env) to specific cognate receptors present at the cell surface. Interestingly, all receptors characterized so far for gammaretrovirus and deltaretrovirus Env are multiple membrane-spanning proteins that belong to the solute carrier (SLC) family. Specific Env binding to SLC is mediated by aminoterminal Receptor Binding Domains (RBD) that can be produced as soluble ligands. SLC comprise over 370 members within 52 families. The presence of certain SLC at the cell surface is likely to condition and reflect cell normal and pathophysiological status and metabolism. However, monitoring SLC cell surface expression has been hindered by the notorious lack of reliable exofacial antibodies. HTLV Env-derived RBD act as exofacial ligands of Glut1, the main glucose transporter and HTLV receptor (Manel et al. Cell 2003) and have been key to assess the role of Glut1 in different hematopoietic environments. At present, our collection of soluble RBD constitutes a new powerful array of ligands to monitor SLC distribution by flow cytometry or microscopy. Our RBD comprise ligands to a dozen of SLC that include Glut1 as well as other transporters such as inorganic phosphate importers, glutamine and other neutral aminoacid importers, the heme exporter, etc. Their use allowed the distinction of metabolically active cell subpopulations in different normal and pathophysiological settings (see for instance Laval et al. J. Immunol. 2013; Petit et al. Lab. Invest. 2013). In addition, we recently showed that Xpr1, the receptor of the xenotropic MLV, a murine leukemia retrovirus that can infect cells from other species, including humans, is the first metazoan inorganic phosphate exporter and that xenotropic MLV Env and RBD can inhibit this function (Giovannini et al. Cell Rep. 2013). Now, we adapted several of these RBD to immunohistochemistry methods, comforting their use as unique metabolic biomarkers.

Workshop Sessions

VIR-WK231.03 - Guinea Pig Cytomegalovirus (GPCMV) epithelial tropism and efficient congenital infection is dependent upon a homolog pentameric glycoprotein complex found in human CMV

K. Yeon Choi[1], Stewart Coleman[1], Jim Choi[1], Alistair McGregor[1]
[1]Texas A&M University Health Science Center, College Station, USA

A vaccine against congenital cytomegalovirus is an important health priority. HCMV viral glycoprotein complexes are necessary for cell entry and are important neutralizing antigens. Glycoprotein gH, complexes with gL and gO for fibroblast infection but forms an additional pentameric complex (128C) for infection of epithelial/endothelial cells in clinical HCMV. Proteins encoded in the UL128-131 locus (UL128, 130 and 131) complexed with gH and gL form the 128C pentameric complex which is a neutralizing target for HCMV epi/endo infection. Consequently, there is considerable interest in 128C as a vaccine strategy. The guinea pig is the only small animal model for congenital CMV but requires the use of GPCMV because of species specificity. We have shown that GPCMV encodes functional glycoproteins homologs (gB, gH, gL, gM, gN, gO) that form specific complexes that are immune targets. Additionally, pathogenic strains of GPCMV (causing efficient congenital infection) encode a homolog locus to UL128-131(GP129-133), which potentially enables formation of a homolog pentameric complex. Transient expression studies demonstrated that GPCMV GP75 (gH homolog) interacts with GP115 (gL homolog), GP129 (UL128 homolog), GP131 (UL130 homolog) and GP133 (UL131 homolog). Additionally, numerous sub-complexes were also formed. Using guinea pig fibroblasts and a newly established epithelial cell line we demonstrate that pathogenic GPCMV was capable of growth on epithelial cells unlike lab adapted virus and that the GP129-133 locus was stable in these cells but was rapidly deleted in fibroblast cells. Furthermore, a GP129 (UL128) mutant defective for epi tropism was restored to full tropism by expression of a wild type GP129. Knockouts of the homolog UL128, UL130 and UL131 ORFs in GPCMV resulted in impaired epi tropism but did not modify the ability of virus to replicate on fibroblasts. We are currently investigating the merit of this complex as part of a GPCMV vaccine strategy.

Workshop Sessions

VIR-WK231.04 - Virulence determinants in the Spanish Influenza virus Hemagglutinin

Alex Silaghi[1], Darwyn Kobasa[1,2]
[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada, [2]Special Pathogens, National Microbiology Laboratory, Winnipeg, Canada

The Spanish Influenza caused the worst pandemic in history with 30-50 million deaths. A virus [A/South Carolina/1/18 H1N1 (Sp)] generated based on genomic sequences obtained from pandemic fatalities is highly virulent in mice, ferrets and non-human primates, through severe lung inflammation, similar to Acute Respiratory Distress Syndrome that was reported in pandemic patients. The hemagglutinin (HA) segment was identified as the main virulence determinant yet the molecular determinants in HA remain unknown and identification of these determinants could benefit pandemic preparedness. Using HA1/HA2 chimeras between Sp HA and A/USSR/90/77 (USSR), a low virulence strain, we found that HA1 is the main determinant in mice, although Sp HA2 is required for full virulence. Mutagenesis studies identified Lysine (K) 60, Alanine (A) 332 in HA1 and Arginine (R) 460 (codon numbering) in HA2 as virulence determinants. Residues 60 and 332 may be related to HA2 functions as mutagenesis of K60 only attenuates Sp HA1-dependent virulence if USSR HA2 is present. K60 is found in a random coil under the Receptor-Binding Domain oppositely from HA2, but it interacts with residue 291 in the Vestigial Esterase region, which is in close proximity to HA2. Sp and USSR contain Aspartic Acid (D) and Glutamic Acid (E) at position 291, respectively, and D291E mutagenesis in Sp did not cause attenuation. However, D291R mutation led to attenuation, suggesting that acid-base interaction at 60-291 may be necessary. A332 is buried in an unexposed pocket in HA1 in close proximity to HA2. Sp HA residues K60, A332 and R460 are rarely found in human H1 isolates the NCBI database, but more commonly found in avian and swine H1 strains. Although these residues are insufficient for virulence transfer, indicating that other residues are involved, these findings are a first step in deciphering the virulence behind the most dangerous human influenza.

Workshop Sessions

VIR-WK232.01 - Current views and future prospects in the transmission of viruses by insect vectors

Stéphane Blanc[1]
[1]*INRA, UMR BGPI, CIRAD TA-A54/K, Campus International de Baillarguet, Montpellier, France*

Understanding the mechanisms controlling vector-transmission of viruses, as well as their ecological/evolutionary consequences, requires integrating information from at least three different viewpoints: virus–vector interactions, host–vector interactions and virus–host interactions. While some of these aspects are reasonably well covered by past and present investigations, others have been bypassed completely, because of technical bottlenecks or conceptual lacunas. It is interesting to confront recent advances in the fields of plant and animal virology to identify the common needs in hitherto poorly documented aspects of vector transmission. These aspects are for example the characterization of the vector molecules responsible for initial viral recognition, the changes in the physiology and/or in the behavior of the vectors in the presence of viruses, the switches in the viral cycle within the hosts that can be induced by the arrival of vectors, and the specific viral population dynamics/genetics events associated with the transmission step. On the basis of detailed studies on a plant virus, we will introduce these different concepts and discuss them in the light of the discoveries not only reported on other plant viruses, but also on arboviruses of animals.

Workshop Sessions

VIR-WK232.02 - What are the risks of emergence of chikungunya outbreak in Central African Republic

Basile Kamgang[1], Carine Ngoagouni[1], Vianney Tricou[1], Emmanuel Nakouné[1], Mirdad Kazanji[1]
[1]*Institut Pasteur de Bangui, Bangui, Central African Republic*

Chikungunya virus, which previously caused only sporadic outbreaks in sub-Saharan Africa, has recently emerged in several urban epidemic foci in Central Africa. This emergence of chikungunya in urban area coincides with introduction of Aedes albopictus. Ae. albopictus originated in Asia was first reported in central Africa in 2000, in Cameroon, with the indigenous species Ae. aegypti is present today in several countries of the region, including the Central African Republic (CAR), where it was recorded in 2009. To determine the consequences of this invasion of Ae. albopictus for epidemiological transmission of chikungunya, we conducted a comparative study in the early and the late wet season in Bangui, and in the other main cities of the country to document infestation by the two species and their ecological preferences. In addition, we explored the geographical origin of populations of Ae. albopictus with two mitochondrial DNA genes, COI and ND5. We also assessed the current circulation of chikungunya virus in CAR by detection anti-CHIK IgM antibodies in different population. Analysis revealed that Ae. aegypti predominates early and Ae. albopictus late in the wet season. Ae. albopictus was the most prevalent species in almost all the sites investigated, except Bouar, where only Ae. aegypti was found, suggesting that Ae. albopictus tends to supplant Ae. aegypti in sympatric areas. Mitochondrial DNA analysis revealed broad low genetic diversity, confirming recent introduction of Ae. albopictus. Phylogeographical analysis with MtDNA COI gene suggested that Ae. albopictus in CAR came from multiple invasions and from multiple population sources. Serological analysis revealed a silent circulation of chikungunya virus, with anti-CHIK IgM antibodies detected in 25.2% from 468 tested samples. Furthermore anti-CHIK IgG antibodies were detected at later stage in 17.2 % of the 58 IgM tested samples. These data may have important implications for vector control strategies in central Africa.

Workshop Sessions

VIR-WK232.03 - Mode of transmission determines the dynamics of primary Parainfluenza Virus infection and protection from reinfection independent of viral and host factors that govern pathogenesis

Crystal Burke[1], John Mason[1], Olga Bridges[1], Guohua Yang[1], Charles Russell[1]
[1]Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, USA

To illuminate respiratory virus infection, reinfection, pathogenesis, and transmission in living animals, we generated a non-attenuated Sendai virus (murine parainfluenza virus 1) luciferase reporter virus. Tracking infection/reinfection in individual living mice has allowed us to test several hypotheses and make numerous discoveries. Major findings include: (1) contact and airborne transmission are associated with virus growth in the nasal cavity while pathogenesis is determined by virus growth and the host response in the lungs; (2) host-based susceptibility maps to the lungs but not the nasal cavity; (3) any previous exposure to infection protects from natural secondary reinfection; (4) greater primary infection in a given respiratory tissue (nasopharynx, trachea, or lung) correlates with the level of reinfection in that same tissue after lethal challenge; and (5) the mode of transmission determines the dynamics and tropism of primary infection which in turn governs the level of seroconversion and protection from lethal challenge. We have discovered four distinct categories of dynamics of respiratory virus infection in individual, living animals after airborne transmission: (1) non productive transmission; (2) tracheal dominant infection; (3) tracheal dominant yet respiratory disseminated; and (4) nasopharyngeal initiated yet respiratory disseminated. Recently, we have been using low- and high-pathogenicity bioluminescent Sendai viruses to investigate the relationship between pathogenesis and transmission. We have discovered that faster, but not more efficient, transmission is promoted by the lethal strain. Overall, these unprecedented studies that visualize the dynamics of infection throughout the respiratory tracts of living animals provide a paradigm for parainfluenza virus infection in the respiratory tract and reveal fundamental insight into respiratory virus transmission.

Plenary Sessions

BAM-PL08.01 - Regulating the regulators: Ribonucleases and the control of sRNAs

Cecilia M. Arraiano[1], José M. Andrade[1], Vânia Pobre[1], Margarida Saramago[1], Inês J. Silva[1], Susana Domingues[1], Rute G. Matos[1], Sandra C. Viegas[1]

[1]*ITQB- Universidade Nova de Lisboa, Oeiras, Portugal*

The continuous degradation and synthesis of RNAs not only give rise to the metabolic changes that are required as cells grow and divide but also rapid adaptation to new environmental conditions. The accessibility of sites for degradation depends on several factors, including RNA higher-order structure, protection by translating ribosomes and polyadenylation status. Furthermore, RNA degradation mechanisms have shown to be determinant for the post-transcriptional control of gene expression. Ribonucleases (RNases) mediate the processing, decay and quality control of RNA. RNases act in different pathways to execute the maturation of rRNAs and tRNAs, and intervene in the decay of many different mRNAs and small noncoding RNAs (sRNAs). RNases can be divided into endoribonucleases that cleave the RNA internally or exoribonucleases that cleave the RNA from one of the extremities. Over the last years, it has become clear that sRNAs are important in almost every facet of gene regulation. RNases are important for their maturation and then control their levels degrading them alone or when hybridized to their targets. For instance exoribonuclease PNPase is the major factor involved in the rapid degradation of small RNAs not bound to Hfq, and RNase III has been shown to degrade several sRNAs in a target dependent pathway. Understanding the “sRNA anatomy” is thus crucial to modulate stability and to differently regulate the targets. This knowledge can allow for the engineering of non-coding RNAs that interact differently with multiple targets. Focusing on the RNase II family of ubiquitous exoribonucleases, (which includes RNase II, RNase R, Dis3/Rrp44, Dis3L1 and Dis3L2) we have constructed many mutants, modulated decay mechanisms and revealed new degradation pathways. Recently we have shown that Dis3L2 degrades preferentially polyuridylated RNAs. In summary, we have demonstrated that RNases act as a global regulatory network extremely important for the regulation of RNA levels and cellular metabolism.

Plenary Sessions

BAM-PL08.02 - C-di-GMP signaling and Escherichia coli biofilm architecture

Regine Hengge[1]
[1]Humboldt-Universität, Berlin, Germany

Bacterial macrocolonies represent biofilms that can produce intricate three-dimensional structures. Macrocolony morphology depends on an extracellular matrix of adhesins, amyloid fibres (e.g. curli) and exopolysaccharides (e.g. cellulose). In *E. coli*, synthesis of curli fibres and cellulose is under control of the stationary phase sigma factor RpoS and the second messenger c-di-GMP. The latter is produced by diguanylate cyclases (DGC, with GGDEF domains) and is degraded by specific phosphodiesterases (PDE, with EAL or HD-GYP domains). Many bacterial species possess multiple GGDEF/EAL domain proteins (29 in E. coli K-12). As shown by fluorescence microscopy of cryosections and scanning EM, *E. coli* macrocolony biofilms exhibit a stratified architecture that develops along gradients of nutrients. In essence, small starved cells literally encased in a nanocomposite of curli fibres and cellulose are found in the top layer of macrocolonies, whereas the outer edges and the lower layer (adjacent to the nutrient-providing agar) consist of vegetatively growing cells with entangled flagella. The intricate spatial arrangement of curli fibres and cellulose in the top layer generates the tissue-like cohesiveness and elasticity that allows the buckling up and folding of macrocolonies into their complex 3D-structure (1). The production of matrix components follows the expression of the key regulator CsgD, which is promoted by a spatially controlled regulatory network of several directly interacting DGCs, PDEs and the transcription factor MlrA (2). Massive matrix production only in the top layer as well as short-range cellular heterogeneity of matrix synthesis in the transition zone between the two strata are essential for macrocolony integrity and morphogenesis. Overall, these studies reveal the microanatomy and physiological heterogeneity of a bacterial biofilm and relate these to the underlying regulatory networks prominently featuring RpoS and c-di-GMP. (1) Serra & Hengge (2014) Environ. Microbiol. (in press) (2) Lindenberg et al. (2013) EMBO J. 32, 2001-2014.

Plenary Sessions

BAM-PL09.01 - Systems wide proteome analysis in Gram-positive bacteria

Dörte Becher[1], Michael Hecker[1]
[1]*Institute for Microbiology, Ernst-Moritz-Arndt University Greifswald, Germany*

Because of their low complexity, bacteria are extremely attractive model systems to bring the genome sequence to real life and to test newly developed methods in proteomics. Despite the fact that 2D gel based proteomics is still a useful approach to gain a global view of bacterial physiology, many proteins escape detection by gel-based proteomics. Thus, it is not surprising that mass spectrometry based proteomics had a great impact on bacterial proteomics. Through the parallel implementation of gel based and gel free approaches the maximal potential of recent technologies can be utilized. Therefore, we have applied state of the art methods as 2D PAGE and GeLC-MS/MS analysis of metabolically labelled proteins for identification and quantification. Newly developed methods for sample preparation like membrane shaving and biotinylation of surface associated proteins were used to enlarge the coverage of accessible proteins. Analysing the single subproteomes from the cytosol, via the membrane proteins up to the surface associated and extracellular proteins we could show that almost the entire set of proteins of a model bacterium can be identified and even quantified by a combination of these techniques. To target dynamic changes of the proteome time line experiments complete these studies and to get deeper insight into the cell physiology of gram-positve bacteria. By combining targeted mass spectrometry for the accurate determination of absolute protein abundance of specific proteins with the resolving power of 2D gels it is even possible to obtain for a large number of proteins the absolute protein amount per cell. Compared to other methods this approach does not require the availability of large mutant libraries or the synthesis of hundreds of synthetic peptides and achieves an accuracy similar to MS measurements. This method benefits a broad systems biology community due to its simple and low-cost strategy.

Plenary Sessions

BAM-PL09.02 - Transcriptome-wide discovery of regulated transcriptional termination in bacteria

Daniel Dar[1], Iris Karunker[1], Rotem Sorek[1]
[1]*Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel*

Regulated transcriptional termination in bacteria was shown to control the expression of key metabolic and virulence related genes, as well as lysis/lysogeny decisions during phage infection. To uncover novel forms of regulated termination we developed a method for direct sequencing of RNA 3' ends in bacteria. We demonstrate that our method can identify the vast majority of termination events in the cell in a genome wide manner and to the single nucleotide resolution. We further show that our approach provides mechanistic insights into the process of termination in E. coli, and enables the detection of the majority of known riboswitches in B. subtilis with high sensitivity and specificity. Furthermore, our approach identified additional novel candidates for regulated termination in genes involved in swarming, antibiotic resistance and core metabolic processes.

Plenary Sessions

MEM-PL08.01 - A chemical-genetic matrix strategy for synthetic control of biological networks

Jan Wildenhain[1], Michaela Spitzer[1,3], Sonam Dolma[2], Almer van der Sloot[4], Susan Moore[4], Gerry Wright[3], Mike Tyers[1,4]

[1]University of Edinburgh, Edinburgh, UK, [2]The Hospital for Sick Children, Toronto, Canada, [3]McMaster University, Hamilton, Canada, [4]University of Montreal, Montreal, Canada

Cell behaviour is governed by a complex network of genetic interactions, such that combinations of genetic mutations can either exacerbate or ameliorate any given phenotype. Chemical biology and synthetic biology represent two complementary approaches for multi-parametric perturbation, both to probe network structure and to control network activity. Predicated on this network concept, we have combined sensitized genetic screens, interaction network data and cheminformatics to discover combinations of compounds that exhibit synergistic bioactivities. A query set of 3,672 small molecules was used to probe 195 different *S. cerevisiae* deletion strains for genotype-specific inhibition of cell growth. This Chemical Genetic Matrix (CGM) of interactions was then integrated with chemical structural properties and extensive genetic interaction datasets to build a second order network activity response (SONAR) graph to identify chemical combinations that mimic synthetic lethal interactions. Of 896 molecules that showed specific bioactivities across deletion strains, we selected 128 compounds and tested all possible combinations. This dataset of 8128 drug combinations verified the predictive approach, and revealed 58 previously unidentified small molecule combinations for which synergism could be verified in dose-response surfaces. Some combinations exhibited selective toxicity against pathogenic fungal species, including *C. albicans, C. parapsilosis, C. neoformans* and *C. gattii*, suggesting that species-specific network sensitivities may exploited in anti-fungal therapy. This CGM approach will be extended through screens against diverse synthetic natural product libraries synthesized in situ from yeast artificial chromosomes that encode re-factored biosynthetic enzymes.

Plenary Sessions

MEM-PL08.02 - Synthetic Genomics: from genetic parts to genomes

Patrick Yizhi Cai[1]
[1]*University of Edinburgh, Edinburgh, UK*

The Synthetic Yeast genome project, or Sc2.0 (www.syntheticyeast.org), aims to design, construct, and replace the native 12Mb genome of Saccharomyces cerevisiae with a fully synthetic version. Sc2.0 chromosomes encode a myriad of designer changes. First, to improve genomic stability, destabilizing elements such as transposons and tRNA genes are removed from the synthetic genome. Second, synonymously recoded sequences called PCRtags permit encryption and tracking of the synthetic DNA. Finally, to enable downstream genetic flexibility, Sc2.0 encodes an inducible evolution system called SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution) that can generate combinatorial genetic diversity on command. To date, ~10% of the genome has been synthesized and we have powered a semi-synthetic yeast entirely dependent on multiple synthetic chromosome arms designed to our specifications. Software and experimental infrastructure developed to facilitate Sc2.0 genome design and construction are applicable to new projects ranging from single gene/pathway design to synthesizing artificial chromosomes. Sc2.0 international partners include Imperial College London, Edinburgh University (UK); Tsinghua University, Tianjin University, GenScript, BGI (China); Pondicherry University, IGIB (India). Sc2.0 has the potential to revolutionize the future of genome structure-function analysis.

Plenary Sessions

MEM-PL09.01 - Staphylococcus aureus and Candida albicans inter-Kingdom interactions

Mark Shirtliff[1]
[1]*Dept. of Microbiology and Immunology, School of Medicine, University of Maryland-Baltimore, Baltimore, USA*

The bacterial species Staphylococcus aureus and the fungal pathogen Candida albicans are among the leading bloodstream pathogens causing high morbidity and mortality and are often co-isolated from an array of infections. In this study, we developed a novel murine model of oral co-colonization to demonstrate the clinical implication of their interaction as they co-exist on host oral tissue. Following inoculation with either species individually or in combination, animals were monitored for clinical signs of disease and three days post-infection, were sacrificed and tongues and kidneys were analyzed. Findings demonstrated that animals orally co-colonized with both species developed advanced oral candidiasis and subsequently exhibited clinical signs of systemic disease with high morbidity and mortality concomitant with the recovery of S. aureus from the kidneys. In contrast, animals inoculated with individual species remained healthy. We identified the C. albicans cell wall adhesin Als3p as a specific receptor crucial for S. aureus adherence to hyphae. These studies revealed extensive invasion by C. albicans hyphae containing Als3p with S. aureus seen within the sub-epithelium of the tissue while the mutant strain lacking this receptor invaded but S. aureus was confined to the tongue tissue surface. When tested in the dual species oral infection animal model, the mutant strain of C. albicans lacking the Als3p receptor failed to mediate systemic S. aureus infection in the co-colonized animals as compared to the successful staphylococcal sepsis accomplished by dual species infection with the wild type and complemented strains of C. albicans containing Als3p. In conclusion, we describe a novel strategy by which S. aureus is able to invade host tissue and disseminate via adherence to the invasive C. albicans hyphae.

Plenary Sessions

MEM-PL09.02 - Farming ants, fungus gardens and actinomycete bacteria

Jacobus J. Boomsma[1], Sandra B. Andersen[1], Hermógenes Fernández Marín[2], David R. Nash[1], Panagiotis Sapountzis[1], William T. Wcislo[2], Sze Huei Yek[1]

[1]Centre for Social Evolution, Department of Biology, University of Copenhagen, Denmark, [2]Smithsonian Tropical Research Institute, Panama

The attine ants and their fungus gardens live in complex symbiosis with multiple further symbionts, both mutualists and parasites, but it is not always clear how obligate these additional interactions are. Resolving these ambiguities is important because signatures of co-adaptation can only be expected when symbionts have predictable roles that affect the survival or reproduction of the farming ants. The cuticular actinobacteria that many attine ants rear on their cuticle are particularly enigmatic because they have both been shown to co-evolve with the ants and to be environmentally acquired, while a number of attine genera appear to have secondary lost them. I will present recent comparative data across and within attine ant genera to evaluate life-history variables that correlate with the maintenance or loss of these cuticular bacteria, and experimental work on the leaf-cutting ant *Acromyrmex echinatior* and its two native *Pseudonocardia* strains in Panama. I will conclude that experimental studies are particularly effective to elucidate signatures of co-adaptation, but that it is crucial to do these experiments with newly hatched workers, rather than with older foragers that no longer have garden maintenance and brood nursing tasks.

Plenary Sessions

VIR-PL05.01 - Herpesviral Noncoding RNAs: Insights into evolution

Joan Steitz[2,4], Yang Guo[1], Kasandra Riley[2,5], Akiko Iwasaki[3,4]
[1]Department of Cell Biology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, USA, [2]Department of Molecular Biophysics and Biochemistry, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, USA, [3]Department of Immunobiology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, USA, [4]Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, USA

In marmoset T cells transformed by *Herpesvirus saimiri* (HVS), a viral U-rich noncoding RNA, HSUR 1, specifically mediates degradation of host microRNA-27 (miR-27). High-throughput sequencing of RNA after crosslinking immunoprecipitation (HITS-CLIP) identified mRNAs targeted by miR-27 as enriched in the T cell receptor (TCR) signaling pathway, including GRB2. Accordingly, transfection of miR-27 into human T cells attenuates TCR-induced activation of mitogen-activated protein kinases (MAPKs) and induction of CD69. MiR-27 also robustly regulates SEMA7A and IFN-ɣ, key modulators and effectors of T cell function. Knockdown or ectopic expression of HSUR 1 alters levels of these proteins in virally transformed cells. Two other T-lymphotropic ɣ-herpesviruses, AlHV-1 and OvHV-2, do not produce a noncoding RNA to downregulate miR-27 but instead encode homologs of miR-27 target genes. Thus, oncogenic ɣ- herpesviruses have evolved diverse strategies to converge on common targets in host T cells

Plenary Sessions

VIR-PL05.02 - How gut microbes enhance enteric virus infectivity

Christopher Robinson[1], Palmy Jesudhasan[1], Julie Pfeiffer[1]
[1]UT Southwestern Medical Center, Dallas, USA

Enteric viruses encounter a vast microbial community in the mammalian digestive tract. We found that gut microbes are required for replication and pathogenesis of two unrelated enteric viruses, poliovirus and reovirus (Kuss et al., Science, 2011). Similarly, Tanya Golovkina's lab demonstrated that the mouse retrovirus MMTV relies on intestinal microbiota for transmission (Kane et al., Science, 2011). Therefore, enteric viruses from three different families (Picornaviridae, Reoviridae, and Retroviridae) benefit from intestinal bacteria. We found that exposure to bacterial surface polysaccharides, including lipopolysaccharide (LPS) and peptidoglycan, enhanced poliovirus stability and cell attachment/viral receptor binding, providing one mechanism by which intestinal microbiota promote enteric picornavirus infection. Using LPS as a model bacterial polysaccharide, we found that poliovirus binds LPS and that the virion stabilization enhancement requires higher LPS concentrations than the virion cell attachment enhancement. Additionally, we have identified a poliovirus mutant, VP1-T99K, with reduced LPS binding. We determined that VP1-T99K poliovirus binds enough LPS to enhance cell attachment, but not enough to stabilize VP1-T99K virions. VP1-T99K poliovirus does not have replication or pathogenesis defects in vitro or in vivo, likely because virion stability is not a major selective pressure over the short time interval preceding the first cycle of viral replication in the gut. However, due to virion instability in feces from lack of microbiota-mediated stabilization, VP1-T99K has a transmission defect in mice. Taken together, these data suggest a model where picornavirus virions bind bacterial surface polysaccharides, enhancing cell attachment to promote infection and enhancing environmental stability to promote transmission to a new host.

Plenary Sessions

VIR-PL05.03 - RNA interference, viral persistence, and small RNAs

Maria-Carla Saleh[1]
[1]*Institut Pasteur, Department of Virology, Paris, France*

How persistent viral infections are established and maintained is widely debated and remains largely misunderstood. We show here that RNA virus persistence in Drosophila is achieved through the combined action of endogenous cellular reverse transcriptase activities and of the RNA interference pathway. Fragments of diverse RNA viruses are reverse transcribed early during infection, resulting in DNA forms embedded within retrotransposon sequences. These viral-retrotransposon DNA chimeras produce transcripts that are processed by the RNAi machinery, which in turn inhibits viral replication. Conversely, inhibition of reverse transcription hinders the appearance of chimeric DNAs and prevents persistence. Our results reveal a cooperative function for retrotransposons and antiviral RNAi in the control of lethal acute infection in order to reach viral persistence.

Plenary Sessions

VIR-PL05.04 - Systems Biology of Viral Infections

Joseph DeRisi[1]
[1]*University of California, San Francisco, USA*

“No abstract available at time of publication”

Thursday, 31 July 2014 13:05 - 13:45 Room 517 C-D

Bridging Plenary Sessions

BR-04.01 - The Ubiquitin Proteolytic System - From Basic Mechanisms thru Human Diseases and on to Drug Development

Aaron Ciechanover[1]

[1]*Cancer and Vascular Biology Research Center, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Between the 50s and 80s, most studies in biomedicine focused on the central dogma - the translation of the information coded by DNA to RNA and proteins. Protein degradation was a neglected area, considered to be a non-specific, dead-end process. While it was known that proteins do turn over, the high specificity of the process - where distinct proteins are degraded only at certain time points, or when they are not needed any more, or following denaturation/misfolding when their normal and active counterparts are spared - was not appreciated. The discovery of the lysosome by Christian de Duve did not significantly change this view, as it was clear that this organelle is involved mostly in the degradation of extracellular proteins, and their proteases cannot be substrate-specific. The discovery of the complex cascade of the ubiquitin solved the enigma. It is clear now that degradation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes such as cell cycle and differentiation, communication of the cell with the extracellular environment and maintenance of the cellular quality control. With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, certain malignancies and neurodegeneration among them, and that the system has become a major platform for drug targeting.

Bridging Plenary Sessions

BR-04.02 - Immunology taught by viruses

Rolf Zinkernagel[1]
[1]*University of Zurich, Zurich, Switzerland*

Immunology as a field of medical enquiry has drifted away often to turn purely academic, because the interest and appreciation of protective immunity in infectious disease medicine has been overtaken by ‘l’art pour l’art’ of so-called ‘basic immunology’. This development deprives much of immunological sciences of the biological basis and understanding that must be linked to co-evolution of infectious agents and hosts’ protective immunity. It is this co-evolutionary context that renders this field so different from studying yeast, bacteria, fibroblasts, lymphocytes or neuronal cells in splendid isolation in in vitro model situations, where everything is possible (and permitted or mistakes forgiven without repercussions) because the co-evolutionary context is ignored by too many. I shall critically review the following parameters:1) Definition of specificity; by phenolic haptens or by protective antigenic sites against infections and explain why crossprotective vaccines are an illusion. 2) The importance of antigen as the (major, only?) regulator of immunity versus the idea of regulatory T cells 3) Protective immunity by vaccines against the classical acute childhood infections (e.g. measles), by neutralising antibodies, whereas vaccines are not efficient against chronic persistent infections (e.g. TB, leprosy, HIV or malaria). 4) The importance of effector T cell class differences induced by phagocytosed antigens and that crosspresentation on to MHC class I is not efficient enough for anti-viral or for tumor immunity. 5) Affinity maturation of antibodies against poorly or cytolytic infections is too slow, and against noncytopathic agents so slow, that HIV or plasmodia escape by mutation. 6) So called immunological memory is an experimental artefact. It is the pre-existent level of protective (neutralizing) antibodies (or the number of pre-activated T cells) that determine protection. Re-stimulation of so called memory B- cells to become antibody secreting plasma cells or T cells takes 2 - 5 days and therefore is generally too slow for rapid efficient protection. In summary I conclude that we cannot do better immunologically than co-evolution if we use the same tools as evolution has been using so far. But we certainly can do better if we use new tools not used by evolution such as antibiotics, antivirals, autoantibodies or education.

Poster Session

BAM-PTH1001 - Bioethanol production from sweet potato varieties using malted Acha (Digitaria exilis) and wild Saccharomyces cerevisae in simultaneous saccharification and fermentation

Anene Moneke[1], Onyetugo Amadi[1], Tochukwu Nwagu[1], Bartho Okolo[1], Atinuke Ogunboye[1]
[1]Microbiology Department, University of Nigeria, Enugu, Nigeria

Sweet potato (Ipomoea batata) is an energy crop widely cultivated in Nigeria. Different varieties (red and white skin) were exploited for ethanol production. Acha (Digitalis exilis) an indigenous cereal crop known for its rapid growth, high yield, unique ability to tolerate poor soil and withstand draught was used as source of enzyme. Acha malt and wild yeast cells isolated from palm wine and identified as Saccharomyces cerevisae (A and B) were used in simultaneous saccharification and fermentation for ethanol production. The interior and peel parts of potato tubers produced varying ethanol yield depending on the yeast strain used, yeast strain A gave an ethanol yield of 116g/l with white skin sweet potato interior and 92.53g/l with the white skin sweet potato peel, red skin sweet potato interior and peel gave an ethanol yield of 90.85g/l and 63.3g/l respectively. On the other hand yeast strain B gave an ethanol yield of 101.95g/l and 88. 68g/l for white skin sweet potato interior and peels, while red skin sweet potato interior and peel gave 110.7g/l and 90g/l ethanol yields respectively. Considering economic situation and emphasis on the use of locally available resources, both yeast strains are able to convert sugars produced efficiently. Further more efficient hydrolysis of starch can be achieved by utilization of indigenous cereal like acha which has high enzyme activity. Interior part of the sweet potato can be available for human food and peels used as alternative waste renewable substrates.

Poster Session

BAM-PTH1003 - First description of multidrug-resistant Klebsiella pneumoniae producing CTX-M-15 - extended spectrum beta lactamase (ESBL) from pharmaceutical wastewaters in south western Nigeria.

Avemaria Obasi[1], Esther Ugoji[1], Cyril Nwachukwu[1], Christian Kohler[2], Kathrin Breitbach[2], André Göhler[2], Veronica Balau[2], Yvonne Pfeifer[3], Ivo Steinmetz[2]

[1]University of Lagos, Lagos, Nigeria, [2]Friedrich Loeffler Institute for Medical Microbiology, University of Greifswald, Greifswald, Germany, [3]Robert Koch Institute, FG13 Nosocomial Pathogens and Antibiotic Resistance, Wernigerode, Germany

Klebsiella Pneumoniae isolates habouring extended–spectrum β-lactamases (ESBLs) have emerged worldwide during the last decade, with the CTX-M-type being particularly important. This presents a major threat to public health leading to increased morbidity, mortality and healthcare expenditure. In this study we analyzed the mechanisms of resistance of Klebsiella pneumoniae isolates from pharmaceutical wastewaters in Nigeria. Bacteria were isolated from 2 pharmaceutical wastewaters, species identification were performed by standard microbiological methods and re-confirmed by VITEK 2 ID GN card. Antimicrobial susceptibilities to 21 antibiotics were tested using Vitek 2 card AST-N223. Phenotypic confirmation of ESBL production was performed by E-Test. Presence of relevant beta-lactamase genes ((blaTEM, blaSHV, blaCTX-M, blaCMY, blaDHA, blaOXA-48, blaKPC, blaVIM,) were tested by PCR and sequencing. Transfer of beta-lactamase genes was tested by broth mate conjugation. Typing of selected isolates was done by enzymatic macrorestriction and subsequent pulsed-field gel electrophoresis (PFGE). Seven Klebsiella pneumoniae isolates with multidrug-resistance to the 3rd and 4th generation cephalosporins and fluoroquinolones were identified. ESBL-phenotype was confirmed in 6 Klebsiella pneumoniae isolates. Presence of ESBL-types (CTX-M-15) (n=5) and SHV-2 (n=1) were identified by PCR and sequencing. Additionally, 4 and 5 isolates harbored TEM-type and SHV-type beta-lactamases, respectively. Using a broth mate conjugation assay, the blaCTX-M-15 and one blaTEM-type gene of two strains could be transferred into a sodium azid-resistant Escherichia coli recipient. Bacterial typing revealed that 3 Klebsiella pneumoniae isolates from 2 different sources show identical macrorestriction patterns (one clone/strain). This study showed multidrug-resistance of Klebsiella pneumoniea isolates with beta-lactamase and ESBL genes from pharmaceutical wastewaters in Nigeria, the identified beta-lactamase and ESBL genes were proven to be transferable and therefore facilitate further spread of resistance determinants in other Enterobacterial species. Continuous identification and further surveillance of the environmental reservoirs of multidrug-resistant bacteria is necessary to prevent further spread.

Poster Session

BAM-PTH1005 - The role of chirality in the efflux pump inhibiting properties of phenothiazine derivatives on the bacterial AcrAB-TolC system

Gabriella Spengler[1], Daniella Takács[2], Ádám Horváth[1], Zsuzsanna Riedl[2], György Hajós[2], József Molnár[1], Katalin Burián[1]

[1]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary, [2]Institute of Organic Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Multidrug resistance of bacteria to antibiotics has become a serious problem in the antibacterial chemotherapy and multidrug efflux pumps are known to play a role in antibiotic and biocide resistance. These membrane transporter proteins are to extrude antibiotics, biocides and other structurally unrelated compounds. Furthermore, antibiotic-sensitive bacteria that are gradually exposed to increasing concentrations of a given antibiotic can develop increasing resistance to that antibiotic. The co-therapy with efflux pump inhibitors (EPIs) could be a solution to improve the effectiveness of the antibiotics. The rational drug design should consider the chirality of the molecules because the racemic compound and its enantiomers may have different efflux pump inhibiting activity. The aim of this study was to elucidate the role of chirality of N-hydroxyalkyl-2-aminophenothiazines as effective efflux pump inhibitors by an automated method that uses the general efflux pump (EP) substrate ethidium bromide (EB) for the assessment of AcrAB-TolC system of wild-type Escherichia coli K-12 AG100. Racemic phenothiazines and their (+) and (-) enantiomers were investigated by this real-time screening method for efflux pump inhibition to characterize the AcrAB-TolC modulating properties of these derivatives. Comparison of effects of enantiomeric pairs revealed that their activities were comparable to that of the racemic derivatives. Moreover, there was no significant difference between the racemic compounds and their enantiomers related to their antibacterial and efflux pump inhibiting effects. We can assume that the chirality of resolved N-hydroxyalkyl-2-aminophenothiazines does not influence the EPI activity of the derivatives on the bacterial AcrAB-TolC efflux pump system of E. coli AG100.

Poster Session

BAM-PTH1007 - Phenotypic study of imipenem-resistant Acinetobacter baumannii strains (IRAB) isolated in an Algerian hospital

Moufida Hamidi[1], Selma Nouar[1], Nafissa Ait Hammou[1]
[1]Biology Laboratory, El Harrach Hospital, Algiers, Algeria

Introduction : Strains of imipenem-resistant Acinetobacter baumannii are responsible of severe opportunistic infections in vulnerable patients causing problems in the antibiotic treatment. Because of the multiple antibiotic resistance and persistence in the environment of these strains, outbreaks of nosocomial infections burst especially in intensive care units. In order to know the mechanisms of resistance to beta-lactam antibiotics, phenotypic study of antibiotic resistance has been undertaken in an Algerian hospital laboratory. Materials and methods : A total of 38 strains isolated mainly from ICU was selected on the basis of resistance to imipenem following antimicrobial susceptibility testing over a period from July 2012 to April 2013. As recommended by the CLSI different resistance mechanisms were investigated: - Detection of extended-spectrum beta lacatamase ESBL : synergy test between ceftazidime and ticarcillin + clavulanic acid, double disc test. - Search of cephalosporinase : cloxacillin test 1mg/ml . - Search of the carbapenemase : modified Hodge test and inhibition test by EDTA. Results: 31% of strains secreting extended spectrum beta-lactamase, the synergy test was positive in 1/ 38 and the double disc test was positive in 11/38. 85% of strains secreting cephalosporinase (Amp C) following the restoration diameters around cephalosporin after the test at 1mg/ml cloxacillin. Research carbapenemase by modified Hodge test was positive in 84 % of cases. 27 (71%) strains secretiong metallo beta lactamase probably with the EDTA test positive. Conclusion : We concluded that most of these strains have a class B carbapenemase (metallobetalactamase) probably associated in some cases with ESBL or cephalosporinases , This proves the complicity and the multitude of mechanisms of resistance to antibiotics that may accumulate Acinetobacter baumannii and be a formidable pathogen in hospitals. These results should be confirmed by genotypic extensive study and research of other non-enzymatic mechanisms (efflux and impermeability).

Poster Session

BAM-PTH1009 - WITHDRAWN - Prevalence drug resistance pattern of Mycobacterium tuberculosis in Adama referral Hospital, Adama,Ethiopia

Mohammed Suaudi Hassen[1], Habte H.Tola[2], Adunga Belawu Mekonin[3]
[1]Oromia Public Health Research Capacity Building and Quality Assurance Laboratory , Adama ,Ethiopia., [2]Ethiopian Health and Nutrition Research Institute, Addis Ababa , Ethiopia, [3]Wellega University, Nekemitt, Ethiopia

Background: Drug resistance types of tuberculosis threaten the National Tuberculosis Control Programme in several countries across the globe. Objectives: The aim of this study was to assess the magnitude of drug resistance pattern of M. tuberculosis in Adama referral hospital, Ethiopia. Methods: A cross sectional survey was conducted among new and re-treated TB patients from February 2012 to November 2013. A sputum samples from 197 patients were collected consecutively, and Drug susceptibility test (DST) was carried out by Haine life science technology line probe assay (LPA) method. Results: Out of 197 study participants 155 (78.7%) were new cases, while 42 (21.3%) were retreated. Of these, 66.5% isolates were sensitive and 4.4% resistance for two first line anti-tuberculosis drugs; (Rifampicin and Isoniazid), while the remaining 33.5% were resistance at least for one. MDR-TB was detected in 6.5 % isolates, of which 4.4% were resistance to two first line anti-TB drugs. Thirty nine (25.2 %) primary drug resistance and 3 (1.9%) primary MDR-TB cases were identified among new cases. Similarly 25 (59.5 %) cases were resistance to at least for one first line TB drug and 8 (19%) was MDR-TB among retreated cases. MDR-TB case was high among treatment failure cases, 4 (9.5%). Conclusion: Drug resistance TB, particularly MDR-TB is an emerging problem in both new and retreatment patients in the study area. Therefore, it is essential to address the problems of drug resistance through strengthen TB control and prevention program like DOTS strategy. Key words: Drug resistant, Multidrug resistant, Tuberculosis, TB treatment

Poster Session

BAM-PTH1011 - Streptomycin resistance due to target gene mutations affecting virulence in Klebsiella pneumoniae

Yu-Kuo Tsai[1], Ci-Hong Liou[2], Jung-Chung Lin[2], Ling Ma[1], Chang-Phone Fung[3], Feng-Yee Chang[2], L. Kristopher Siu[1]
[1]Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli, Taiwan, [2]Division of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, [3]Section of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University, Taipei, Taiwan

Streptomycin was introduced as a therapeutic agent in early 1940s and its resistant strains were often arised by a mutation in the rpsL gene, which encodes the ribosomal protein S12, and easily selected in vitro. However, in this study no streptomycin resistance was found to be caused by rpsL mutations in all 127 clinical strains of Klebsiella pneumoniae isolated from patients with liver abscess. By screening 107 spontaneous mutants of streptomycin resistance from a clinical strain of K. pneumoniae, a point mutation located within the rpsL was detected in each of these strains. Thirteen different mutants of the S12 protein were obtained, including nine streptomycin-dependent mutants. The virulence of all four streptomycin-resistant mutants was further evaluated. In comparing to the parental strain, the K42N, K42T and K87R mutants showed a reduction in growth rate, and the K42N and K42T mutants became susceptible to normal human serum. In the mice LD50 (the bacterial dose that caused 50% death) assay, the K42N and K42T mutants was ~1000-fold less lethal (~2×10^5 cfu) and the K87R mutant was ~50-fold less lethal (~1×10^4 cfu) than the parental strain (~2×10^2 cfu). The K42R mutant showed non-observable affects on above assays, while this mutant exhibited a small cost in an in vitro growth competition experiment. In summary, most of the K. pneumoniae strains with streptomycin resistance caused by rpsL mutation are less virulent than its parental strain in the absence of streptomycin.

Poster Session

BAM-PTH1013 - Enzymatic activity of quinolinic acid phosphoribosyltransferase from Mycobacterium tuberculosis H37Rv and inhibition of its activity by pyrazinamide

Hyun Kim[1], Shigetarou Mori[1], Emiko Rimbara[1], Keigo Shibayama[1]

[1]Department of Bacteriology II, National Institute of Infectious Diseases, Tokyo, Japan

Emergence of resistance to anti-TB drugs, in particular multidrug-resistant tuberculosis (MDR-TB), is a public health problem and poses a serious threat to global control of TB. There is an urgent need for new countermeasures against TB. Therefore, this study was aimed to define the functions of poorly characterized enzymes that may provide targets for designing new drugs to eradicate Mycobacterium tuberculosis infections, and elucidated the function of M. tuberculosis enzyme with unidentified activity to discover potential drug targets for treating TB. Quinolinic acid phosphoribosyltransferase (QAPRTase, EC 2.4.2.19) is a key enzyme in the de novo pathway of nicotinamide adenine dinucleotide biosynthesis and a target for the development of new anti-tuberculosis drugs. QAPRTase catalyzes the synthesis of nicotinic acid mononucleotide from quinolinic acid (QA) and 5-phosphoribosyl-1-pyrophosphate (PRPP) through a phosphoribosyl transfer reaction followed by decarboxylation. The crystal structure of QAPRTase from M. tuberculosis H37Rv (MtQAPRTase) has been determined; however, a detailed functional analysis of MtQAPRTase has not been published. Here, we analyzed the enzymatic activities of MtQAPRTase and determined the effect on catalysis by the anti-TB drug pyrazinamide (PZA). The optimum temperature and pH for MtQAPRTase activity were 60 °C and pH 9.2. MtQAPRTase required bivalent metal ions and its activity was highest in the presence of Mg2+. Kinetic parameters revealed as the values of Km and kcat for QA and PRPP. When the amino acid residues of MtQAPRTase, which may interact with QA, were substituted with alanine residues, catalytic activity was undetectable. Further, PZA, which is an anti-tuberculosis drug and a structural analog of QA, markedly inhibited the catalytic activity of MtQAPRTase. The structure of PZA may provide the basis for the design of new inhibitors of MtQAPRTase. These findings provide new insights into the catalytic properties of MtQAPRTase.

Poster Session

BAM-PTH1015 - Molecular insight into transcriptional regulation of AmpC beta-lactamase

Grishma Vadlamani[1], Misty Balcewich[1], Trushar Patel[1], Lynda Donald[1], Thomas Reeve[1], Jorg Stetefeld[1], David Vocadlo[2], Brian Mark[1]

[1]University of Manitoba, Winnipeg, Canada, [2]Simon Fraser University, Burnaby, Canada

Chromosomal AmpC beta-lactamase expression is a major cause of resistance against beta-lactam antibiotics in Gram-negative bacteria, including Pseudomonas aeruginosa and Enterobacteriaceae. When challenged with beta-lactams, these microbes respond by producing AmpC via the action of the transcriptional regulator AmpR. The genes for AmpR and AmpC form a divergent ampR-ampC operon, to which AmpR binds and controls the transcription of both genes. AmpR is modulated by catabolites of the peptidoglycan-recycling pathway. In the absence of beta-lactams, AmpR binds the peptidoglycan precursor UDP-MurNAc-pentapeptide and represses ampC transcription. Exposure to beta-lactams however, causes peptidoglycan recycling catabolites (1,6-anhydroMurNAc-peptides) to accumulate in the cytosol, which competitively bind AmpR and convert it into an activator of ampC transcription. The molecular interactions between AmpR, its DNA operator, and regulatory ligands, have not been described. Using small angle X-ray scattering, we found that AmpR from Citrobacter freundii (bound to a 21 bp DNA fragment of its operator) changes conformation upon binding to UDP-MurNAc-pentapeptide. Mass spectrometry analysis reveals that the AmpR:DNA complex binds four UDP-MurNAc-pentapeptide molecules, which may explain how AmpR regulates ampC expression levels: as activator ligands accumulate, they displace the UDP-MurNAc-pentapeptide ligands, increasing ampC transcription in a step-wise manner until all four sites are occupied. A 2.1Å crystal structure of the AmpR effector binding domain bound to UDP-MurNAc-pentapeptide reveals that the pentapeptide portion of the repressor molecule binds into a deep regulatory pocket on the domain, which supports a previous claim that 1,6-anhydro-MurNAc-pentapeptide is the true activator of AmpR, and not the related tripeptide species. Together, this work offers key insights into the regulatory mechanism of AmpC beta-lactamase expression, which plays a critical role in antibiotic resistance in Gram-negative bacteria.

Poster Session

BAM-PTH1017 - Suppression of stop codon UGA in acrB can contribute to antibiotic resistance in Klebsiella pneumoniae ATCC10031

Wakano Ogawa[1,3], Minako Mizusawa[1], Motoyasu Onishi[1], Tomofusa Tsuchiya[2], Teruo Kuroda[1]
[1]Okayama University, Okayama, Japan, [2]Ritsumeikan University, Kusatsu, Japan, [3]Daiichi University of Pharmacy, Fukuoka, Japan

Introduction: Emerging antibiotic-resistant Klebsiella pneumonia strains is a serious problem at clinical sites worldwide. We previously reported that K. pneumoniae MGH78578 exhibited higher resistance against various antimicrobial agents than K. pneumoniae ATCC10031. We investigated the reason why the antimicrobial-resistant levels were different between these strains. Methods: K. pneumoniae GS3 was isolated by Zorzópulos's method with a few modifications. The anti-AcrB antibody to detect AcrB from K. pneumoniae was gifted by Dr. A. Yamaguchi (Osaka University). Nucleotide substitution was performed by the method of Tomic et al with a few modifications. Results: K. pneumoniae MGH78578 possesses five plasmids. Of them, pKPN5 carries many antibiotic-resistant genes. To investigate the contribution of pKPN5 to the drug-resistant phenotype, we transformed K. pneumoniae ATCC10031 with pKPN5. The MICs of various antibiotics were significantly elevated in K. pneumoniae ATCC10031/pKPN5. Meanwhile, K. pneumoniae GS3, a pKPN5-removed strain from K. pneumoniae MGH78578 showed decreased resistance to kanamycin, tetracycline, chloramphenicol, and ampicillin. Therefore, we concluded that pKPN5 played a prominent role in the multidrug-resistant phenotype in K. pneumoniae MGH78578. However, the difference of resistant levels to dyes and detergents between these strains was impossible to explain with only pKPN5. By sequencing, we identified a nonsense mutation on acrB from K. pneumoniae ATCC10031, and concluded that the lack of AcrAB caused the hypersensitivity to various antimicrobial agents in K. pneumoniae ATCC10031. On the other hand, we appeared that the intact AcrB was produced in K. pneumoniae ATCC10031 despite the stop codon, though its quantity was small. Therefore, we concluded that the stop codon in acrB was overcome to some extent in K. pneumoniae ATCC10031 and the AcrB slightly elevate resistance to antimicrobial agents.

Poster Session

BAM-PTH1019 - Plasmids carrying tet genes in clinical and environmental samples

Livia Balsalobre[1], Milena Dropa[1], Mariana Ponte[1], Samara Alves[1], Glavur Matté[1], Maria Helena Matté[1]
[1]*University of São Paulo, São Paulo, Brazil*

The extensive use of tetracycline class of antibiotics has contributed to the emergence of resistance bacteria isolated from both environmental and clinical sources, leading to the selection of a great number of genetic determinants called "the tetracycline resistome". Aiming to verify the presence of mobile structures and the plasmidial location of tet genes, the elements Tn1721, Tn10 and IS26 were searched in 335 strains, divided in environmental strains: 23 Klebsiella pneumoniae and 95 Escherichia coli; clinical strains: 95 Klebsiella pneumoniae and 122 Escherichia coli. Transformation experiments using One Shot® Top 10 Electrocompetent cells were performed in selected strains. Sixty-eight strains were resistant to TET, 25 were carrying tetA, of which 18 demonstrated the gene associated with Tn1721 while 7 presented the entire mobile structure searched; 27 strains possessed the tetB gene of which 25 were associated with Tn10 and possessed the entire structure; 20 strains had tetD gene and presented the entire structure associated with the gene. Five strains were selected for transformation experiments: of four strains two were carrying tetA and the other two tetB, both groups had strains isolated from human stool and sewage samples; and one strain isolated from a nosocomial sample was carrying tetD. All plasmids were confirmed to be from IncF incompatibility group over 30kb and the presence of the respective tet gene was checked by PCR. The presence of the tet genes in plasmids demonstrates the ability to disseminate these genes through horizontal transfer facilitated by the transposons detected in the same strains, which also encourages new studies regarding the distribution of tet genes in food, water, soil and even clinical samples. Since we are facing an antibiotic resistance era it is important to monitor antibiotics previously determined as obsolete, consequently determining the role of this class of antibiotics in the worldwide resistance scenery.

Poster Session

BAM-PTH1021 - Antimicrobial resistance profiles and virulence factors of Escherichia coli isolates from urinary tract infections in Mexico

Flor Ramirez Castillo[1], Francisco Márquez-Díaz[2], Philippe Garneau[3], Francisco Avelar-González[1], Alma Guerrero-Barrera[1], Josée Harel[3]

[1]Universidad Autonoma de Aguascalientes, Laboratorio de Biología Celular y Tisular, Aguascalientes, Mexico, [2]Departamento de Infectología, Centenario Hospital Miguel Hidalgo, Aguascalientes, Mexico, [3]Centre de Recherche en Infectiologie Porcine, Faculté de médecine vétérinaire, Université de Montréal, Canada

Uropathogenic Escherichia coli (UPEC) is a major pathogen causing urinary tract infections (UTI), one of the most common bacterial infections worldwide. Antibiotic resistant E. coli complicate the management of the infection. This study determined the virulence genes, phylogroups and antimicrobial resistant profile of 110 E. coli isolates patients with urinary tract infections from Mexico. Antibiotic susceptibility towards 20 different antibiotics was performed. The presence of plasmid-media quinolone resistance genes as well as the mutations in GyrA and ParC proteins was investigated. An E. coli specific microarray was used to characterize the virulence and antimicrobial resistance genes profiles of the 18 strains. Sixty-seven percent of the isolates were multi-drug-resistant. A frequent occurrence of resistance was observed toward fluoroquinolones (46%) and cephalosporins (31%). Ten qnrA, 1 qnrB, and 7 aac(6')lb-cr genes were confirmed. GyrA mutations at codons Ser-83 and Asp-87, and ParC mutations at codons Ser-80 were observed in 94% (17/18) of the strains tested. Moreover, other antimicrobial resistance genes such as aph3 (strA), sulII, mphA, tet(B) and class 1 integron were also found in quinolone resistant isolates. Cephalosporin resistant isolates showed positive PCRs for blaCTX-M (9.1%), blaTEM (15.4%), blaPSE (1.8%), and blaOXA (8.1%). Sequence of blaCTX-M showed the presence of blaCTX-M-15, blaCTX-M-3, and blaCTX-M-12. Seven major phylogroups and cryptique clades were found, with phylogroup D (23.6%) and B2 (15%) being the most common. Among virulence genes, strains that carry the fyuA, chuA, and yfcV genes combination, carried also the higher proportion of virulence genes, and were associated with the phylogroups B2, D and F (P = 0.023). Antibiotic susceptibility test must be carried out prior treatment on pathogens isolates of patients with UTIs in order to avoid the spread of resistant bacteria. This study highlights the high occurrence of UPEC multi-drug resistant to antibiotics usually used in UTIs in Mexico.

Poster Session

BAM-PTH1023 - Combating quinolone resistance in bacteria using sRNA screens

Kamya Bhatnagar[1], Alex Wong[1]

[1]*Department of Bology, Carleton University, Ottawa, Canada*

Antibiotic resistant bacteria are increasingly commonly and, are responsible for major challenges to healthcare. Therapeutic and public-health efforts to combat resistance have met with mixed success, and new approaches are needed. Here, we propose to develop new methods aimed at reversing antibiotic resistance. We intend to focus on gyrase-mediated quinolone resistance in Escherichia coli. We are developing a small RNA (sRNA) screen to identify genes that reverse quinolone resistance in gyrA mutants. Bacterial sRNAs modulate gene expression through antisense interactions with mRNAs and either activate or repress translational efficiency. Therefore, by randomizing the antisense domain of a known sRNA, an artificial library of sRNAs will be obtained, allowing us to identify new auxiliary genes whose knockdown reverses gyrase mediated antibiotic resistance. This approach should allow for the development of new therapeutic approaches for combating antibiotic resistance.

Poster Session

BAM-PTH1025 - Investigating compensatory evolution in quinolone resistant Escherichia coli

Ahlam Alsaadi[1], Alex Wong[1]
[1]Carleton University, Ottawa, Canada

Antibiotic resistance is a major threat to public health, undermining our ability to treat infectious disease. Often, isolates bearing resistance mutations suffer a cost of fitness, that is, a lower growth rate than their susceptible counterparts. Nonetheless, fitness can be ameliorated by secondary site mutations, known as compensatory mutations. These mutations restore fitness to the normal level without eliminating resistance. Despite the potential importance of compensation for public health strategies to combat antibiotic resistance, relatively little is known about the molecular mechanisms of compensation. Here, we investigate mechanisms of compensation for quinolone resistance mutations in Escherichia coli. We found substantial costs of resistance for two genotypes, derived from MG1655 (K-12): a gyrA D87G mutant, and a marR R94C mutant. Subsequent selection in the absence of antibiotics has led to an improvement in fitness. Whole-genome sequencing will be used to identify potential compensatory mutations. This work will provide insight into the mechanisms of compensation of the costs of quinolone resistance in Escherichia coli.

Poster Session

BAM-PTH1027 - Whole genome sequencing of members of the *Staphylococcus sciuri* group

Sae Tsubakishita[1], Kyoko Kuwahara[2,3], Takashi Sasaki[3], Tadashi Baba[2,3], Tatsufumi Takahashi[4], Naoya Kikuchi[4], Keiichi Hiramatsu[2,3]

[1]Department of Veterinary Science, Rakuno Gakuen University, Ebetsu, Japan, [2]Department of Microbiology, Juntendo University, Tokyo, Japan, [3]Department of Infection Control Science, Juntendo University, Tokyo, Japan, [4]Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu Japan

Methicillin-resistant *Staphylococcus* (*S.*) *aureus* (MRSA) is one of the most important multidrug-resistant pathogen of human and animals. MRSA is born when methicillin-susceptible *S. aureus* (MSSA) exogenously acquired a methicillin-resistance gene '*mecA*' carried by a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*). SCC*mec* is excised from the chromosome and site-specifically integrated in *orfX* gene near the replication origin (*oriC*) by the action of site-specific recombinase encoded by *ccr* gene. SCC*mec* has been identified not only in *S. aureus* but also widely in other staphylococci. SCC*mec* is transmissible among staphylococcal species, but its origin has not been clarified since the discovery of MRSA in 1961. Recently, we found the origin of *mecA* on *S. fleurettii* chromosome. *S. fleurettii* classified as a species of *S. sciuri* group which are commensal bacteria of animals. To investigate the origin of SCC*mec*, we performed the whole-genome sequencing of *S. fleurettii* JCSC9018, *S. sciuri* subsp. *carnaticus* JCSC9016 and *S. lentus* JCSC9019, which were methicillin-resistant strains of *S. sciuri* group. The analyses were carried out with hybrid assemblies using Roche454, Illumina and Sanger method. The chromosome sizes ranged from 2.6 to 2.8 Mbp. *S. fleurettii* JCSC9018 and *S. sciuri* subsp. *carnaticus* JCSC9016 harbored 5 distinct sizes of plasmid. The *mecA* of *S. fleurettii* JCSC9018 was approximately 235 kbp downstream of *orfX*, which was not associated with *ccr* genes. On the other hand, the *mecA* genes of *S. sciuri* subsp. *carnaticus* JCSC9016 and *S. lentus* JCSC9019 were located inside the SCC*mec* inserted just downstream of *orfX*. In comparison with *S. sciuri* subsp. *carnaticus* JCSC9016 and *S. lentus* JCSC9019, *S. fleurettii* JCSC9018 chromosome and plasmids contain numerous predicted mobile genetic elements, including insertion elements, transposons mediating resistance to antibiotics and *ccr* genes. These findings may contribute to elucidation of the birth of SCC*mec* on the *S. fleurettii* chromosome.

Poster Session

BAM-PTH1029 - Mutations in global regulatory proteins confer resistance to fluoroquinolone and aminocoumarin antibiotics by altering DNA topology in Salmonella enterica

Stefani Kary[1], Charles Dorman[2], Andrew Cameron[1]
[1]Department of Biology, University of Regina, Canada, [2]Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin, Ireland

Fluoroquinolone and aminocoumarin antibiotics, such as ciprofloxacin and novobiocin, are widely used for the treatment of *Salmonella* infections. These antibiotics cause relaxation of DNA supercoiling by inhibiting DNA gyrase, which is normally deleterious to cell function. The global regulatory proteins FIS, CRP, and RpoS contribute to the maintenance of DNA supercoiling levels in the cell, leading us to test whether loss of these regulatory proteins could exacerbate or possibly reduce antibiotic sensitivity in *Salmonella*. We measured growth and DNA supercoiling in Salmonella enterica Δ*fis*, Δ*crp*, and Δ*rpoS* mutants at sub-inhibitory concentrations of ciprofloxacin (15 and 25 ng/mL) and novobiocin (15 and 25 μg/mL). Surprisingly we found that the loss of global regulatory proteins made cells more resistant to antibiotics, which correlated with higher levels of DNA supercoiling in mutants compared to wild-type. The degree of antibiotic resistance differed between mutants; for example, Δ*crp* and Δ*rpoS* doubled faster and reached higher density than wild type in 15 ng/mL ciprofloxacin, while only Δ*crp* was capable of growing in 25 ng/mL ciprofloxacin. Because osmotic pressure directly influences DNA supercoiling levels, we tested antibiotic resistance at moderate (0.5%, and 1% NaCl in LB) and low (0% NaCl in LB) osmotic pressures. Curiously, increased osmotic pressure made all cell types more resistant to novobiocin but more sensitive to ciprofloxacin. Importantly, all three mutants consistently grew faster than wild type when exposed to 15 or 25 μg/mL novobiocin at low salt concentrations, indicating that sub-inhibitory concentrations of antibiotics can actually improve bacterial fitness in some conditions.

Poster Session

BAM-PTH1031 - KHP30-like phages among clinical isolates of Helicobacter pylori

Iyo Takemura-Uchiyama[1], Jumpei Uchiyama[2], Hiroaki Takeuchi[2], Keiji Gamoh[2], Yoshihiko Sakaguchi[3], Shin-ichiro Kato[2], Takako Ujihara[4], Masanori Daibata[2], Shigenobu Matsuzaki[2]

[1]Kochi University Graduate School, Kochi, Japan, [2]Research and Education Faculty, Kochi University, Kochi, Japan, [3]Interdisciplinary Research Organization, University of Miyazaki, Miyazaki, Japan, [4]Scientific Research Center, Kochi University, Kochi, Japan

Aim: Helicobacter pylori bacteriophage (phage) KHP30 has been isolated from strain NY43, in which is maintained as an episome (i.e., pseudolysogeny). The phage KHP30 is genetically homologous to all the other reported H. pylori phages. KHP30-like phage sequences were found in some H. pylori genomes. Ecological aspects of H. pylori and KHP30-like phages are still in mystery. We here estimated the distribution of KHP30-like phage in H. pylori strains, and KHP30-like phage integration was studied. Material and methods: Phage was screened by both culture-based method and PCR targeting the KHP30-viron genes (i.e., orfs 11, 13, 14 and 15), using clinical isolates. After a passage culture of the strain NY43, the phage harboring of the clones was examined. Using newly-sequenced and previously-reported genomes, the KHP30-like phage integration was examined. Results: (I) The active KHP30-like phages were present at only 1.1% among the tested H. pylori isolates (2/177 strains). (II) Only 21.4% of the NY43 clones maintained phage-harboring trait (21/98 clones). The KHP30-lost clones grew faster than KHP30-harboring clones. (III) Based on the results I & II and the biased bacterial isolation in the clinical laboratory (i.e., larger colonies are usually picked up), the phage was estimated to be initially distributed more than 19.6%. (IV) Screening the integrated phage sequence (orf11) among the isolates, the KHP30-like phage sequence was detected at 98.3% (174/177 strains). (V) Studying the phage integration, the KHP30-like phages were assumed to have integrated into any of four genome plasticity regions, and then have been recombined possibly by transposase and restriction-modification system. (VI) Based on the results IV & V, the KHP30-like phage was considered to be highly associated with the host evolution. Conclusion: The KHP30-like phage was estimated to be moderately distributed in H. pylori isolates initially, and they have contributed to the H. pylori genetic evolution.

Poster Session

BAM-PTH1033 - Targeting intracellular bacteria with bacteriophages

Anita Nieth[1], Cyprien Verseux[2], Winfried Römer[1]
[1]BIOSS-Centre for Biological Signalling Studies, University of Freiburg, Germany, [2]Tor Vergata University, Rome, Italy

Bacterial resistance to antibiotics has become a severe problem during the past decades and multiple-resistant bacterial strains are seriously threatening public health. Hence, alternative treatments are urgently needed and in this context, bacteriophages are experiencing a renaissance of interest. Bacteriophages have been regularly used as antibacterial treatment in the former Soviet Union, but were not considered worth investigating since the advent of chemical antibiotics in the West. Mycobacterium tuberculosis, the causative agent of tuberculosis and responsible for yearly over 1 million deaths worldwide, has also developed multidrug-resistant strains. Mycobacteriophages could be a promising alternative or supplement to side-effect rich and little effective second-line antibiotics for treatment of these resistant strains. But as pathogenic mycobacteria lead an intracellular life-style and bacteriophages do not pass biological membranes, they need to be vectorized in order to reach the inside of infected cells in a Trojan horse-like manner. The main objective of the here presented project is the in vitro-development of a liposome-based bacteriophage therapy against pathogenic mycobacteria. We have encapsulated bacteriophages into liposomes by different techniques and bacteriophage-loaded liposomes are taken up by macrophages which are the main target of Mycobacterium tuberculosis. Pathogenic mycobacteria persist within macrophages in a specialized mycobacterial phagosome. They inhibit phagosome-lysosome fusion but allow fusion with early endosomes. Indeed, our results show that liposome-delivered bacteriophages co-localize with early and late endosomes as well as lysosomes and hence seem to enter the cell via the endosomal-lysosomal pathway. Like this they have the chance to directly encounter their target bacteria upon uptake.

Poster Session

BAM-PTH1035 - Estimation of the level of antiphage antibodies in human sera using ELISA technique

Maciej Zaczek[1], Marzanna Lusiak-Szelachowska[1], Beata Weber-Dabrowska[1,2], Barbara Owczarek[1], Beata Dziedzic[1], Ryszard Miedzybrodzki[1,2], Wojciech Fortuna[1,2], Andrzej Gorski[1,2,3]

[1]Laboratory of Bacteriophages, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland, [2]Phage Therapy Unit, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland, [3]Department of Clinical Immunology, Transplantation Institute, Medical University of Warsaw, Warsaw, Poland

Since bacteriophages were discovered almost a hundred years ago there have been attempts to make use of their properties for therapeutic purposes. Nowadays, due to the antibiotic treatment crisis there is growing interest in phage therapy, and therefore it is necessary to conduct investigations, including those that go beyond only the antibacterial effect of phages. The aim of our project was to assess human serum antibacteriophage activity. Investigations included the antibacteriophage activity of sera of patients treated with phages in the Phage Therapy Unit of the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy and healthy subjects. Two groups of patients (treated with the S. aureus A3/R phage or S. aureus 676/Z phage) were examined. Sera of 20 healthy blood donors came from the Blood Donation Center, Clinical Military Hospital in Wroclaw, Poland. We found that sera of examined patients undergoing phage therapy indicated higher levels of antiphage antibodies during treatment (oral or local administration) in about half of the cases. Furthermore, in some cases that level was even insignificantly lower than before treatment. Patients treated with S. aureus 676/Z phage showed a greater immune response during phage therapy ($p<0,05$) than those treated with S. aureus A3/R phage. In some cases a level of antiphage antibodies did not occur together with a level of phage neutralization by human sera (K rate) measured by neutralization test. Studies on the way of phage application, level of antiphage antibodies and phage neutralization by sera in patients subjected to phage therapy have enormous practical potential. It is necessary to continue the research for better understanding of the effect of phage treatment. This study is conducted within grant " Innovative bacteriophage preparation for the treatment of diabetic foot" (POIG.01.03.01-02-048/12) funded by The National Centre for Research and Development.

Poster Session

BAM-PTH1037 - Bacteriophages in human environment

Aneta Kurzepa-Skaradzinska[1], Kinga Wapinska[1], Marta Kuzminska-Bajor[1], Grzegorz Skaradzinski[1]
[1]*Wroclaw University of Environmental and Life Sciences, The Faculty of Food Science, Department of Biotechnology and Food Microbiology, Wroclaw, Poland*

Bacteriophages are considered to be most ubiquitous biological beings on our planet. They are present everywhere in the environment, including ecosystems with extreme environmental conditions, as well as human organisms. Human environment is full of bacteria being potential hosts for bacteriophages. In our studies, we investigated the presence of bacteriophages in human environment. We selected 10 objects in human daily environment which are considered to be the main sources of bacteria: (1) fridge (2) mobile phone (3) computer keyboard (4) coin (5) tv remote (6) toilet (7) sponge for the dishes (8) waste container (9) trolley in supermarket (10) button for door opening in public transport. We took samples from three of each objects. From each sample we isolated bacteria and bacteriophages. Bacteria were identified with BBL Crystal Identification System (BD Biosciences, USA). 52 of isolated bacteria strains were gram-positive, 8 were gram-negative. We isolated 3 bacteriophages specific for isolated bacteria. Two of them (TO1+6f; TO1+7f) were specific for bacteria isolated from toilet (Enterobacter cloacae) and one bacteriophage (LO5+1f) was specific to bacteria isolated form fridge (Staphylococcus uberis). Bacteriophage specific for bacteria isolated from fridge was also isolated from fridge. One of bacteriophages specific for bacteria in toilet was also isolated from toiled (TO1+6), second phage specific to this bacteria was isolated from sponge for the dishes (TO1+7). We also studied influence of different temperatures and pH on activity of isolated phages. Bacteriophages TO1+6f and LO5+1f showed the highest activity after incubation in low temperatures (-20˚C, +4˚C), while bacteriophage LO5+1f showed the highest activity in the highest temperature (+50˚C). Optimal pH for phages TO1+7f and LO5+1f was neutral and slightly alkaline. Interestingly, phage TO1+6 retain high titer in pH=11.

Poster Session

BAM-PTH1039 - Functional diversity of the rhizosphere microbiome in petroleum-hydrocarbon contaminated soils

Fahad Alotaibi[1], Terrence Bell[1], Etienne Yergeau[2], Mohamed Hijri[1], Marc St-Arnaud[1]
[1]Department of Biological Sciences, Montreal, Canada, [2]National Research Council of Canada, Montreal, Canada

Plant-associated bacteria are important for the growth and health of their host, but little is known about the functional diversity of these bacteria, particularly in the context of phytoremediation. We studied bacterial functional diversity in the rhizosphere of willows (Salix spp.) growing in petroleum-hydrocarbon contaminated soils, as well as in non-contaminated soils. Our hypothesis was that functional gene composition would vary based on plant identity and contaminant level, as was observed previously when looking at the taxonomic composition of the microbial communities (bacterial 16S rRNA and fungal ITS). In this study, we used high-throughput 454 pyrosequencing of two key functional genes related to petroleum hydrocarbon biodegradation, namely biphenyl dioxygenase (BphA) and alkane monoxygenase (alkB), as well as nitrogen fixation genes (nifH), which play a key role in determining the amount of nitrogen available to both microorganisms and plants. Preliminary analysis of our sequence data suggests that distinct bacterial populations of petroleum-hydrocarbon degrading bacteria and N-fixers where present across willow rhizospheres and bulk soil. This project is part of GenoRem, a collaborative initiative that is funded primarily by Genome Canada, that aims to gain an integrated understanding of the relationships between fungi, bacteria, plants, and soil to enable the design of treatments that promote effective bioremediating communities.

Poster Session

BAM-PTH1041 - Next-generation sequencing analysis of pathogenic bacteria in water samples from the Hokkaido University campus

Jednipit Borthong[1], Ryo Nakao[1], Yongjin Qiu[1], Aiko Ohnuma[1], Manabu Igarashi[1], Chihiro Sugimoto[1], Orasa Suthienkul[2], Kimihito Ito[1]

[1]Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan, [2]Department of Microbiology, Faculty of Public Health, Mahidol University, Bangkok, Thailand

Bacteria are abundant in a wide range in community of aquatic environments, playing important roles for ecological systems. Most of them are harmless in nature but some of them pose potential risks for human and animal diseases, and become to the problematical concern in worldwide levels. Although the culture-based methods and molecular techniques such as PCR-based assays are commonly used worldwide to confirm the presence of pathogens in aquatic reservoirs. These methods have faced the limitations such as time-consuming, labor intensiveness, and miss-identification due to low discriminatory power between closely related bacteria. In order to establish the effective method for detecting pathogenic bacteria causing water-borne diseases, a highly specific and sensitive assay should be developed. Recently, the advance in sequencing technologies has enabled metagenomic analysis for a variety of environmental samples including water. Therefore, the present study explored the possibilities of metagenomic approach for detecting pathogens in water samples from Sakusyukotoni stream, which is located in the Hokkaido University campus. The genetic material was extracted and a highly variable region of 16S rRNA gene was consequently amplified. The 454 pyrosequencing was used to discover the communities of bacteria including pathogenic ones. The results indicated that the bacterial genera related with infectious diseases were detected from this natural environment following Acinetobacter, Aeromonas, Arcobacter, Bacillus, Brevudimonas, Campylobacter, Chitinophaga, Chryseobacterium, Clostridium, Corynebacterium, Enterococcus, Erwinia, Helicobacter, Legionella, Mycobacterium Pseudomonas, Serratia, Sphingomanas, Staphylococcus, Stenotrophomonas, and Streptococcus. Additionally, typical bacteria genera were also discovered from these samples. In conclusion, this assay demonstrated the high possibility of detecting pathogenic bacteria and allows us to educate the presence of non-culturable bacteria in aquatic environments. We highly recommend the use of metagenomic strategy for detecting pathogenic bacteria in aquatic environments.

Poster Session

BAM-PTH1043 - Degradation of polystyrene by microorganisms

Claudia Figueroa[1], Alma Cruz[1], Gerardo Ramírez[1]
[1]*UAM, Mexico City, Mexico*

Polystyrene (PS) is one of the most used synthetic plastics due its excellent thermal insulation properties, however, it is poorly biodegradable. Synthetic plastics are not usually biodegradable due to the complexity of their structure, high molecular weight and hydrophobic nature; however some microorganisms have the ability to degrade plastics, like polystyrene. The aim of the present study was to evaluate the ability of Bacillus subtilis, Rhodococcus fascians, Pseudomonas aeruginosa, Micrococcus luteus and Aspergillus niger on polystyrene degradation. In order to evaluate the polystyrene degradation potential of the microorganisms, the CO2 produced during four days of each of the evaluated strains, was quantified by the Sturm test. Minimum media supplemented (MM) with PS as test media, MM supplemented with PS without inoculum as a negative control. The cumulative CO2 generated during four days of test with each of the strains was used in order to calculate the percent of degradation (OECD) and percent of mineralization. Subsequently, it was quantified the CO2 produced by the growth of Rhodococcus fascians during three months. It was observed that Rhodococcus fascians and Pseudomonas aeruginosa have higher rates of mineralization (≥ 1%) in four days. It was observed that the percent of mineralization of PS by the growth of a microbial consortium was 1.5 % in three months. Rhodococcus fascians was chosen for carrying out degradation of polystyrene for three months. It was observed that the cumulative CO2 produced by the microorganism by MM was 15 mg in three months. However, the microorganism has the ability to use the supplemented PS as a carbon source and generated 30 mg of CO2 in three months. That confirms the potential of PS degradation of Rhodococcus fascians. The evaluated strains have the ability to use PS as carbon source. Rhodococcus fascians and Pseudomonas aeruginosa showed the highest degradation rates.

Poster Session

BAM-PTH1045 - The abilities of four species of Nigerian aquatic phycomycetes to utilize petroleum and petroleum products as sole carbon sources

Collins Odu[1], Chike Ogbonna[2], John Egbere[3], Chukwu Chukwu[4]

[1]Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria, [2]Applied Microbiology and Biotechnology Research Unit, Dept of Plant Science and Technology,University of Jos, Jos, Nigeria, [3]Department of Microbiology, University of Jos, Jos, Nigera, [4]Molecular Biology Department, FCVMLT, VOM, Jos, Nigeria

Studies were carried out on the abilities of four species of aquatic phycomycetes isolated from crude oil polluted marine , brackish and fresh water environments in Nigeria to utilize refined petroleum and petroleum products as sole sources of carbon. The said species of fungi included Brevilegnia indica,Protoachyla paradoxa,Saprolegnia bhargavi and Thraustotheca clavata. They were grown on fungal culture media which contained mineral salts solution, refined petroleum, kerosene and diesel as sole sources of carbon and agar as a solidifying agent. Two concentrations of each the resulting oil agar media were used vis 1% and 2%. The resultant medium was then used to culture each of the test fungi in triplicates. The resultant culture plates were then incubated at 25oC and left for daily observation. The test fungi that grew on each medium were observed for their abilities to emulsify the refined petroleum or its products (diesel and kerosene). Control experiments were also set up using Malt Extract Agar medium Brevilegnia indica grew on 1% petroleum and diesel growth media but did not grow on kerosene medium. Protoachyla paradoxa grew minimally on diesel medium at 1% and 2% compositions. Saprolegnia bhargavi did not grow on the petroleum agar medium or petroleum products media. Thraustotheca clavata grew minimally on the kerosene medium at both 1% and 2% compositions. The four test fungi emulsified the diesel agar medium at both 1% and 2% compositions. Brevilegnia indica emulsified only petrol at 1% while Non of the isolates emulsified the kerosene medium at both 1% and 2% compositions. Oil globules were also observed in the vegetative hyphae of the test fungi which grew on the experimental culture media. This means that such fungi either degraded or accumulated the petrol or petroleum products in their systems.

Poster Session

BAM-PTH1047 - Investigation of the recent microbial degradation of the skin of the Chinchorro mummies of ancient Chile

Alice DeAraujo[1], Archana Vasanthakumar[1], Marcela Sepulveda[2], Ralph Mitchell[1]
[1]Harvard University, Cambridge, USA, [2]University of Tarapacá, San Miguel de Azapa, Chile

Chinchorro mummies are the oldest recovered mummies dating back to 7020 B.C. These mummies, which were recovered from the Atacama Desert, are currently stored, with minimal environmental control, in the Universidad de Tarapacá, 15 kilometers from the Pacific coast in northern Chile. Accelerated deterioration of some Chinchorro mummies has recently been documented. Blackening and oozing of some areas of their remaining skin is posing a threat to the collection. The local climate has changed in recent years. Incoming fog from the Pacific coast has become denser and consequently, the humidity has increased. We hypothesized that the changes occurring in the environment, coupled with the presence of opportunistic microbes, might have caused the recent and unexpected discoloration of the mummies' skin. We have isolated microorganisms from diseased Chinchorro mummy skin samples and identified them as common environmental microorganisms by sequencing the 16S and 18S Internal Spacer gene. Human skin is primarily composed of collagen and keratin. Preliminary tests showed the ability of some isolates to utilize collagen and/or keratin as the sole carbon source when incubated in basal salts medium. Due to the sensitivity and cultural value of the Chinchorro mummies, we are currently analyzing and quantifying collagenase activity of the isolates grown on pig skin (which is highly similar to human skin). Our investigation is an important step towards gathering microbiological data, studying the biochemical processes underlying, and investigating the environmental conditions required for the microbial degradation of the Chinchorro mummies' skin. The assembly of such data will enable us to identify methods of proper storage in controlled environments, so as to minimize biodeterioration of this significant archeological collection.

Poster Session

BAM-PTH1049 - Isolation and oxygen-tolerant domestication of obligate anaerobic isoflavone biotransforming bacteria

Xiu-Ling Wang[1], Meng Li[1], Yan-Jing Xie[1], Lu Yang[1]
[1]College of Life Sciences, Agricultural University of Hebei, Baoding, China

Isoflavone biotransforming bacteria (IBB) are specific gastrointestinal bacteria responsible for the production of different isoflavone metabolites which are more biologically active than isoflavones. Studies in humans have suggested that individuals capable of producing isoflavone metabolites might be associated with a reduced risk of certain diseases. However, with the exception equol and dihydrodaidzein, all the other isoflavone metabolites can not be synthesized yet. In recent years, numerous IBB have been isolated and identified. These isolated IBB make it possible to biosynthesize different isoflavone metabolites. However, all of the reported IBB can grow and exert their biotransforming activities only under anaerobic conditions. In order to improve the oxygen-tolerant capacity of the obligate anaerobic IBB, we carried out a long-term oxygen-tolerant domestication process. At present we have successfully obtained two oxygen-tolerant bacterial strains, i.e. bovine rumen bacterium Sharpea sp. strain Aeroto-Niu-O16 capable of bioconverting isoflavones daidzein and genistein to dihydrodaidzein and dihydrogenistein respectively (AMB, 2011, 92:803-813) and cock intestinal bacterium Clostridium sp. strain Aeroto-AUH-JLC140 (submitted). The two obtained oxygen-tolerant strains share many characteristics in common, such as change in bacterial shape and in biochemical traits (from indole negative to indole positive and from amylohydrolysis positive to negative). However, obvious differences between these two oxygen-tolerant mutants also exist (for example, strain Aeroto-AUH-JLC140 obtained the capability to produce H2S gas). Moreover, in order to further improve the oxygen-tolerant capacity and yield of the product by strain Aeroto-Niu-O16, we carried out polyethylene glycol (PEG)-assisted protoplast fusion between non-heat treated protoplasts of strain Aeroto-Niu-O16 and heat-inactivated protoplasts of Escherichia fergusonii strain M6. One fusant, which we named AUH-RHZ-1, was obtained. The fusant strain showed more similarity or identity to the non-heat treated parental strain Aeroto-Niu-O16 (submitted).

Poster Session

BAM-PTH1051 - New biohybrid materials for bioremoval of oil from water surface

Svetlana Vasilieva[1], Elena Lobakova[1], Polina Kashcheeva[2], Galina Dol'nikova[1], Ekaterina Ivanova[2], Elena Belousova[2], Aleksey Dedov[2]

[1]Moscow State University after M.V. Lomonosov, Moscow, Russia, [2]Russian State University of Oil and Gas after I.M. Gubkin, Moscow, Russia

Immobilized microorganisms have been used for oil treatment for several years. Successful immobilization of bacteria with maintaining their biodegrading activity in case of nutrient lack is often elusive. The new bio-hybrid materials (BGM) obtained by immobilization of oil-degraders on different polymeric matrixes were investigated. Polymeric matrixes of different chemical compositions: polypropylene (PP), polyester (PE), copolymer methylmethacrylate-acrylonitrile (CMMA) were used with oil adsorbing capacity ranging from 15 to 40 g/g (oil/matrix). Nutrient lack is limiting factor for oil–biodegradation in the marine environment. We used water plants (duckweed) - Wolffia arrhiza and Lemna minuscula – as the sources of nutrient for maintaining bacterial viability and oil-degrading activity. Dried whole cells and cell walls of the plants were incorporated into CMMA during the fiber formation. The additive (AD) content ranged from 15 to 30% of CMMA+AD weight. Both artificial and indigenous bacterial associations efficiently immobilized on surface and inner fibers of all polymeric matrixes under lab or field conditions. The bacterial adsorptive capacity of the matrixes was 0,2 - 0,7 g/g (cels/matrix) and the best results were obtained with CMMA+AD. Oil-degradation experiments were carried out in artificial sea water (lacking N and P) containing crude oil (1% vol.). BGMs containing the association of Rhodococcus qingshengii and Leucobacter aridicollis - were tested. After 10 days of treatment, oil content in water decreased by 90-98% as compared to control. After 25 days, oil content in the BGMs made from PP, PE and CMMA were 80-90% and in the case of CMMA +AD – 20-25%. Evidently, under nutrient deficiency conditions the oil-degrading bacteria more vigorously oxidized the oil adsorbed in BGM with the duckweed plant cell structures incorporated. The latter served as a nutrient source for bacteria and contributed to enhancing their oil-destruction ability.

Poster Session

BAM-PTH1053 - Inducible trichloroethylene degradation system in Rhodococcus jostii RHA1

Naoto Araki[1], Kanako Chino[1], Kenta Yonezuka[1], Daisuke Kasai[1], Masai Eiji[1], Masao Fukuda[1]
[1]Nagaoka Univ. Tech., Niigata, Japan

Trichloroethylene (TCE), which had been widely used for industrial purposes, is a major environmental pollutant. A number of aerobic and anaerobic bacteria have been isolated worldwide for the bioremediation of TCE. Most of the aerobic bacteria utilize hydroxylating oxygenases (HOs) such as methane, phenol, and toluene monooxygenases and toluene dioxygenase for cometabolic TCE decomposition. To express these HOs, inducing substances specific for respective HOs are required. In this study, we examined the TCE degradation system of an aerobic biphenyl-degrading bacterium, Rhodococcus jostii RHA1. While RHA1 cells grown on succinate in the absence of TCE depleted 10 mg/L TCE within five days, those grown in the presence of TCE depleted 10 mg/L TCE within three days. Transcriptome analysis using RNAs extracted from RHA1 cells incubated for 24 hours in the presence or absence of 0.5 mM TCE revealed that biphenyl degradation genes (bhp/etb genes) were upregulated in the presence of TCE. A double mutant of bphAc and etbAc encoding ferredoxin component of biphenyl dioxygenase lost the TCE degradation activity. When biphenyl dioxygenases BphA and EtbA were expressed individually in a heterologous host, Rhodococcus erythropolis IAM1399, both the recombinants degraded TCE. These results indicate that both BphA and EtbA are responsible for TCE degradation in RHA1. In RHA1 two sets of bphS and bphT encoding a sensor kinase (BphS) and a response regulator (BphT) of a two-component regulatory system, respectively, positively control the transcription of the bhp/etb genes including the genes for BphA and EtbA. Neither of the double mutants of bphS nor bphT degraded TCE suggesting that the transcription of BphA and EtbA is induced by the BphS-BphT regulation system in response to TCE. These results reveal the unique TCE degradation system in the strain RHA1.

Poster Session

BAM-PTH1055 - Profiling sulfate-reducing bacterial communities involved in bioremediation

Christopher Bartlett[1], Robin Slawson[1], Neil Thomson[2]
[1]Wilfrid Laurier University, Waterloo, Canada, [2]University of Waterloo, Waterloo, Canada

Improved techniques for remediating groundwater systems are required for the more than 500,000 contaminated sites in North America. Many of these sites are the legacy of historical industrial operations, inappropriate disposal practices and accidental releases. The most widely observed contaminant at many of these sites is petroleum hydrocarbons (PHCs). PHC products remain a staple source of energy worldwide and are an integral part of our daily lives. Recently, remediation efforts that involve the sequential application of treatment technologies have gained widespread interest. One specific sequential technology application or treatment train employs the aggressive nature of a chemical oxidation followed by bioremediation for polishing. When persulfate is used as the chemical oxidant its natural degradation by-product is sulfate, an electron acceptor. Hence, in this situation, the focus is on ways to optimize the mass removal behaviour of a treatment train that involves sulfate-reducing bacteria. Persulfate is predicted to have a multitude of effects on a microbial community, both positive and negative. It is hypothesized that the production of sulfate will enhance the sulfate-reducing community and subsequently increase biodegradation potential. However, the use of a strong oxidant like persulfate may also have detrimental effects on a microbial community. In order to test this hypothesis, both bench- and field-scale systems are being implemented to gather data for the analyses of enhanced bioremediation. Culture- and molecular-based microbiological techniques are being used to examine diversity, richness and abundance of sulfate-reducing communities involved in bioremediation. Preliminary data shows a decrease in toluene concentration (a PHC) following exposure to a persulfate-induced SRB community.

Poster Session

BAM-PTH1057 - Biodegradation of linear alkyl sulfonate by a native bacterial consortium isolated from Peruvian Rimac River

Susana Gutierrez Moreno[1], Fernando Abilio Merino Rafael[1], Manuel Hospinal Santiani[1], Elim Paredes Huánuco[1], Yessenia Cristhel Manrique Guzmán[1]

[1]*National University of San Marcos, Lima, Perú*

The household and industrial detergents cause serious problems in aquatic life avoiding the fish oxygenation, destroying fish gills and kill the watercourse, preventing its self-purification. The Rimac River is the most important waterway source of drinking water for Lima and Callao. For that reason, the objective of this project was isolate a detergent degrading microbial population from Rimac River. We took three samples of 500 mL of water and three samples of 500 g of sludge from three Rimac River sectors. These samples were inoculated on a mineral medium as American Society for Testing and Materials (ASTM) Method. After the microbial growth three tests were performed using several concentration of detergent (5, 25 and 50ppm). To measure detergent concentration, we used MBAS (Methylene Blue Active Substance) method adapted from Standard Methods for the Examination of Water and Wastewater (APHA, 1985). The consortium of bacteria belonging to the families Corynebacteriacea, Pseudomonadaceae, Enterobacteriaceae and Micrococcaceae, isolated from sector Nº 3 in a mineral medium with 30 ppm of detergent, showed the greatest LAS biodegrading (84%) using 5ppm of LAS. Therefore, that consortium was used to test the biodegradation ability on several commercial LAS detergent at 30ppm each one: Alkyl aryl sodium sulphonate, Sodium dodecyl benzene sulphonate and Sodium fenil sulphonate. The consortium got decrease 75% of the Alkyl aryl sodium sulphonate concentration, 59% of the Sodium dodecyl benzene sulphonate and 56% of the Sodium fenil sulphonate. In conclusion, the sector N° 3 bacterial consortium is able to biodegrade over 50% of three kinds of LAS, having better biodegradability on Alkyl aryl sodium sulphonate.

Poster Session

BAM-PTH1059 - Carbazole degradation in soil microcosm by tropical bacterial strains capable of angular dioxygenation and mineralization

Lateef Salam[1], Mathew Ilori[1], Olukayode Amund[1]
[1]*Department of Microbiology, University of Lagos, Akoka, Lagos, Nigeria*

Carbazole, an N-heterocyclic aromatic hydrocarbon is a persistent environmental pollutant that is recalcitrant, mutagenic and toxic. Previously, three bacterial strains isolated from tropical hydrocarbon-contaminated soils and phylogenetically identified as Achromobacter sp. strain SL1, Pseudomonas sp. strain SL4 and Microbacterium esteraromaticum strain SL6 displayed angular dioxygenation and mineralization of carbazole in batch cultures. Soil microcosm study was conducted using an agricultural soil amended with 100 mg/kg carbazole to examine the survival and carbazole degradation ability of the isolates in soil. Physicochemical analysis of the soil indicate moisture content and water holding capacity of 9.36 and 40%, while grain size determination shows 89% sand and 11% clay and silt, respectively. Strain SL4 has the highest survival rate (1.8 x 107 cfu/g) after 30 days of incubation in sterilized soil while there was a decrease in population density in native (unsterilized) soil when compared to initial populations. Gas chromatographic analysis after 30 days of incubation showed that in sterilized soil amended with carbazole, strains SL1, SL4 and SL6 reduced the initial concentration to 33.04, 17.85 and 31.46 mg/kg corresponding to 66.96, 82.15 and 68.54% carbazole removal. The combination of the three isolates as inocula in sterilized soil reduces the initial concentration to 12.87 mg/kg corresponding to 87.13% carbazole removal. In native soil amended with carbazole, autochthonous bacterial population reduced the initial concentration of carbazole to 80.81 mg/kg corresponding to 19.19% carbazole removal. However, inoculation of strains SL1, SL4 and SL6 into the native soil results in carbazole reduction to 8.36, 12.71 and 10.87 mg/kg after 30 days of incubation corresponding to 91.64, 87.29 and 89.13% carbazole removal. This study established the survivability (>106 cfu/g detected after 30 days) and carbazole degradation ability of these bacterial strains in soil and further confirm the results obtained in batch culture experiments.

Poster Session

BAM-PTH1061 - *In situ* Raman imaging of secondary metabolites in antibiotic-producing bacteria

Rimi Miyaoka[1], Masahiro Ando[2], Masahito Hosokawa[2], Tetsushi Mori[1,2], Hiro-o Hamaguchi[2,3], Haruko Takeyama[1,2]
[1]Department of Life Science and Medical Bioscience, Waseda University, Tokyo, Japan, [2]ASMeW, Waseda University, Tokyo, Japan, [3]National Chiao Tung University, Hsinchu, Taiwan

A wide variety of secondary metabolites, which have broad functions including antibacterial, antifungal, antiviral, and other activities, have been isolated from different microbes. Due to continued demand for the finding new pharmaceutical, rapid and efficient screening technique is required for the cost-effective production of biologically active metabolites. In this study, we report the first application of Raman microspectroscopy to *in situ* detection of antibiotics such as amphotericin B (AmB) produced by actinomycetes *Streptomyces nodosus*. Raman spectroscopy provides the characteristic information on the molecular structure and does not require any sample pretreatment such as dye labeling or genetic manipulation. Moreover, in combination with optical techniques, Raman microspectroscopy can provide high spatial-resolved distribution of biomolecules within single-cells in less-invasive and non-label manner. All Raman spectroscopic measurements were carried out with a laboratory-built confocal Raman microspectrometer. For Raman imaging, cell area was scanned at 0.5 μm pitch using a piezoelectric stage. In the case of AmB detection, the Raman spectrum of AmB shows the specific resonance Raman scattering bands due to the hydrophobic polyene domain. These two specific bands were able to be distinguished from other Raman bands derived from cellular major components such as protein, lipid and nucleic acids. The Raman image of *S. nodosus* revealed the localization and the accumulation of AmB dominantly occurred in the center area of cellular aggregates. Our result indicated that the Raman imaging technique allows the characterization of localization and molecular association state of intact secondary metabolites produced in live microbial cells. Therefore, we considered that our approach has a potential for the assessment for the productivity of antibiotics and the screening of new antibiotics from environmental bacteria.

Poster Session

BAM-PTH1063 - Periodontopathic-bacterial metabolite retention in the rat gingival tissue leads to cellular dysfunction in the jugular blood: A possible correlation with apoptosis, inflammation, and ageing

Marni Cueno[1], Noriko Matsukawa[2], Takamitsu Tsukahara[2], Kuniyasu Ochiai[1]
[1]Nihon University School of Dentistry, Tokyo, Japan, [2]Kyoto Institute of Nutrition and Pathology, Inc., Kyoto, Japan

Periodontal diseases attributed to bacterial infections are among the most common human chronic diseases affecting more than 80% of the adult population in the middle and advanced aged-individuals. Butyric acid (BA) is a major extracellular metabolite produced by anaerobic periodontopathic bacteria and is commonly deposited in the gingival tissue, however, the systemic effects of gradual BA accumulation has never been elucidated. Here, we determined the BA effects in blood mitochondrial function and cytosolic cellular processes. We injected BA into the rat gingival tissue and collected jugular blood at 0, 60, and 180 min after injection. Blood mitochondrial and cytosolic heme, hydrogen peroxide, and catalase activities were measured. Subsequently, NAD and NADP pool levels, NADPH oxidase (NOX) activation, sirtuin-1 (SIRT1) amounts, and caspase activities (CASP1, CASP3, CASP4, CASP8, CASP9) were determined using blood cytosol. We established that BA has prolonged retention in the gingival tissue which consequentially induced heme accumulation causing blood mitochondrial oxidative stress. Similarly, we found that blood cytosolic heme levels, NADP pool, and NOX2 amounts were all increased after BA injection insinuating blood cytosolic oxidative stress was likewise induced. In addition, we observed that BA induced a decrease in both the NAD pool and SIRT1 levels, whereas, all caspase activities measured were all increased in the rat jugular blood. Our results would insinuate that prolonged BA retention in the rat gingival tissue induces heme accumulation causing cellular oxidative stress which consequentially leads to activation of various caspase enzymes and indirectly results in decreased SIRT1 amounts. CASP3, CASP8, and CASP9 activties are associated to apoptosis, CASP1 and CASP4 activities are linked to inflammation, whereas, the available NAD pool and SIRT1 amounts are correlated to the ageing process. Thus, we propose that BA retention in the jugular blood simultaneously activates apoptosis and inflammation while concurrently contributing to the ageing process.

Poster Session

BAM-PTH1065 - Production of growth promoting factors by Sphingomonas sp. (GF9) for different species of bacteria after changing the culture conditions

Shinya Mitsuhashi[1], Ryogo Takai[1], Mohammad Nazrul Islam Bhuiyan[1], Kengo Shigetomi[1], Yoichi Kamagata[1], Makoto Ubukata[1]

[1]Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

Numerous investigators have attempted to improve the methods used for culturing bacteria from the environment. However, more than 99% of these bacteria can still not be cultured using laboratory media. Some “uncultivable bacteria” grow due to growth promoting factors that are provided by other species of bacteria. However, the symbiosis mechanisms between these “uncultivable bacteria” and these other “provider” bacteria are not fully understood. Catellibacterium nectariphilum (designated AST4) was isolated from activated sludge. This bacterium did not show any significant growth in a nutrient broth, but its growth was clearly induced by adding the culture supernatant of a Sphingomonas sp. (GF9). The filtered culture broth of GF9 also stimulated the growth of other bacterial species, including ASTN45 and ASN212. Each of these bacteria, AST4, ASTN45, and ASN212 was stimulated in culture by different factors from GF9. Thus, it is important to identify these factors to explore the mechanisms underlying symbiosis. Although we have attempted to isolate these growth promoting factors, isolation has not yet succeeded because the amounts of these factors were very small. Thus, to increase the production of these factors, we focused on the relationships between the amounts of these factors and the culture conditions used for GF9. GF9 was cultured in a jar fermenter at 30°C. The amount of each factor was monitored by determining their growth promoting activities on AST4, ASTN45, and ASN212. We investigated the effects of 3 agitation speeds, several concentrations of peptone and yeast extract, other types of peptone, and the incubation periods. From these experiments, we decided upon better culture conditions to increase the production of these factors. In particular, a high agitation speed resulted in increased amounts of these factors. These study results suggest that the “provider” GF9 species may change its productivity of these factors in response to environmental changes.

Poster Session

BAM-PTH1067 - Streptomyces avermitilis NBRC 14893T has complestatin and oxazolomycin analog biosynthetic gene clusters, which do not exist in the reported genome sequence of S. avermitilis MA-4680T

Hisayuki Komaki[1], Natsuko Ichikawa[2], Mitsuo Sekine[2], Yuko Kitahashi[2], Nobuyuki Fujita[2], Ken-ichiro Suzuki[1]
[1]Biological Resource Center, National Institute of Technology and Evaluation (NBRC), Kisarazu, Japan, [2]NBRC, Tokyo, Japan

The complete genome sequence of Streptomyces avermitilis ATCC 31267T, known as an avermectin producer, was reported in 2001, and is now published as that of S. avermitilis MA-4680T in GenBank/EMBL/DDBJ. In our PCR-based nonribosomal peptide synthetase (NRPS) gene analysis for actinomycetes, NRPS genes that are not present in the S. avermitilis complete genome sequence were unexpectedly amplified from S. avermitilis NBRC 14893T. To investigate the reason of this unexpected result, we sequenced whole genome of S. avermitilis NBRC 14893T using Genome Sequencer FLX and Illumina HiSeq1000. Consequently, the genome size was estimated at approximately 10 Mb, and 93 scaffold sequences were obtained. Some of the scaffold sequences were not present in the published S. avermitilis genome sequence, suggesting NBRC 14893T-specific. Approximately 40 kb of NRPS gene cluster and 65 kb NRPS/polyketide synthase hybrid gene cluster, previously unreported in S. avermitilis, were discovered in the NBRC 14893T-specific genome sequences. Bioinformatic analyses suggested that the two gene clusters were likely involved in the synthesis of demethylcomplestatin and a novel oxazolomycin analog, respectively. According to strain transfer histories among culture collections, the original type strain S. avermitilis MA-4680T had been deposited to NRRL, and the NRRL strain had been subsequently deposited to KCC and ATCC. NBRC 14893T is derived from the KCC strain, while the complete genome-elucidated strain is from ATCC 31267T. It is hard to imagine that pure culture stocks acquire additional DNA coding large biosynthetic gene clusters. Furthermore, plasmid loss and dynamic genome reconstitution frequently occur in the genus Streptomyces. Therefore, the deletion of the two gene clusters we discovered here had probably occurred after the type strain of S. avermitilis was transferred from NRRL to ATCC.

Poster Session

BAM-PTH1069 - Differential carotenoid and astaxanthin production by Phaffia rhodozyma mutants

Lina M. Castelblanco Matiz[1], Odilia Pérez Ávalos[1], Ana C. Ramos Valdivia[1], Fernando J. Esparza García[1], Carlos M. Cerda García-Rojas[2], César M. Flóres Ortiz[3], M. Teresa Ponce Noyola[1]

[1]*Departamento de Biotecnología y Bioingeniería CINVESTAV-IPN, México City, México,* [2]*Departamento de Química CINVESTAV-IPN, México City, México,* [3]*Laboratorio de Biogeoquímica FES-Iztacala UNAM, México City, México*

Astaxanthin is one of the most important carotenoids with a high biotechnological interest, mainly in the aquaculture industry because it is responsible for the orange – red color of animal´s flesh such as salmon and trout. It is the main product of carotenogenesis in the yeast Phaffia rhodozyma and in mutant strains the carotenoid profile is modified. In this work, we characterized the carotenoid profile during growth kinetics of P. rhodozyma wild type and its mutant strains XR4 (red), XP26 (pink), XY21 (yellow) and XW10 (white), previously obtained by nitrosoguanidine treatment. The strains were grown in 3 L bioreactors (Applikon®, Holland) at 20 oC for 72 h at 500 rpm. Growth was measured by dry weight, carotenoids were extracted as described by Sedmak and carotenoid profile characterization was carried out by HPLC in a HP 1100 series with diode array detector (DAD) using an Allsphere® ODS-1 (C-18) column. The volumetric and specific carotenoid content were partially growth associated in all P. rhodozyma strains. P. rhodozyma XR4 had the highest biomass production values while XP26 strain had the lowest. Wild type; XR4; XP26; XY21 and XW10 strains reached final specific carotenoid content of 4.62, 10, 0.95, 2.4 and 3.77 mg g-1, respectively. The more pigmented mutant XR4 enhanced 116% the carotenoid production while XP26, the less pigmented strain, decreased 79% both respect to the wild type. In XR4, XY21 and XW10 strains, astaxanthin, β-carotene and phytoene were the main identified carotenoids respectively, whereas in XP26 strain, HDCO was the carotenoid with the highest ratio. In XR4 strain astaxanthin reached 83% of total carotenoids, while in wild type and XP26 strains this pigment only reached 67% and 50% respectively. Due to its high values of total carotenoids and astaxanthin production, Phaffia rhodozyma XR4 mutant strain is a potential natural source of astaxanthin.

Poster Session

BAM-PTH1071 - Identification of ‘Cryptic’ antibiotics by constitutively active pleiotropic regulators

Martin Daniel-Ivad[1], Nabeela Hameed[1], Sheila Pimentel-Elardo[1], Tomas Gverzdys[1], Justin Nodwell[1]
[1]*Department of Biochemistry, University of Toronto, Toronto, Canada*

Antibiotic resistance is a worldwide problem that is growing in severity while antibiotic discovery remains stagnant with limited success bringing novel antibiotics to market. The *Streptomyces* genus of gram positive, soil-dwelling bacteria has provided up to 80% of the clinically relevant antibiotics. Through comprehensive antibiotic screening, it was believed that this genus had exhausted its potential to provide new leads for novel antibiotics. However, the publication of the *S. coelicolor* genome uncovered that it has the genetic capacity to synthesize several more molecules, and that potentially ~80% of its secondary metabolites have yet to be discovered. The challenge in leveraging these molecules lies within the expression of their assembly machinery, as under normal culturing conditions these ‘cryptic’ molecules remain undetected. Here we present a platform where heterologous expression of AfsQ1*, a constitutively active pleiotropic response regulator, in wild isolates of *Streptomyces* can be used to identify ‘cryptic’ antibiotics. We showed that when AfsQ1* was expressed in 6 wild *Streptomyces* a strain gains the ability to inhibit the growth of several clinically significant multidrug resistant pathogens. LC-MS analyses shows that 2 molecules are produced with AfsQ1* expression, suggesting that AfsQ1* can induce cryptic metabolite production. Both of these ‘cryptic’ antibiotics have been purified from the producer strain to elucidate the structure of the molecules. NMR data suggests that one of the ‘cryptic’ antibiotics is potentially novel, while LC-MS/MS of the other molecule suggests that it is a rare poorly characterized lasso peptide known as siamycin-I. In conclusion this strategy is a viable screening platform to discover novel antibiotics to help curb the antibiotic resistance epidemic.

Poster Session

BAM-PTH1073 - Mycobacterial DNA-binding protein 1 induces phenotypic tolerance to isoniazid in mycobacteria

Makoto NIki[1], Mamiko Niki[1], Mayuko Osada-Oka[2], Yuriko Ozeki[1], Sohkichi Matsumoto[3]
[1]Osaka City Univ, Medicine, Osaka, Japan, [2]Kyoto Pref Univ, Food & Environment, Kyoto, Japan, [3]Niigata Univ, Medicine, Niigata, Japan

Tuberculosis remains one of the most deadly infectious diseases worldwide and is a leading public health problem. Although isoniazid (INH) is a key drug for the treatment of tuberculosis, tolerance to INH necessitates prolonged treatment, which is a concern for effective tuberculosis chemotherapy. INH is a prodrug that is activated by the mycobacterial enzyme, KatG. Here, we show that mycobacterial DNA-binding protein 1 (MDP1/HupB/HLP/LBP), which is a histone-like protein conserved in mycobacteria, negatively regulates katG transcription and leads to phenotypic tolerance to INH in mycobacteria. Mycobacterium smegmatis deficient for MDP1 exhibited increased expression of KatG and showed enhanced INH activation compared with the wild-type strain. Expression of MDP1 was increased in the stationary phase and conferred growth phase-dependent tolerance to INH in M. smegmatis. Regulation of KatG expression is conserved between M. smegmatis and Mycobacterium tuberculosis complex. Artificial reduction of MDP1 in Mycobacterium bovis BCG was shown to lead to increased KatG expression and susceptibility to INH. MDP1 has similar activity of ferritin like protein and is a ferroxidase-iron storage protein. Thus MDP1 enzymatically converts H2O2 into water and oxygen in the presence of Fe2+. It is conceivable that both KatG and MDP1 play a significant role in H2O2 detoxification in mycobacterial cells. MDP1 may compensate for reduced KatG activity, and therefore their expression levels are reciprocally regulated. This regulation of H2O2 detoxification in mycobacteria induces phenotypic tolerance to INH in mycobacteria.

Poster Session

BAM-PTH1000 - A phylogenetic study of cellulase synthase and cellulase and cellobiase activities in exopolysaccharide-producing filamentous cyanobacteria from the Atacama Desert

Alexandra Galetovic Carabantes[1], Nicole Fernández[1], Tamara Caimanque[1], Héctor Olivares[1], Jorge Araya[2], Benito Gómez-Silva[1]

[1]Biochemistry Laboratory, Biomedical Department, Universidad de Antofagasta, Antofagasta, Chile, [2]Parasitology Laboratory, Medical Technology Department, Universidad de Antofagasta, Antofagasta, Chile

Cyanobacteria from environments subjected to dryness synthetize exopolysaccharides (EPS) as a tolerance strategy to desiccation. Since cellulose is a raw material for biofuels production, we have focused our attention in search of the answer to the following question: Do EPS from the Atacama cyanobacteria contain cellulose molecules? Two approaches were implemented: the use of enzymatic assays to show that EPS contain cellulose-like molecules and to verify the presence of a gene for a key enzyme in the cellulose biosynthetic pathway. Cyanobacteria were sampled from sediments and waters from lakes, ponds and rivers. EPS from five Atacama filamentous cyanobacteria were isolated, purified and used as substrate for commercial cellulase and cellulase plus cellobiase; these assays provided 12-113 and 205-356 micromoles of glucose per min, respectively. The presence of the cellulose synthase gene, a key enzyme for cellulose biosynthesis, was demonstrated in the genome of seven cyanobacteria strains from the Atacama Desert. PCR fragments of about 900 bp were sequenced and the corresponding phylogenetic tree showed association of cellulose synthase from the Atacama strains with Calothrix PCC7507, Synechococcus sp. PCC7002, Nostoc sp. PCC7120, Nostoc punctiforme PCC73102 and with eukaryotic cellulase synthase of Arabidopsis thaliana. Thus, our results confirm the notion that Atacama cyanobacteria have the genetic capability to synthesize cellulose-like molecules which are incorporated into their EPS. Supported by grants CONICYT/FIC-R 4603 and Universidad de Antofagasta CODEI-5394.

Poster Session

BAM-PTH1002 - Physiological and genomic characterization of a Caldibacillus debilis strain isolated from a aerotolerant thermophlic Lignocellulosic enrichment culture

Scott Wushke[1], Justin Zhang[1], Brian Fristensky[1], Nazim Cicek[1], David Levin[1], Richard Sparling[1]
[1]*University of Manitoba, Winnipeg, Canada*

Caldibacillus debilis GB1 was isolated from a thermophlic aerotolerant cellulolytic enrichment culture, which also included a strain of cellulolytic Clostridium thermocellum. In a defined co-culture with C. thermocellum DSM 1237, it allows cellulose degradation and fermentation under aerobic conditions. Due to GB1's potential as co-culture partner with C. thermocellum in lignocellulosic biofuel production, GB1 was further characterized. It grows under both aerobic and anaerobic atmospheres on cellobiose producing formate, acetate, ethanol, CO2, and trace lactate as end products. This fermentation profile contrasts with the type strain DSM 16016, which grew only under an aerobic atmosphere, did not produce ethanol but produced lactate as a major-end product. The genome of C. debilis GB1 was sequenced and characterized and a comparison was done against the DSM 16016 genome to identify potential causes for the differences in physiological profiles. Genome sequencing identified a 3340752-bp bacterial chromosome and 5386-bp plasmid. GB1 genome closely resembles the type strain DSM 16016 in both gene complement and synteny. Gene type and copy number were identical in core metabolic pathways including: glycolysis, tricarboxylic acid cycle, respiratory pathways, pyruvate metabolism and end-product synthesis. While the genes in core metabolic pathways were highly conserved there is multitude of point mutations potentially affecting the expression of key genes. Furthermore, there are several differences in copy number of cellobiose associated phosphotransferase system subunits in GB1, suggesting cellobiose flux may be a contributing factor for physiological differences as well.

Poster Session

BAM-PTH1004 - The prevalence and susceptibility pattern of betalactamase producing Staphylococcus Aureaus at Oauthc, Ile-Ife, Osun State

Udoh Jark, Saturday[2], Awoyemi G.M[2], Adefusi Francisca[1], Hassan-Olajokun Rachael[1], Odetoyin Babatunde[1], Olaniran Olarinde[1]

[1]Obafemi Awolowo University, Ile Ife ,Nigeria, [2]Obafemi Awolowo University Teaching Hospital, Ile Ife, Nigeria

Introduction: Staphylococcus aureus infections remain a threat to both immunocompetent and immunosuppressed patients despite advances in anti-bacterial therapy. Their infections on patients often results to high morbidity complication rates, also they possess plasmid and penicillinase capable of developing antibiotic and so limit the choice of therapeutic agents available against them. The objectives is to know the new trend prevalence and antibiogram profile of Staphylococcus aureus in a case beta-lactamase production with the view to reveal penicillin-resistant strain in our society. Setting Methods: A total of 250 strains of Staphylococcus aureus isolated from clinical materials obtained from inpatients and outpatients of the OAUTHC Ile-Ife were used. Samples of clinical specimens collected between May and October 2008 from various sites of body inoculated on the appropriate media and incubated at 37oC and 20% carbon dioxide for 24hrs after which growth were examined and isolations were identified biochemically beta-lactase test detection by starch paper technique and anti-biotic sensitivity test was carried out. Results: Among the 250 Staphylococcus aureus isolated, 250(80%) were positive for beta-lactamase production with sputum and having greatest prevalence of (100%) each. High resistance was observed against penicillin (100%) followed by ampicillin(96%), Tetracycline(70%), Streptomycin(46%), Gentamicin(39%), chloramphenicol (37.5%), cloxacilin(43%), Enthromycin(17.5%), ceftaximidine(12.5%) and pefloxacin(2.5%). Susceptibility was observed maximum against Preflotaxin(97.5%) followed by Ceftaxide(87%), Enthromycin(82.5%), Cloxaxillin(66%), chloramphenicol(62.5%) and Gentamicin(61%). The production of beta-lactamase is associated with resistance of isolates to Ampicillin x2 43.956, p<0.05, Gentamicin x2=25.34, P<0.05. Penicillin x2= 41.67, P<0.05. Conclusion: This study suggests Pefloxacin as the best anti-staphylococcal drug whereas anti-biotic susceptibility test in conjuction with the test on beta-lactamase production will help as a barometer for anti-biotic policy formations. Keywords: Prevalence, Susceptibility patterns, betalactamase, Staphylococcus aureus.

Poster Session

BAM-PTH1006 - Comparative study of the cefazolin activity on enterobacteria by the diffusion susceptibility testing method versus determination of minimal inhibitory concentration

Adel Tlemsani[1], Chafika Kessous[1]
[1]Biology Laboratory, Ain Taya Hospital, Algiers, Algeria

Introduction. objectives: The sensitivity of bacteria to antibiotics remains largely studied in our country with the diffusion of antibiotic disk on agar medium, but the determination of the minimum inhibitory concentration (MIC) is still the reference method. Our study aimed to compare the sensitivity of enterobacteria strains overlooked the cefazolin by using the diffusion susceptibility testing and MIC determination on solid medium. Materials and methods: We studied 49 strains of enterobacteria with inhibition diameters for cefazolin are between 18 and 24mm, for each of them a diffusion susceptibility testing and determination of mic was performed on solid medium as recommended by the CLSI 2011, a reference strain E. coli ATCC 25922 was tested under the same conditions. Results: on 49 studied strains 12 (24.49 %) of them showed a discrepancy between the two techniques, among these 41.66 % corresponded to susceptible strains by the mic but intermediate with diffusion susceptibility testing and 33% resistant by the MIC but intermediate with diffusion susceptibility testing .

Poster Session

BAM-PTH1008 - Emergence of expanded spectrum beta lactamases among quinolone resistant Enterobacteriaceae in Yaounde, Cameroon

Emilia Lyonga[1,2], Michele Toukam[1], Celine Nkenfou[3], Gonsu Hortense[1], Martha Mesembe[2], Agnes Eyoh[1,2], George Ikomey[1,2], Eustace Berinyuy[1], Marie-Claire Okomo- Assoumou[1,2]

[1]Faculty of Medicine and Biomedical Scs, University of Yaounde 1,Cameroon, [2]Centre for the Study and Control of Communicable Diseases, Yaounde, Cameroon, [3]3University of Yaounde I, Higher Teachers Training College, Yaounde, Cameroon

Introduction Cross resistance to quinolones and Beta-lactams is frequent in Enterobacteriaceae due to the extensive use of these antibiotics clinically and in the food industry. Use of one of these categories of antibiotics may consequently select for resistance in both categories. The aim of this study was to determine the emergence of expanded spectrum beta lactamases (ESBL) among quinolone resistant Enterobacteriaceae in Yaounde, Cameroon. Method Two hundred and eighty-one Enterobacteriaceae species were isolated from different clinical specimens in a tertiary hospital in Yaounde over a period of ten months. Identification was done using standard bacteriological procedures including API 20E. Susceptibility testing was performed using the Kirby Bauer Disc Diffusion method for fifteen antibiotics made up of seven quinolones and eight beta lactams: Nalidixic acid, pipemidic acid, norfloxacin, ciprofloxacin, ofloxacin, sparmofloxacin, moxifloxacin, ampiciline, cefuroxime, cefaclor, gentamicin, cefotaxime, ceftazidime, ceftriaxone, and amikacin. All the isolates were screened for ESBL by using two third generation cephalosporins as recommended by the Clinical and Laboratory Standard performance guideline. All isolates resistant to one or both were considered positive (screening) for ESBL and were confirmed using the double disc synergy test. Results Out of the 281 enterobacteriaceae isolates the most prevalent enterobacteriaceae genera were in the order: Escherichia 98/281(34%) with 29/98(29.6%) being quinolone resistant and 9/98(9.18%) producing ESBL; Klebsiella 92/281(32.7%) with 29/92(31.52%) being quinolone resistant and 9/92(9.78%) producing ESBL; Enterobacter 23/281(8.10%) with 4/23(17.37%) being quinolone resistant and 1/23(13.04%) producing ESBL; Proteus 22/281(7.8%) with 2/22(9.09%) being quinolone resistant and 1/22(4.55%) producing ESBL; Serratia 21/281(7.5%) with 6/21(28.57%) being quinolone resistant and 0% ESBL production; others 25/281(8.9%) with 4/25(20%) being quinolone resistant and 0% ESBL production. Conclusion The prevalence of ESBL among quinolone resistant enterobacteriaceae was high. Guidelines for the use of these antibiotics are therefore required.

Poster Session

BAM-PTH1012 - Class 1 integrons of a multidrug-resistant nosocomial Achromobacter xylosoxidans Isolated in Akita Prefecture, Japan

Jun Yatsuyanagi[1], Shioko Saitou[1], Masahiro Suzuki[2], Junko Isobe[3], Masanori Watahiki[3]
[1]Akita Prefectural Institute of Public Health, Akita, Japan, [2]Aichi Prefectural Institute of Public Health, Nagoya, Japan, [3]Toyama Institute of Health, Toyama, Japan

Background: Achromobacter xylosoxidans is a rare but important nosocomial pathogen whose multidrug resistant (MDR) mechanism remains poorly understood. The aim of this study was to characterize the class 1 integron genes of MDR nosocimial A. xylosoxidans strains isolated in one hospital in Akita Prefecture, Japan. Methods:Fourteen A. xylosoxidans strains isolated in Akita Kumiai General Hospital between July 2003 and September 2006 were examined for antibiotic resistance by the KB method. Class 1 integron genes were amplified by using primers designed within the 5' and 3' conserved sequences (CS) of class 1 integron, blaIMP, and aacA4 genes. The DNA sequences of the amplicons were determined by direct sequencing. The effect of Phe-Arg-beta-naphthylamide, a known efflux pump inhibitor, on the MIC of 18 antibiotics was examined by using a commercially available panel to assess involvement of the efflux pump in the MDR mechanism of the A. xylosoxidans strains. Results: The fourteen A. xylosoxidans strains were all resistant to ABPC, CET, CAZ, CTX, CFP, CFX, IPM, EM, FOM, KM, NFLX, and TC. Of the 14 strains, seven strains harbored two class 1 integrons (5CS-aacA4-3CS and 5CS-blaIMP-1-aadA5-3CS), four strains harbored two class 1 integrons (5CS-aacA4-aadA5-3CS and 5CS-blaIMP-1-aadA5-3CS), and three strains harbored one class1 integron each (5CS-aacA4-blaIMP-1-aadA5-3CS, 5CS-blaIMP-1-aacA4-aadA5-3CS, and 5CS-blaIMP-1-aadA5-3CS). Among the 18 antibiotics included in the panel, only the MIC of MINO showed a concentration dependent decrease after the addition of a sub-lethal concentration of Phe-Arg-beta-naphthylamide. Conclusions: The results suggested that class 1 integrons in these MDR A. xylosoxidans evolved via recombination of aacA4, aadA5, and blaIMP-1 gene cassettes. Our results also suggested that the efflux pump did not play an important role in the MDR mechanism of the A.xylosoxidans strains. Further study should be conducted to elucidate the MDR mechanisms of these A.xylosoxidans strains.

Poster Session

BAM-PTH1014 - Antimicrobial spectra of activity chart based on north american susceptibility data

Marc-Alexandre Duceppe[1], Dr. Daniel J.G. Thirion[2]
[1]McGill University Health Centre, Montreal, Canada, [2]McGill University Health Centre, Montreal, Canada

Experts and guidelines recommend adapting empiric antibiotic therapy according to local epidemiological patterns. However, local susceptibility data are not always available, not adequately summarized and not easily retrievable for most practitioners. Because antimicrobial activity changes over time, updated tools are required to facilitate transfer of published data into practice. A tool on antimicrobial spectra of activity based on local data can help practitioners and expert groups make decisions for optimal antimicrobial use. Objective: To create a comparative chart of antimicrobial spectra of activity with available Canadian data. Methods: A systematic approach was used to review current literature regarding similar charts and Canadian / North American susceptibility data for antimicrobials. A search for primary literature was conducted through EMBASE, Pubmed and OVID Medline. Public, governmental and associations' surveillance networks were scanned for available data. Trials and data were included if their methods were based on Clinical and Laboratory Standards Institute methods and breakpoints. Predetermined cut-offs were used for the interpretation of susceptibility data. Presented microorganisms and antimicrobials were chosen according to their clinical relevance and frequency of encounter in clinical practice. Results: Forty-five studies were identified and 39 were removed because of non-applicable data. To better represent susceptibility patterns across Canada, data from the Canadian Antimicrobial Resistance Alliance, the Canadian Nosocomial Infection Surveillance Program, the Toronto Invasive Bacterial Disease Network, the Institut national de santé publique du Québec and the CANWARD 2007-2011 study were selected. An attempt was made to identify characteristics of the infection (community acquired or health-care related). This information was unavailable in all selected studies. North American data were used preferably to complete missing susceptibility information. We present a methodology to summarize local antimicrobial susceptibility data for healthcare practitioners. This tool can be used to aid the selection of empiric antimicrobials according to recommendations and for teaching purposes.

Poster Session

BAM-PTH1016 - Proteomic analysis of swarming pseudomonas aeruginosa cells exposed to antibiotic

Laure Cockenpot[1], Eric Deziel[1]
[1]*Institut National de la Recherche Scientifique, Montreal, Canada*

Pseudomonas aeruginosa is a pathogenic Gram-negative bacterium expressing many resistance systems that can accommodate multiple classes of antibiotics. Interestingly, the swarming motility behaviour of P. aeruginosa has been associated with increased resistance to most classes of antibiotics. While the mechanism at play is still unknown, we have noted that the outer membrane of swarming cells is less permeable than their vegetative counterparts (Lai, Tremblay, Déziel. 2009. Environ. Microbiol. 11:126). Comparing different modes of action of several drugs, the ones that need to cross the outer membrane, such as the aminoglycosides antibiotics, are not effective against swarming colonies. On the other hand, cationic peptides which act directly on the outer membrane maintain their bactericidal activity. Therefore, we hypothesize changes in the outer membrane could be a mechanism of resistance involved in the loss of susceptibility to antibiotics of swarming cells. To determine which proteins are involved in the increased drug resistance of swarming P. aeruginosa cells, the protein profiles of swarming cells exposed to antibiotics was assessed. The outer membrane proteins were extracted from swarming cells exposed to tobramycin, an aminoglycoside, or without drug. These proteins were then separated using the two-dimensional gel electrophoresis method (2-DE). After analysis, we observed that additional proteins are expressed when swarming P. aeruginosa cells are growing in the presence of tobramycin. Briefly, we found different protein expression patterns between the exposed and non-exposed swarming cells; some being expressed only when an antibiotic was present. Further investigations are ongoing in order to better understand the protein expression profiles and determine the implications of the differently expressed proteins.

Poster Session

BAM-PTH1018 - Antimicrobial susceptibility and plasmids presence in Salmonella enterica subsp. enterica serovar Heidelberg from clinical and environmental sources isolated in Chile

Carmen Aravena[1], Andrea Villegas[1], Barbara Valencia[1], Alda Fernández[2], Pamela Araya[2]
[1]School of Medicine, Universidad de Valparaíso, San Felipe, Chile, [2]Laboratory of Bacteriology, Instituto de Salud Pública, Santiago, Chile

Introduction. Salmonella Heidelberg is a causative agent of food-borne infections and in a lesser extent bacteremia. In Chile, an outbreak caused by this bacterium occurred in 2011. This prompted us to characterize this serovar. Objective. To determine the antimicrobial susceptibility, plasmids presence and detection virulence gene invA in Salmonella Heidelberg strains which were isolated in Chile over the period 2006-2011. Materials and methods. Sixty-one S. Heidelberg isolates from clinical (32) and environmental (29) sources collected by Public Health Institute in Chile (2006-2011). Antimicrobial susceptibility measured by minimum inhibitory concentration (MIC) was determined using MICEvaluator TM, (Oxoid). The presence of plasmids was studied using kit (Omega, BioTek Inc.). Also, invA and blaCMY-2 genes were detected by polymerase chain reactions (PCR). Results. S. Heidelberg isolates were 100% susceptible to chloramphenicol, sulfamethoxazole-trimethoprim and gentamicin and 91.8% susceptible to ampicillin and amoxicillin-clavulanic acid. 33% isolates were resistant to tetracycline (MIC> 256 μg/ml) and 52% showed reduced susceptibility to ciprofloxacin (MIC = 0.25 μg/ml). Three isolates (two clinical and one environmental source) showed resistance to ceftiofur, ceftriaxone, amoxicillin-clavulanic acid and ampicillin, one of these was positive for blaCMY-2 gene. 13% of S. Heidelberg isolates showed no plasmids presence, 75% showed one to seven plasmids with different sizes (≤ 4 kbp) and 12% showed over one plasmid with variables sizes (≥ 4 to 48.5 kbp). All isolates studied were positive for invA with an amplicon of 411 bp. Conclusions. The most of Salmonella Heidelberg isolates were susceptible to the antimicrobials tested. Three isolates were resistant to broad-spectrum cephalosporins and one of them from clinical sources showed blaCMY-2 gene encoding a cephalomycinase, associated with plasmid probably. All isolates studied here have the invA gene. The presence of plasmids in S. Heidelberg could be used as a molecular tool for studies in outbreaks. Work supported by DIUV-Regional (71/2011).

Poster Session

BAM-PTH1020 - High throughput sequencing to investigate the impact of Cefprozil on the cultivable bacterial communities of the human gut microbiota

Amin Ahmed Ouameur[1], Frédéric Raymond[1], Maxime Déraspe[1], Ève Bérubé[1], Richard Giroux[1], Maurice Boissinot[1], Ann Huletsky[1], Jacques Corbeil[1], Michel G. Bergeron[1]
[1]Centre de recherche en infectiologie de l'Université Laval, Canada

Culture-based methods have been used to understand the effects of antibiotics on the cultivable fraction of human gut microbiota. However, these approaches have generally focused on specific indicator bacteria. Here, high throughput sequencing was used to analyze the in-vivo effects of antibiotics on the diversity of cultivable aerobic and anaerobic bacterial communities of the human gut microbiota. Ten healthy individuals were given second-generation cephalosporin Cefprozil and stool samples were collected before administration (day 0) and on the last day of antibiotic administration (day 7). These samples were cultured in liquid media (supplemented BHI) under aerobic (with 5% CO2) or anaerobic atmospheres and sequenced using the Hiseq1000 sequencer (Illumina). The metagenomic Ray Meta 2.0 assembler was used to profile bacterial taxa and resistance genes for each sample. In our culture conditions, Bacteroidaceae and Enterobacteriaceae are the most abundant families in the majority of anaerobic and aerobic cultures, respectively. Multivariate analysis showed that there is a significant difference in the relative abundance of several taxa in day 7 compared to day 0. We observed an increase in Clostridiaceae, Erysipelotrichaceae and Enterococcaceae in aerobic cultures while only an increase in Clostridiaceae was observed in anaerobic cultures. A significant decrease in Veillonellaceae, Streptococcaceae and Bifidobacteriaceae was observed in aerobic cultures, while 12 taxa were decreased in anaerobic cultures, including Eubacteriaceae, Ruminococcaceae, Porphyromonadaceae, Lachnospiraceae, Actinomycetaceae and Sutterellacea. The beta-lactamase-encoding gene blaCMY was enriched in 6 of 10 aerobic cultures at day 7. In conclusion, the combination of culture-based methods with high throughput sequencinghas shown to be reliable to analyze the effects of antibiotics on the cultivable intestinal microbiota.

Poster Session

BAM-PTH1022 - Glycopeptides and Daptomycin susceptibility in methicillin-resistant Staphylococcus aureus isolates from a tertiary care centre of North India

Kashi Nath Prasad[1], Avinash Singh[1,2], Aparna Tripathi[1], Satyendra Kumar Singh[1], Ravi Prakash Rai[1], Mohibur Rahman[1], Janmejai Kumar Srivastava[2]

[1]*Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India,* [2]*Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow, India*

Increased vancomycin minimum inhibitory concentrations (MICs) in Staphylococcus aureus and their association with glycopeptide treatment failure is a well-known problem. The present study determined glycopeptides and daptomycin susceptibility among methicillin resistant S. aureus (MRSA) isolates. It also investigated the prevalence of heterogeneous vancomycin intermediate S. aureus (hVISA) and assessed their agr polymorphisms. A total of 776 MRSA isolates recovered from different clinical specimens during 2009 to 2012 were studied for glycopeptides (vancomycin and teicoplanin) and daptomycin susceptibility by E-test method. Prevalence of hVISA was studied in randomly selected 200 isolates by simplified population analysis, gradient plate and macro E-test methods, and confirmed by PAP-AUC. hVISA was identified in 23 (11.5%), 20 (10.0%) and 18 (9.0%) isolates by simplified population analysis, gradient plate and macro E-test methods respectively. Only 9 (4.5%) isolates were confirmed as hVISA by PAP-AUC. Three of these 9 hVISA strains were non-susceptible to daptomycin. Vancomycin MIC geometric mean (GM) of MRSA isolates was 0.923, 0.944, 1.134 and 1.294 mg/L in the year 2009, 2010, 2011 and 2012 respectively and the trend was significantly higher over the years ($P < 0.0001$). Similarly teicoplanin MIC GM was 1.47, 1.49, 1.8 and 2.04 mg/l in the year 2009 to 2012 respectively ($P < 0.0001$). MIC shifts were not observed for daptomycin over the years ($P > 0.232$). All identified hVISA strains carried agr I type allele and lacked vanA and vanB genes.

Poster Session

BAM-PTH1024 - Study of resistance to β-lactam antibiotics in Gram-negative bacilli isolated from effluents of two hospitals in Tizi Ouzou, Algeria.

Khadidja Yousfi[1], Abdelaziz Touati[1], Sadjia Bekal[2], Brigitte Lefebvre[2], Cécile Tremblay[2], Djamila Bougdour[1]
[1]Laboratoire d'Écologie Microbienne, université d'A. Mira, Béjaia, Algérie, [2]Laboratoire de Santé Publique du Québec, Sainte-Anne-De-Bellevue, Canada

There is a concern that hospital effluent potentially containing antimicrobial compounds, antimicrobial resistant (AMR) bacteria and genetic determinants of resistance may contribute to the emergence, dissemination and persistence of AMR bacteria in municipal wastewaters. These bacteria may be directly discharged into receiving waters (rivers, soils) without pretreatment. The spread of these bacteria in the natural environment can have significant consequences in public health and ecological disturbance. The main risk is that resistance genes can be transferred to human and animal pathogens. The aim of the present study was to characterize the mechanisms of resistance to β-lactam antibiotics in Gram-Negative Bacilli isolated from effluents of two hospitals in the Wilaya of Tizi Ouzou, Algeria. The identification of isolates is performed by API 20E for strains of Enterobacteriaceae and rrs or rpoB gene sequencing for non-enteric Gram negative Bacilli. Resistance phenotypes were established on the basis of the results of double-disk diffusion technique. MICs were determined by the micro-dilution method. A total of 47 strains of Gram-negative bacilli were isolated. The identified strains were: Escherichia coli, Morganella morganii, Enterobacter coloacae, Citrobacter freundii, Klebsiella oxytoca, Proteus vulgaris, Kluyvera sp., Acinetobacter baumannii, Pseudomonas aeruginosa, Aeromonas veronii, Aeromonas hydrophila, and Shewanella xiamenensis. Analysis of resistance phenotypes revealed the presence of ESBLs (34/47) among Enterobacteriaceae strains (Escherichia coli, Klebsiella oxytoca, Kluyvera sp.,...), and non-enteric ones (Aeromonas sp., Acinetobacter baumannii) in addition to overproduction of cépahlosporinases (8/47). Interestingly, Shewanella xiamenensis strain was resistant to cefotaxim, ceftazidim and aztreonam. The presence of such resistant strains in hospital effluents should be of a major public health concern because of their resistance transfer potential to other pathogenic or environmental bacteria.

Poster Session

BAM-PTH1026 - Antibiotics resistance and extended spectrum beta-lactamases (ESBLs) production by Salmonella species isolated from HIV/AIDS seropositive and seronegative subjects in Akwa Ibom State, Nigeria

Dora Udoh[1], Simon Utsalo[2], Anne Asuquo[2]
[1]University of Uyo, Uyo, Nigeria, [2]University of Calabar, Calabar, Nigeria

The occurrence of antibiotic resistant strains and extended-spectrum beta-lactamases (ESBLs) producers among salmonellae have been reported in some parts of Africa but such information especially from HIV/AIDS patients is rare in Akwa Ibom State, Nigeria. The present study investigated the occurrence of antibiotics resistant and ESBLs producing strains of Salmonella isolated from HIV/AIDS seropositive and seronegative patients in Akwa Ibom State. Ninety two Salmonella strains comprising 79 from HIV seropositive and 13 from HIV seronegative subjects were isolated from blood and stool samples of the subjects using standard microbiological techniques. Isolates were identified using conventional biochemical method and Microgen- Gram Negative Identification System (Microgen Bioproducts, USA). Isolates were tested for their susceptibility to 10 commonly used antibiotics using Kirby-Bauer agar disc diffusion method with commercially produced antibiotic discs (BB, USA) and the production of ESBLs by double disc diffusion method (BB, USA). Salmonellae that produced ESBLs were selected for plasmid analysis using gel electrophoresis while plasmid curing was performed using 0.10mg/ml acridine orange. A total of 48(60.8%) of the isolates from HIV seropositive subjects expressed resistance to Tetracycline, 35(44.3%) to Ampicillin-Sulbactam, 44(55.7%) to Gentamicin, 42(53.1%) to Ciprofloxacin and 63(79.8%) to Sulfamethoxazole – Trimethoprim. Six (46.2%) isolates from HIV negative subjects expressed similarly high resistance to Tetracycline, 8(61.5%) to Ampicillin-Sulbactam and 7(53.8) to Sulfamethoxazole – Trimethoprim. A total of 28 (30.4%) of the isolates produced ESBLs against Amoxicillin + Clavulanic acid, 23 (25%) against Cefotaxime + Clavulanic acid and 26(28.3%) against Cefotaxidime + Clavulanic acid. Thirty four Salmonella isolates produced ESBLs and 14 exhibited plasmids ranging ≥ 23.13kbp and above out of which 9 of the plasmids were cured. ESBL-producing salmonellae have become a significant public health problem in Nigeria with particular implications especially in immunocompromised conditions.
Keywords: HIV/AIDS, Antibiotics, Salmonella, ESBLs, Plasmids, Nigeria

Poster Session

BAM-PTH1028 - Genomic characterisation of tigecycline resistance mechanisms in Streptococcus pneumoniae

Andréanne Lupien[1], Hélène Gingras[1], Philippe Leprohon[1], Marc Ouellette[1]
[1]*Centre de Recherche en Infectiologie, Université Laval, Canada*

Tigecycline (TGC) is a broad spectrum antibiotic acting on the 30S ribosomal subunit by blocking prokaryotic translation. Developed primarily in response to the increased prevalence of multidrug-resistant bacteria such as Acetinobacter baumanii and Staphylococcus aureus, it has a good activity against many anaerobes, Gram-negative bacteria, and Gram-positive bacteria, including Streptococcus pneumoniae. Even if the resistance rate toward this antibiotic is still low worldwide, the emergence of TGC non-susceptible A. baumannii and Enterobacteriaceae has now challenged the lifespan of this last-resort antibiotic. The aim of this project was to characterize TGC resistance mechanisms in S. pneumoniae, using two S. pneumoniae R6 TGC-resistant mutants selected in vitro by whole genome sequencing (WGS) and resistance reconstruction. The S. pneumoniae R6M1TGC and R6M2TGC mutants were selected in broth until they were resistant to 8ug/mL TGC. Both mutants also demonstrated cross-resistance to tetracycline (TC) and minocycline (MI). The efflux pump inhibitor reserpine didn't sensitize both mutants to TGC, TC and MI, suggesting that no efflux pumps were involved in antibiotic resistance. WGS of R6M1TGC and R6M2TGC revealed the presence of seven commonly mutated genes, from which four were linked to TGC and TC resistance in both mutants by resistance reconstruction. Indeed, the introduction of the mutations in the ribosomal protein S10 and the 16s ribosomal RNA genes, sequenced from R6M1TGC and R6M2TGC, in a R6 sensitive background decreased the susceptibility to TGC and TC in the transformant strains. Moreover, the introduction of a R6 wild-type allele in the ribosomal protein S3 gene and spr1784, coding for a RNA methyltransferase, into R6M1TGC and R6M2TGC genome sensitized both mutants to TGC and TC. This study highlighted new mechanisms of resistance to TGC and we think is the first report of TGC resistance mechanisms in S. pneumoniae.

Poster Session

BAM-PTH1030 - Generalized transduction in vitro among Staphylococcus spp., using a novel phage S6

Jumpei Uchiyama[1], Iyo Takemura-Uchiyama[2], Yoshihiko Sakaguchi[3], Shin-ichiro Kato[1], Keiji Gamoh[1], Naoaki Misawa[4], Masanori Daibata[1], Shigenobu Matsuzaki[1]

[1]Research and Education Faculty, Kochi University, Kochi, Japan, [2]Kochi University Graduate School, Kochi, Japan, [3]Interdisciplinary Research Organization, University of Miyazaki, Miyazaki, Japan, [4]Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

Background: Methicillin-resistant Staphylococcus aureus (MRSA) causes serious infections in clinical and community settings. The recent study has shown that the methicillin-resistant gene is originated from animal-derived methicillin-resistant Staphylococci. One of the possible lateral transfer of the methicillin-resistant gene is the generalized transduction in the environment. No study has been conducted on the generalized transduction among Staphylococcus spp. In this study, the generalized transduction from non-aureus Staphylococci spp. to S. aureus, and vice versa, was examined using a newly-isolated phage S6. Methods and materials: (I) Morphology, genome size, and nucleosides of genomic DNAs of phage were examined. (II) Phage propagated on the staphylococci harboring a plasmid pCU1 or MRSA strain was cultured with the recipient cells, and the transductants were selected by antibiotics (20 μg/ml chloramphenicol, or 5 μg/ml oxacillin). Results: Phage S6 is a novel giant staphylococcal phage containing ca. 270 kbp dsDNA genome. In the genome DNA, thymine was totally replaced with uracil for nucleic acid base. Using phage S6, the transduction was observed from S. aureus to non-aureus Staphylococcus spp., and vice versa. The transduction of methicillin resistance was also observed from MRSA strain COL to S. aureus strain RN4220. Thus, the generalized transduction can occur among Staphylococcus spp. Conclusion: The generalized transduction mediated by S6-like phage may have contributed on the emergence of the MRSA

Poster Session

BAM-PTH1032 - Bacteria and bacteriophages in the myocardium are related with severity of myocardial rejection

Marcia M Reis[1], Rafael Ferreira[1], Renata N Ikegami[1], Joyce T Kawakami[1], Suely AP Palomino[1], Pablo Pomerantzeff[1], Sandrigo Mangini[1], Edimar Bocchi[1], Maria de Lourdes Higuchi[1]
[1]*Heart Institute of Clinical Hospital, Medical School, University of São Paulo, São Paulo, SP, Brazil*

Purpose- Cardiotropic bacteria and many viral DNAs were described in Dilated Cardiomyopathy (DCM). In previous study we found in DCM biopsies, human viral and bacterial antigens and at electron microscopy, viral microparticles in the cytoplasm of bacteria , suggesting that these are bacteriophages. In the present paper we studied if these infectious agents proliferated in the donor heart after heart transplantation (HT), during episodes of myocardial rejection (MR) and caused resistance to pulse therapy (PT). Methods - 13 patients with DCM had their endomyocardial biopsies (EMBs) revised in 60 days post HT to determine by immunohistochemistry the % antigens' area against Borrelia burgdorferi (Bb), Mycoplasma pneumoniae (Mp), HBs, HCV, PVB19, and HHV6. EMBs were grouped in: G1 – donnor heart zero time after HT; G2- absent or mild MR, G3 – moderate MR, G4 - healing MR and G5 -persistent MR. Results – The mean % area of bacterial antigens in groups G1, G2, G3, G4 and G5 were: of Bb 2.51, 18.73, 17.22, 7.26 and 24.93 and of Mp: 22.13, 1.95, 1.82, 1.1 and 2.17. There was an increase in Bb and viral levels in G3 than in G1 ($P <0.01$) and decrease after PT in G4. In G5 occurred increase in Bb ($P <0.01$) and Mp ($P=0.09$) levels; there were Mp correlations with levels of many viral antigens: HBs, HCV and HHV6 and also correlations between viruses HHV6 vs PVB19 ($r=0.7$, $P<0.05$) and HHV6 vs HCV ($r=0.90$, $P<0.001$). In G4, correlation occurred only between Bb vs HHV-6 ($r=0.78$, $P=0.02$). Conclusion - Beyond autoimmunity, MR seems to be a myocarditis associated with Bb and HHV-6, which regresses with PT. In persistent MR occurs increase in levels of Bb and Mp in correlation with many viruses, suggesting that these are bacteriophages and may have a role in PT resistance in treatment of MR.

Poster Session

BAM-PTH1036 - Isolation and characterization of Salmonella bacteriophages isolated from zoonotic specimens

Beata Dziedzic[1], Beata Weber-Dabrowska[1,2], Marta Kiejzik[1], Maciej Zaczek[1], Agnieszka Kopciuch[1], Barbara Owczarek[1], Ewa Jonczyk-Matysiak[1], Aneta Kurzepa-Skaradzinska[4], Wieslaw Londzin[5], Andrzej Gorski[1,2,3]
[1]Laboratory of Bacteriophages, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland, [2]Phage Therapy Unit, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland, [3]Department of Clinical Immunology, Transplantation Institute, Medical University of Warsaw, Warsaw, Poland, [4]Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences, Wroclaw, Poland, [5]Skotan SA, Chorzow, Poland

Samples from the human and animal environment have been proved to contain specific bacteriophages for many pathogens. Salmonella is the major cause of foodborne diseases worldwide and farm animals are considered to be the main reservoir of this pathogen. The use of lytic phages to fight foodborne pathogens such as Salmonella is considered to be a promising alternative for using antibiotics in animal farms. A total of 111 samples from farm animal environments were collected in order to investigate the presence of Salmonella phages. Raw samples as well as those incubated in peptone water were used. For the examination of lytic activity of newly isolated bacteriophages we used the collection of 260 Salmonella sp. strains which included reference strains obtained from the Collection of Salmonella Microorganisms, Gdańsk, Poland and strains isolated from people and food by Sanitary and Epidemiological Stations in Poland. As a result of this study no Salmonella strains were found in the samples but 37 new Salmonella phages were isolated. For further investigations 7 phages have been tested so far for their lytic activity. The broadest host range was exhibited by the 4N/3043 phage of S. Enteritidis and S. Typhimurium strains 98.69% and 90.19% respectively. It also lyses most other Salmonella serovars with the host range varying from 60% (S. Hadar, S. Virchow) to 100% (S. Goldcoast, S. Agona) and single examined strains of other serovars. We isolated also 8N/270/S phage which was able to lyse 100% of S. Virchow and S. Infantis strains but no strains of S. Typhimurium. The results of the above studies suggest that newly isolated phages have the potential to reduce the number of Salmonella in animal environments. This study is conducted within grant “Use of bacteriophages to develop antibacterial preparations in veterinary" (POIG.01.04.00-24-133/11) funded by The Polish Agency for Enterprise Development.

Poster Session

BAM-PTH1038 - Comparative study of the effect of virgin olive oil and butter on the intestinal microbiota and diverse parameters related to metabolic syndrome

Isabel Prieto[1], Marina Hidalgo[1], Antonio Cobo[1], Ana Belén Segarra[1], Manuel Ramírez[1], Antonio Gálvez[1], Francisco Vives[2], Magdalena Martínez-Cañamero[1]

[1]University of Jaén, Jaén, Spain, [2]University of Granada, Granada, Spain

It is clear now that intestinal microbiota varies according to diet and has a role in the development of metabolic syndrome. However, there are few comparative studies using fat with different degree of saturation and none after following a diet enriched with virgin olive oil. Our objective was to demonstrate that a high virgin olive oil diet has a distinctive effect on intestinal microbiota in comparison with a high butter diet and that this effect has a correlation with the physiological benefits exerted by virgin olive oil. For three months, we have fed mice with two high fat diets (virgin olive oil and butter) and with a standard diet. Different metabolites and hormonal systems related to the glucose control, lipid metabolism, energy balance, body weight, blood pressure regulation and endothelial function were monitored. At the end of the experiment, DNA was extracted from faeces and libraries were constructed in order to analyse the different gut microbiomes by large-scale pirosequencing the V3-V5 regions of the 16S rRNA gene on the 454 platform. Butter enriched diet produced higher accumulation of visceral adipose tissue, body mass before sacrifice and blood pressure levels. Diet supplemented with virgin olive oil was associated to lower levels of triglycerides in plasma, a higher HDL/LDL ratio and lower values in blood pressure. These values correlated with the percentage of different microbial groups, opening the door to the possibility that certain beneficial effects of virgin olive oil are produced through modulation of intestinal microbiota. Additional experiments will be presented to prove this hypothesis. Acknowledgements: This work has been supported by the Andalusian Government (Consejería de Economía, Innovación y Ciencia. Proyecto de Excelencia Ref. AGR 6340).

Poster Session

BAM-PTH1040 - Spatial and temporal metagenomic classification of sediment prokaryotic communities inhabiting a hyper-saline estuary, the Laguna Madre (Texas, USA)

Meghan Guzman[1], Erin Schuenzel[1], Kristine Lowe[1]
[1]University of Texas - Pan American, Edinburg, USA

The Laguna Madre is a rare, subtropical, high-salt, estuary in Texas, USA. The Laguna Madre is only 1 of 5 hypersaline estuaries in the world and provides habitat to many rare, endangered, and migratory organisms. The macro-ecology of the ecosystem has been the focus of numerous past studies but little is known regarding the microbiology of the estuary. For this study, we determined the spatial and temporal composition of prokaryotic communities inhabiting the sediment of the Laguna Madre. Sediment samples were collected from 8 locations in the Laguna Madre in 2010, 2011, and 2012. Genomic DNA (gDNA) was extracted from the sediment using a phenol/chloroform protocol. The gDNA was amplified by PCR using 16S rRNA primers then sequenced using an Illumina MiSeq next-generation sequencer to identify the major taxonomic groups of prokaryotes in the sediment. Sequence data of the 8 locations were compared to elucidate spatial differences (site-to-site) and temporal changes (year-to-year) in the sediment community composition. Results showed the presence of several different phyla (e.g., Actinobacteria, Proteobacteria, Firmicutes, and others) comprising the sediment community and both spatial and temporal differences were observed. The study provides the first metagenomic analysis of sediment prokaryotes in this rare ecosystem and demonstrates that the sediment is inhabited by a diverse and dynamic microbial community.

Poster Session

BAM-PTH1042 - Taxonomic diversity in different culture periods and relationship between microbial communities with soil chemical composition

Yeon-Ju Kim[1], Nguven Ngoc Lan[1], Deok-Chun Yang[1]
[1]Kyung Hee University, Yongin-si, South Korea

The total of 212,397 archaeal 16S rRNA, 171,589 bacterial 16S rRNA and 126,617 28S rRNA sequences reads were obtained from all 33 soil samples with average of 6436, 5199 and 3836 reads per sample, respectively. Based on the relationship between microbial communities with soil chemical composition, pH and exchangeable Ca was decreased and varied in second cultivation in continuous cultured soil. Exchangeable Mg and Na were higher in first than second cultivation, but available P2O5 was low. Exchangeable Na and available NO3-N had highly positively correlation with electrical conductivity (EC). Organic matter (OM) and available P2O5 were positively related and negatively related with exchangeable Mg that also had highly correlated with exchangeable Ca. So in cultivation of ginseng, phosphorus should be supplied adequately. We differentiated microbial diversities in first and re-cultivation, we found Methanomicrobia, Verrucomicrobia, Pseudeurotiaceae, Chaetomiaceae, Bionectriaceae, Mollisia, Lachnum and Montagnulaceae exhibited higher amount in first cultivation. Whereas Nitrososphaera, Acidobacteriaceae, Anaerolinaceae, Plantomycetes, Cyanobacteria, Mortierella, Phyllosticta and Hypocreaceae presented higher in re-cultivation. Caldilineae, Burkholderiales, Plantomycetes, Verrucomicrobia, Pseudeurotiaceae, Chaetomiaceae were bad indicator with high occupancy in low yield of ginseng. And Cenarchaeales, Nitrososphaera, Acidobacteriaceae, Solibacteraceae, Rhizobiales, Cyanobacteria, Rhodospirillades, Hypocreaceae, Phyllosticta and Bionecteriaceae. Methylophilaceae were also higher in 6 years old soil as bad indicators. As a result we report the microbial community in different ginseng field and microbial relationship with soil elements.

Poster Session

BAM-PTH1044 - Isolation and characterization of soil microbes degrading lobster shell wastes to chitin derivatives

Gayathri Ilangumaran[1], Balakrishnan Prithiviraj[1]
[1]*Dalhousie Univeristy, Truro, Canada*

The lobster industry contributes substantially to the economy of Atlantic Canada. Lobster processing industries generate large amount of shell wastes and pose environmental and financial challenges in waste management. Chitin, a biopolymer of N-acetyl-D-glucosamine (GlcNAc) is one of the major constituents in lobster shells. Chitin and its deacetylated derivative chitosan are known for their wide applications in research and development and are commercially extracted through a series of acid and alkali treatment of crustacean shells. The project explores the use of microorganisms as an eco-friendly approach to produce chitin derivatives from lobster shells. Microbes from soil samples were isolated and screened on culture media containing lobster shell powder as the sole carbon source. Significant levels of deproteinization, demineralization and chitinase activities indicated degradation of the lobster shell in vitro. Among the 10 different soil isolates examined, S223 and S224 were chosen the best and their culture conditions were optimized for maximum degradation efficiencies. The two isolates differ in colony morphology, pigmentation and enzymatic degradation. Gram staining and preliminary microbiological investigations revealed that they belong to Actinobacteria. Characterization of these microbes at better taxonomical resolutions and enzyme metabolism are currently in progress. The study strongly suggests that the natural soil microbial diversity could better assist the sustainable degradation of the tough chitin rich shells. The digested lobster shell extracts are used in model plant Arabidopsis to study the level of immune elicitation and simultaneous disease resistance against Pseudomans syringae and Botrytis cinerea by their application. Keywords: Chitin, chitosan, biodegradation, plant immunity

Poster Session

BAM-PTH1046 - Ferulate metabolism in Rhodococcus jostii RHA1

Hiroshi Otani[1], Young E. Lee[1], Jie Liu[1], Lindsay D. Eltis[1]
[1]*Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada*

Ferulate, a component of the plant cell wall, has a number of commercial applications. The soil bacterium Rhodococcus jostii RHA1 is able to utilize ferulate as sole organic growth substrate. A vanA mutant was unable to grow on ferulate, indicating that RHA1 first converts this compound to vanillate then further catabolizes it via the beta-ketoadipate pathway. Transcriptomic and bioinformatics analyses revealed a cluster of genes that were highly up-regulated during growth on ferulate and that are likely involved in ferulate catabolism. These genes are predicted to encode four enzymes (FerLMNO), a major facilitator transporter (FerT) and a MarR-family transcriptional regulator (FerR). Purified FerL catalyzed the thioesterication of ferulate to feruloyl-CoA, the first reaction of ferulate catabolic pathway. FerL also catalyzed the thioesterication of p-coumarate, dihydroferulate and caffeate. A ferL mutant was unable to grow on ferulate. Four transcriptional start sites of the ferulate catabolic regulon were identified using either RNA-seq or 5'RACE. All four possess a conserved inverted repeat sequence separated by a 5-bp spacer (aatcATTGA-5n-TCAATcatt) that overlaps the –35 consensus element. We hypothesize that these inverted repeats are bound by FerR. Bioinformatic analysis further predicted that feruloyl-CoA is an effector of FerR, antagonizing the binding of FerR to DNA. Overall, our studies reveal a novel ferulate catabolic pathway. Knowledge of this catabolism should facilitate the development of biocatalysts to augment the production of ferulate from plant biomass.

Poster Session

BAM-PTH1048 - Biodegradation of aromatic-aliphatic copolyesters and polyesteramides by esterase activity producing microorganisms

Cenek Novotny[1], Hana Sezimova[2], Katerina Malachova[2], Zuzana Rybkova[2], Ashutosh Verma[2], Lenka Malinova[3], Irena Prokopova[3], Jiri Brozek[3]

[1]Institute of Microbiology ASCR, Prague, Czech Republic, [2]University of Ostrava, Ostrava, Czech Republic, [3]Institute of Chemical Technology, Prague, Czech Republic

Biodegradability of two types of synthetic polymers, polyesteramides prepared by anionic ring-opening copolymerization of ε-caprolactone and ε-caprolactam and aromatic-aliphatic copolyesters (PETP/LA) synthesized by solvolysis of poly(ethyleneterephthalate) with water solutions of lactic acid, by microorganisms producing extracellular esterase and lipase was investigated. Pseudomonas aeruginosa, Candida guilliermondii and Aspergillus fumigatus exhibiting strong esterase and lipase activities on agar plates were selected to be used in six-week degradation experiments carried out at 28°C. PETP/LA samples exhibited mass reductions of up to 5-10% in both the presence of the three microorganisms and in abiotic controls where the polymers were exposed only to Nutrient Broth or malt extract-glucose medium. Scanning electron microscopy revealed breaks in PETP/LA fibres when polymers were exposed to A. fumigatus and C. guilliermondii, attributable to microbial action. Polyesteramides were resistant to biodegradation. Degradation of both polymer types resulted in a 5-10-fold increase of toxicity of culture supernatans measured with Vibrio fischeri and Sinapis alba tests, as compared to the biotic and abiotic controls. No genetic toxicity was detected with Salmonella typhimurium His- test. The study suggested that the ecotoxicity of compounds produced by biodegradation of polymers should be monitored. Supporting projects: Czech Science Foundation 106/09/1378, Ministry of Agriculture CR QJ1210165, EU projects: BIOCLEAN 312100, IET CZ.1.05/2.1.00/03.0100, OPVK CZ.1.07/2.3.00/30.0019.

Poster Session

BAM-PTH1050 - Improvement of activity and immobilization of organophosphorus hydrolase for the decontamination of nerve agents

Kosuke Nakayama[1], Satoshi Ishikawa[1], Takeshi Ohmori[2], Yasuo Seto[2], Kazuyoshi Kawahara[1]
[1]Kanto Gakuin University, Yokohama, Japan, [2]National Research Institute of Police Science, Kashiwa, Japan

Organophosphorus hydrolase (OPH) produced by Sphingobium fuliginis(formally Flavobacterium sp.) ATCC 27551 can hydrolyze a variety of organophosphorus compounds with P-O, P-S, P-F, and P-CN bonds. The gene that encodes OPH was cloned, and the OPH enzyme was produced in Escherichia coli. OPH can hydrolyze several nerve agents such as sarin, tabun, and soman as well as organophosphorus pestisides, but it has only weak activity toward O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate (VX). In the present study, therefore, some amino acids in OPH were substituted to improve the hydrolytic activity of OPH toward nerve agents, especially VX. We focused the position of substitution to Leu at 136, Tyr at 254, His at 257 from N-terminal, because these amino acids were reported to influence the activity of OPH. Site-directed mutagenesis was performed by using DNA primers designed for nucleotide substitution and commercially available mutagenesis kit. The activities of the crude enzyme preparations of mutant enzymes were compared with that of the wild-type enzyme, and the enzyme with the substitution form Tyr to His at 254 was proved to exhibit the highest activity. It could hydrolyze most of the nerve agents almost completely, and approx. half of VX during 20 min when the enzyme was activated with $CoCl_2$. Other mutant enzymes showed the similar activity with or weaker activity than that of the wild-type enzyme. In the next experiment, the mutant OPH with His-Tag at N-terminal was purified using a nickel-chelating column, and immobilized to CNBr-activated Sepharose 4B. The immobilized enzyme showed only attenuated activity toward paraoxon or some nerve agents, suggesting that the chemical linkages formed during immobilization disturbed the activity. The immobilization without chemical reaction is now under investigation using other material such as porous silica for the practical use of OPH.

Poster Session

BAM-PTH1052 - Degradation of antimicrobial resistance determinants during windrow composting and stockpiling of manure from cattle administered sub-therapeutic antimicrobials

Shanwei Xu[1], Srinivas Sura[2], George Wang[3], Alanna Smith[1], Rahat Zaheer[1], Shaun Cook[1], Andrew Olson[1], Allan Cessna[2], Francis Larney[1], Tim McAllister[1]

[1]Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Canada, [2]Agriculture and Agri-Food Canada, Saskatoon, Canada, [3]University of Lethbridge, Lethbridge, Canada

Veterinary antimicrobials in livestock manure could select for antimicrobial resistant microbes in the environment. Windrow composting or stockpiling has been shown to reduce the viability of pathogens and the levels of antimicrobial residues in manure. However, the impact of these manure management practices on the persistence of genes coding for antimicrobial resistance is less well known. In this study, manure from beef cattle administered either 44 mg of chlortetracycline kg^{-1} feed (dry-weight basis) (CTC), 44 mg of chlortetracycline + 44 mg of sulfamethazine kg^{-1} feed (CTCSMZ), 11 mg of tylosin kg^{-1} feed (TYL) or no antimicrobials (Control) was windrow composted or stockpiled over 101 days. Temperature remained ≥ 55°C for 39 days in compost windrows and 5 days in stockpiles. Quantitative PCR was used to measure the levels of 16S rDNA and 11 resistance determinants for tetracycline (tet), erythromycin (erm), and sulfamethazine (sul) during the experimental period. The concentration of 16S rDNA decreased ($P < 0.05$) over the experimental period with a ranking of Control > CTC = TYL > CTCSMZ in both windrows and stockpiles. Similarly, levels of all resistance determinants decreased after 101 days, with the reductions of Log_{10} copies (g dry matter)$^{-1}$ in ranges of 0.8-2.2, 1.2-2.8, and 0.5-1.1 for tet, erm, and sul genes, respectively. Adminstration of antimicrobials did not increase the abundance of resistance genes with the exception of tet(M), tet(C) and sul(2) in CTCSMZ ($P < 0.05$) as compared to Control. More ($P < 0.05$) reductions in 16S rDNA and resistance determinants were observed in the composted than stockpiled manure. Our findings conclude that windrow composting may be more effective than stockpiling in reducing the introduction of genes coding for antimicrobial resistance into the environment upon land application of manure.

Poster Session

BAM-PTH1054 - Cell dispersion culture for the effective growth of Humicola insolens and efficient enzyme production

Matsumoto Hitoshi[1], Keisuke Koganei[1], Yoshiyuki Koyama[2], Sanshiro Saito[3], Hisashi Kataoka[3], Jun Ogihara[1], Takafumi Kasumi[1]

[1]Department of Chemistry and life Science, Kanagawa, Japan, [2]National Food Research Institute, Ibaraki, Japan, [3]J-oil Mills, Inc, Kanagawa, Japan

Enzymes from Humicola sp. hold promise for biomass degradation, especially of lignocellulosic materials such as rice straw, wood chips, and corn stover. However, the strain is difficult to disperse; hence, an aggregated form of the fungus in liquid culture media is generally used, resulting in poor control of the growth process and low enzyme production. This has greatly limited the utilization of this strain, in spite of its potential as an enzyme producer. We have therefore developed a method for promoting effective growth and efficient enzyme production (cell dispersion culture) using a medium containing extruded soybean meal as a nitrogen source. A patent application for this method has been submitted. Surprisingly, the addition of extruded soybean meal improves mycelium dispersion and enzyme production of H. insolens, and the dispersive effect is applicable to other fungi such as Trichoderma and Aspergillus sp. In contrast, defatted soybean meal not treated by an extrusion process has little effect on cell dispersion. The efficacy of dispersion may therefore be related to the three-dimensional structure of the soybean meal resulting from the manner of processing. Extruded soybean meal has a specifically organized structure due to the heat and high-pressure treatment provided by the extruder used after drying and milling. In contrast, untreated soybean meal has no such organized structure because it does not go through an extrusion process. The detailed mechanism by which the dispersive function affects the structure of extruded soybean meal is presently being investigated. Use of the cell dispersion culture process significantly improves the enzyme activity of H. insolens; cellulase levels were 5.8 times higher and xylanase levels were 3.5 times higher than those obtained using ordinary culture methods. In addition, enzyme productivity was markedly enhanced when the fungus was cultured using a jar fermenter.

Poster Session

BAM-PTH1056 - Efficient function and enzymatic bioconversion of GH10 xylanase from Gloeophyllum trabeum

Ho Myeong Kim[1], Hyeun-Jong Bae[2]
[1]Department of Bioenergy Science and Technology/Chonnam National University, Gwangju, South Korea, [2]Department of Bioenergy Science and Technology, Bio-Energy Research Center/Chonnam National University, Gwangju, South Korea

Recently, many studies have explored the development of hydrolytic enzymes, which play an important role in degradation of lignocellulosic biomass for bioethanol production. We conducted the cloning of xylanase gene (Xyl10g) from Gloeophyllum trabeum, and then expressed in Pichia pastoris GS115. The purified Xyl10g has a molecular weight of approximately 50 kDa, and exhibits maximum specific activity at 70°C and pH 4.0 to 7.0. Xyl10g showed many advantages despite low specific activity compared to XynA, including efficient bioconversion of lignocellulosic biomass. According to immunogold labeling analysis, purified recombinant Xyl10g can efficiently degrade highly substituted xylans as well as unsubstituted and low-substituted xylans. However, XynA cannot efficiently attack highly substituted xylans. Also, Xyl10g can efficiently hydrolyze popping-pretreated corn stover and newspaper waste, and showed synergistic effects in the presence of Cel5B (endoglucanase) and BglB (β-glucosidase). These results suggest that GH10 Xyl10g is a suitable candidate for lignocellulose degradation.

Poster Session

BAM-PTH1058 - *Lysinibacillus sphaericus* and *Geobacillus sp* biodegradation of petroleum hydrocarbons and biosurfactant production.

Lina Manchola[1], Jenny Dussán[1]
[1]University of Andes, Bogotá , Colombia

Petroleum oil is a major driver of worldwide economic activity, but it has also created contamination problems during the storage and refining process of this commercial fuel. Also unconventional resources are natural resources which require greater than industry-standard levels of technology or investment to exploit. In the case of unconventional hydrocarbon resources, additional technology, energy and capital have to be applied to extract the gas or oil. Bioremediation of petroleum spillovers are considered of great importance due to their contaminating effects on human health and the environment. For this reason it is important to reduce total petroleum hydrocarbons (TPHs) in a contaminated soil. In addition, biosurfactant production is a desirable property of hydrocarbon-degrading microorganisms. Seven strains belonging to *Lysinibacillus sphaericus* and *Geobacillus sp* were selected to evaluate their ability to biodegrade TPHs in the presence of toxic metals, their potential to produce biosurfactants and their ability to improve the biodegradation rate. The seven bacterial strains examined in this study, were able to use crude petroleum-oil hydrocarbons as sole source of carbon and energy, their ability to degrade crude oil was not affected by the presence of toxic metals such as chromium and arsenic. At the same time, the strains were able to reduce toxic metals concentration. Biosurfactant production was confirmed using the drop-collapsed method for all strains and they were characterized as an anionic and cationic biosurfactants. Biosurfactants showed an increase in biodegradation rate both in liquid minimal salt medium and landfarming treatments. The final results in open field showed an efficiency of 93% in TPHs degradation by the selected consortium compared to soil without consortium, proposing a *L. sphaericus* and *Geobacillus sp* consortium as a good solution to remediate contaminated soils.

Poster Session

BAM-PTH1060 - Growth promoting compound produced by Sphingomonas sp. (GF9) for vigorous growth of uncultured Catellibacterium nectariphilum (AST4T)

Mohammad Nazrul Islam Bhuiyan[1], Shinya Mitsuhashi[2], Kengo Shigetomi[2], Yoichi Kamagata[2], Makoto Ubukata[2]
[1]Graduate School of Agriculture, Hokkaido University, Sapporo-City, Japan, [2]Research Faculty of Agriculture, Hokkaido University, Sapporo-City, Japan

Researchers have tried to improve methods to recover bacteria from environment by providing conditions such as pH, temperature, and nutrition. However, more than 99% of bacteria are still uncultured under laboratory media. Therefore, we focused on the relationship between the “uncultured bacteria” and “other microbes”. In the natural environment, the “other microbes” may provide growth factors to “uncultured bacteria”. Catellibacterium nectariphilum, designated AST4T, is a gram negative, ovoid to rod-shaped and white to beige color bacterium. This bacterium didn’t show significant growth on nutrient broth, but growth was clearly enhanced by the addition of supernatant from Sphingomonas sp. (GF9). In this study, we tried to isolate these unknown compounds, which promoted the growth of AST4T in artificial culture. After harvesting 66.0 liter of GF9 in jar fermentor, the broth was centrifuged and filtered to separate the mycelium from the liquid phase (supernatant). This supernatant was extracted using 1:3 ratio with 1-butanol (3 times). The aqueous phase was evaporated and the resulting dried samples were extracted using MeOH. The MeOH soluble phase was evaporated, dried and subjected to HP20 resin and was eluted with 50% MeOH (0.1% AcOH). The active fraction was fractionated on sephadex LH20 with 50% MeOH (0.1% AcOH). The resulting active fractions were further clean up by solid phase extraction (ODS), and dried fraction was subjected to HPLC (ODS) for isolation of active compounds. After bioassay, we found two active peaks on HPLC. These active peaks were subjected to LC-ESI-MS and comprehensive NMR for structure elucidation. The purified active compound showed growth activity at 1.0 ng/ml. These results may contribute the exploitation of the new functions of the previously inaccessible bacteria.

Poster Session

BAM-PTH1062 - Effect of biotic and nano elicitors on sanguinarine gene expression in Papaver somniferum L. suspension cell cultures

Mansoor Omidi[1], Mahdiyeh Khodayari[1], Ali-Akbar Shahnejat-Booshehri[1], Darab Yazdani[2], Mohammad-Reza Naghavi[1]

[1]University of Tehran, Karaj, Iran, [2]Iranian Institute of Medicinal Plants, ACECR, Karaj, Iran

To develop an optimal bioprocess for benzophenanthridin alkaloids production in papaver somniferum L. various metabolites engineering are studied to increase yields, in which one of them is elicitation. Elicitors have effectively stimulated the expression of genes involved in secondary metabolites biosynthesis pathway in cell and organ suspension cultures. The effects of two biotic elicitors (yeast extract and chitosan) and two nano-elicitors (nano TiO2 and nano-Ag), were studied at different time points on the most important genes expression involved in sanguinarine biosynthesis pathway in shoot meristem and root suspension cell cultures. The highest increasing in gene expression was observed in early hours treatment with nano eliciotrs, in which that the most expression changes was remark in bbe1 and DBOX, and the minimal one was in tydc7 and DIOX2 in both root and meristem suspension cell cultures. According to the nano particles size, they cause the rapid rises in gene expression and alkaloid production in the shortest possible time. This study showed that the kind of explants has the different effect on expression of genes in cell suspension cultures which maybe regarded to explants origin and its cellular memory.
Keywords: Gene expression, Nano-elicitor, Biotic elicitor, Explants.

Poster Session

BAM-PTH1064 - Relationship between Monascus pigment production of Penicillium purpurogenum and phylogenetic clade based on rDNA ITS sequences

Noriyuki Iwabuchi[1], Teppei Arai[2], Ryo Kojima[2], Takafumi Kasumi[2], Jun Ogihara[2]
[1]Department of Applied Biological Science, Fujisawa, Japan, [2]Department of Chemistry and Life Science, Fujisawa, Japan

The production of pigments as secondary metabolites by microbes is known to vary by species and by physiological conditions within a single strain. The fungus strain Penicillium purpurogenum IAM 15392 has been found to produce Monascus pigment homologs, violet pigment (PP-V) and orange pigment (PP-O), when grown under specific culture conditions in PP-V production media or PP-O production media, respectively. In this study, we analyzed PP-V and PP-O production capability in seven strains of P. purpurogenum in addition to strain IAM15392 under specific culture conditions. The pigment production pattern of five strains cultivated in PP-V production medium was similar to that of strain IAM15392, and all violet pigments produced by these five strains were confirmed to be PP-V. Strains that did not produce pigment were also identified. In addition, two strains cultivated in PP-O production medium produced a violet pigment identified as PP-V. The ribosomal DNA (rDNA) internal transcribed spacer (ITS) region sequences from the eight P. purpurogenum strains were sequenced and used to construct a neighbor-joining phylogenetic tree. The phylogenetic tree showed four distinct clusters. Pigment production of P. purpurogenum was shown to be related to phylogenetic placement based on rDNA ITS sequence. Based on these results, two hypotheses for the alteration of pigment production of P. purpurogenum in evolution were proposed.

Poster Session

BAM-PTH1066 - Identification of bioactive compounds with anti-malarial and GSK-3 inhibitory activities from soil actinomycete isolated from Sabah, Malaysia rainforests

Ping Chin Lee[1], Fauze Mahmud[1], Siew Eng How[1], Jualang Gansau[1], Hasidah Sidek[2], Mohd Noor Embi[2]
[1]Universiti Malaysia Sabah, Malaysia, [2]National University of Malaysia, Malaysia

Sabah, Malaysia also known as North Borneo is one of twelve mega biodiversity region in the world. More than 60 % of its landmass is still covered with forested area. Different types of forests can be found in Sabah such as the virgin Dipterocarp forests and coastal forests. It has diverse species of flora and fauna including microbial diversity that offers huge potential yet to be discovered particularly as a major source for drugs. Actinomycetes are prolific producers of a diversity of bioactive secondary metabolite that have contributed to huge number of important drug discovery. Their distributions and secondary metabolites production are influenced by temperature, soil humidity, pH and nutrient contents; mostly from plants. We report here the identification of a few potential bioactive compounds from actinomycetes isolated from soils collected in different type of forests in Sabah. We have screened for their potential as Glycogen Synthase Kinase-3 (GSK-3β) inhibitor and antimalarial drug. A total of 598 actinomycetes and 352 microfungi were isolated from 157 soil samples using HVA and PDA, respectively. In the screening, 14 actinomycete and 12 microfungi extracts showed positive GSK-3 inhibitory activity; 21 bacterial and 6 microfungi extracts were toxic against mammalian GSK-3β. The extracts from two of the actinomycetes strains were chosen for further fractionation and purification using a bioassay-guided purification approach. Identification of the bioactive compounds were performed using HPLC, LC-MS, GC-MS and DAS-TOFF-MS.

Poster Session

BAM-PTH1068 - Actinomycetes and polyketide synthases in contrasting terrestrial environments

Patrick Hill[1], Christopher Boddy[2]
[1]Department of Biology, University of Ottawa, Ottawa, Canada, [2]Department of Chemistry, University of Ottawa, Ottawa, Canada

Actinomycetes are the major producer of most known polyketide antibiotics. Discovering new polyketide antibiotics involves screening large collections of either isolates or metagenomic clones from the environment for novel antibiotic activity or polyketide sequences. Effective bioprospecting for polyketides means finding the most chemical diversity with the fewest samples. This study characterized environmental DNA from contrasting environments with primers that were specific to the Eubacterial and Actinobacterial 16S ribosomal subunit and type I and II polyketide synthases (PKSI and PKSII). Environmental samples were from contrasting land uses (forest, grassland, cultivated land, street surfaces, compost) from arctic, temperate and tropical climates. Unlike the Eubacteria whose community structure is controlled by soil pH, actinobacterial community structure in soils was controlled by land use. Actinobacterial 16S amplicons from cultivated soil were more diverse than amplicons from forest or grassland soils. Eubacterial 16S amplicons from street dust were enriched in Actinomycetes as compared to soil. Soil Amplicons from PKSI primers were mainly non-actinomycetal and contained several novel AT domain motifs. PKSI amplicons from street sediments were actinomycetal. PKSI amplicons clustered by environment, with well defined clusters for soil, street sediment, worm guts and vermicompost. Type II PKS amplicons were cosmopolitan, both geographically and between contrasting environments, e.g. identical sequences were found in street sediments from Faisalabad, Pakistan and forest soils in Canada and Eurasia. PKSII diversity was greater in cultivated than forest or grassland soils. Results suggest that bioprospectors should identify novel bacterial and actinomycetal habitats rather than exhaustively sample any one habitat such as soil.

Poster Session

BAM-PTH1070 - Marine algae-actinobacteria association: a particularly promising renewable system for the production of new antibacterial metabolites

Mouloud Kecha[1], Ibtissem Djinni[1], Andrea Defant[2], Ines Mancini[2]
[1]University Abderahamane MIRA ,Bejaia, Algeria, [2]University of Trento, Italia

This study was designed to investigate whether culture conditions (media, seawater concentration and pH), could lead Streptomyces sundarbansensis strain,(isolated from marine brown algae Fucus sp. collected from Algerian coastline) to produce bioactive secondary metabolites. The most favorable condition for production the anti-MRSA compound 1 [2-hydroxy-5-((6-hydroxy-4-oxo-4H-pyran-2-yl)methyl)-2-propylchroman-4-one] was determined. The profile of metabolites present in the crude extracts was carried out by HPLC analysis equipped with a Diode Array Detector- Evaporative Light Scattering Detection (DAD-ELSD) or online coupled to ElectroSpray Ionisation-Mass Spectrometry (ESI-MS). Compound 1 resulted the most abundant secondary metabolite by culture on SCA medium in freshwater or 50% seawater at pH 7 or 9 using agar state fermentation method. The study has shown the efficiency of HPLC/ESI-MS technique in the analysis of polyketides produced by the strain under investigation. It was possible to establish the best culture conditions for obtaining the most bioactive compound 1, previously isolated by the same strain. Marine algae-actinobacteria associations are a particularly promising renewable system for the production of new antibacterial metabolites. Based on the promising bioactivity of the chemically characterized compound 1, the analytical methodology here applied has resulted as an effective approach for establishing its optimized production.

Poster Session

BAM-PTH1072 - Identification of toxin-antitoxin systems in P. aeruginosa, their role in persistence

Marco Garavaglia[1], Patrick Scheu[4], Urvish Trivedi[3], Stephan Heeb[1], Kendra Rumbaugh[3], Kenn Gerdes[2], Paul Williams[1]

[1]Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK, [2]Centre for Bacterial Cell Biology, Newcastle University, Newcastle upon Tyne, UK, [3]Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, USA, [4]Institute for Microbiology and Molecular Biology, Justus-Liebig-University, Giessen, Germany

Bacterial type II Toxin–Antitoxin (TA) protein pairs are encoded by adjacent, co-transcribed genes. These toxins mostly act as RNases, decreasing the half-lives of mRNAs and consequently the global translation rate in bacterial cells. The activity of these proteins is modulated by antitoxins that counteract the growth inhibitory effect of their cognate toxins by direct protein-protein interactions. Toxins are far more stable than their relative antitoxins as the latter are rapidly degraded by intracellular proteases, in response to environmental and physiological stresses (e.g. amino acid starvation). Even though TA systems were originally described as plasmid addiction modules, numerous genes encoding TA pairs have also been found in bacterial chromosomes. This finding suggests that plasmid maintenance may not be the only cellular process in which TA systems are involved. In support to this hypothesis, TA systems have recently been shown to modulate persistence in E. coli and S. typhimurium. The term persistence, in this case, defines a multidrug tolerance mechanism that only takes place in a small fraction of a bacterial population. Persistence is totally unrelated to antibiotic resistance. Indeed, the first is not a genetically defined trait: persister and non-persister cells are genetically undistinguishable. In addition, Lon protease has been reported to activate TA loci-encoded RNases in E. coli by degrading the antitoxins. Consistently, Lon plays a role in the regulation of persistence in this bacterium. Interestingly, TA systems are widely conserved amongst bacteria, including in major pathogens. In this study four yet uncharacterized TA systems were identified in Pseudomonas aeruginosa and a genetic approach was used to investigate the possible involvement of these proteins in the regulation of persister cell formation in this opportunistic human pathogen. Our data suggest that TA systems may play a role in the regulation of P. aeruginosa persistence, both in vitro and in vivo.

Poster Session

BAM-PTH1074 - Oxacillin and cefoxitin predictive surrogacy of susceptibility for commonly utilized agents against Methicillin-susceptible Staphylococcus aureus

Nayon Kang[1], Seth Housman[1], David Nicolau[1]
[1]Hartford Hospital, Hartford, USA

BACKGROUND: Susceptibility testing with the use of surrogate agents is common among clinical microbiology laboratories. One such example is oxacillin and cefoxitin for many β-lactams against methicillin-susceptible Staphylococcus aureus (MSSA). Any discordance between agents raises a concern for their surrogate predictive value (SPV). Recent surveillance data demonstrate increasing ceftriaxone MICs for MSSA. The current ceftriaxone MIC90 of 4μg/mL dictates the use of ceftriaxone 2gm every 12-24 hours. This study aimed to reassess the SPV of oxacillin or cefoxitin for MSSA susceptibility against ceftriaxone and other cephalosporins. METHODS: Minimum inhibitory concentrations (MIC) were tested for cefazolin, cefoxitin, ceftaroline, ceftriaxone, nafcillin, and oxacillin by broth microdilution against 1238 MSSA isolates. Oxacillin and cefoxitin MICs were compared with the other drugs. MIC breakpoints were determined by the FDA or CLSI. A pharmacodynamic (PD) breakpoint for ceftriaxone was defined as 2μg/mL for 1gm q24h. Discordance was reported as very major errors (VME) when percent of susceptible isolates were resistant to comparator, major errors (ME) when percent of resistant isolates were susceptible to comparator, and minor errors when categorical disagreements resulted in an intermediate for drug and either susceptibility or resistance for the other. RESULTS: Oxacillin had a good SPV for nafcillin, cefazolin, and ceftaroline with excellent agreement between S, I, and R categories. Its SPV for ceftriaxone produced 5% minor error. 79% of MSSA had a ceftriaxone MIC of 4μg/mL, creating discordance when categorized according to PD breakpoint. Oxacillin had 14% VME against cefoxitin. As a surrogate, cefoxitin produced ME ≥ 13% for nafcillin, cefazolin, ceftaroline, and ceftriaxone. CONCLUSION: Cefoxitin has a poor SPV for susceptibility of agents tested against MSSA. Oxacillin produces a reliable SPV for all agents except ceftriaxone when categorized by PD breakpoint, such that additional MIC testing for ceftriaxone is advocated instead of the oxacillin surrogate results.

Poster Session

MEM-PTH3001 - Vegetarian agar for insulation of Candida species

Ivica Zurak[1]
[1]*Hospital University Centre Zagreb, Zagreb, Croatia*

Of all mycosis infections, Candidacies occupy primary or secondary place, caused by various members of Candida species. Candida species can be isolated even from cultures of peoples and animals, with no visible symptoms of Candidacies. However, this Candida may under suitable conditions enormously multiply and caused Candidacies. Predisposing factors can be found in peoples with impaired immunological systems. Uncontrolled and prolonged use of antimicrobial therapy resulted in bacterial resistance to antibiotics which are used as selective additives to agar media in fungal isolation procedure. The result is the increase in bacterial colonies grows faster and better surpasses the growth of fungi. Fruits and vegetables are rich in vegetable care in protein, vitamins, carbohydrate, ferment and electrolytes. The aim of this study was to examine various types of fruits and vegetables in preparation for feeding insulation Candida species. We decided on the type of fruit and vegetables that have shown a good port Candida species. After fragment with mixer and underwent maceration in mixture electrolyte solution, which contain: sodium chloride, cupric sulfate, magnesium sulfate, ferric ammonium citrate and potassium metabisulfite. Add 20g/l agar, heated to boiling, cooled and poured into Petri dishes, may be used without autoclave sterilization. Specimen can be directly plated to the surface to the agar and incubated at 24-37oC for 18-24h, or more. The agar medium is conductive to grow and isolation of Candida species from a clinical specimen that is contaminated with various bacteria. In parallel, we used the classical method of isolation using Sabouraud agar. Vegetables and fruits in particular offer great opportunities isolation of Candida species, especially those that are sensitive to antibiotics and selective additions that today use in Sabouraud agar. Result is important in the exchange Sabouraud agar and treatment of Candidacies infections.

Poster Session

MEM-PTH3003 - Staphylococcal interactions with Acanthamoeba and ThP1 macrophage-like cells: a symbiosis with environmental and clinical implications

Mihaela Cardas[1], Graham Mitchell[1], Selwa Alsam[1]
[1]*School of Biological Sciences, University of Essex, Colchester, Essex, UK*

Staphylococcus aureus is a major cause of serious hospital- and community-acquired infections. *Acanthamoeba* is a protozoan pathogen known to harbour microbial pathogens and resembles human macrophages in some physiological properties. The present study was aimed to determine if products of the major autolysin gene (*atl*), polysaccharide intercellular adhesion gene (*ica*) and fibronectin binding protein A and B genes (*FnBPAB*) are involved in attachment, invasion and survival of *S. aureus*, BH1CC, within pathogenic *Acanthamoeba castellani* T4 genotype, non-pathogenic *Acanthamoeba astronyxis* T7 genotype and ThP1 macrophage-like cells. *Ica* encodes the biosynthetic enzymes for poly-N-acetylglucosamine production in *S. aureus*, shown to confer resistance to killing mediated by innate host immune mediators, *atl* gene products have been identified as functioning in adhesion/invasion in staphylococcal internalization by human host cells, and *FnBPAB* products were demonstrated to be involved in adherence to a wide range of mammalian cells and implicated in various infections. The findings revealed that *ica* and FnBPAB genes are important in bacterial attachment with ThP1 macrophage-like cells, but all the named virulence factors are crucial for bacterial survival within both *Acanthamoeba* and Thp1 cells. Additionally, fluorescence microscopy studies revealed that after 1 h co-culture, amoebae and ThP1 macrophage-like cells contained *S. aureus* mutants within phagolysosomes. Overall, these findings suggest that *S. aureus* may use the same virulence factors to interact with both *Acanthamoeba* and macrophages.

Poster Session

MEM-PTH3005 - Candidiasis diagnosed at the University Hospital of Constantine, Algeria

Malika Djaballah[1], Fendri Allaoua-Hichem[1], Beldjoudi Wafa[1], Belaribi Imen[1], Rikhouah Hassina[1], Kaouha Zouleikha[1], Djebari Amina[1]

[1]Laboratory of Parasitology and Mycology Constantine Hospital University, Constantine, Algeria

Our study took place throughout the year 2012. Its purpose is to identify cases of candidiasis at the parasitology laboratory of the University Hospital of Constantine. Patients from different services and consultations. We recruited 1073 samples for which we practiced direct examinations and cultures. Course surface with frequent and mild to profound suffering are rare but serious. The results: Found 11.74% are positive, The onyxis candidiasis represent the majority of diseases diagnosed with a rate of 37.3% Followed by digestion with 35.70%. The sex ratio was 0.36. Of the 10 blood cultures received only 3 were positive.

Poster Session

MEM-PTH3007 - γ subunit of G protein Ste18p controls the mating process of Candida albicans in a positive way

Yuan Sun[1], Hui Lu[1], Malcolm Whiteway[1]
[1]Biology Department and Genome Centre of Concordia University, Montreal, Canada

Heterotrimeric G proteins have been confirmed as central elements in the pheromone response pathways of many fungi. Disruption of STE18 gene which encodes a γ subunit of a heterotrimeric G protein in fungal pathogen Candida albicans is shown to block pheromone-induced gene expression and mating process. Ectopic expression of either the Gα or the Gβ subunit of the heterotrimeric G protein is able to restore pheromone-induced gene expression and mating capacity. Ste18p contains the C-terminal CAAX box characteristic of γ subunits, and deletion of CAAX box residues causes mating deficiency without affecting of STE18 gene expression.

Poster Session

MEM-PTH3009 - Comparison of qPCR with bronchoalveolar lavage fluid and serum for the diagnosis of invasive pulmonary aspergillosis

Shuzhen Zhang[1], Ruoyu Li[1], Jin Yu[1]

[1]*Department of Dermatology and Venereology, Peking University First Hospital; Research Center for Medical Mycology, Peking University, China*

Invasive pulmonary aspergillosis is increasing because of the extensive application of immunosuppressants and antibiotics and is an important cause of death. Early diagnosis and treatment is the key to reduce morality. However, the diagnosis of invasive pulmonary aspergillosis is remain challenging. QPCR, as one of the new molecular diagnostic methods, has been in research used for the diagnosis of invasive aspergillosis in the recent years, and had a promising prospect for its sensitivity and rapidness. In our study, we compared the diagnostic efficiency of Taqman-qPCR targeting 28SrDNA with bronchoavleolar lavage fluid and serum. Total of 93 inpatients were included in our study between Mar.2011 and July.2013 at Peking University First Hospital in Beijing, China. The qPCR by using bronchoavleolar lavage fluid has a better area under the curve (0.975, 95%CI: 0.946-1.005) than serum (0.692, 95%CI:0.473-0.910) (Z= 2.527, p=0.012). According to receiver operating characteristic curve(ROC curve), the optimal Ct cutoffs of qPCR are 39.35 and 39.34 for bronchoavleolar lavage fluid and serum respectively.The sensitivity, specificity, positive predictive vaule(PPV), negative predictive value(NPV) of qPCR with bronchoavleolar lavage fluid are 100%, 90.6%, 56.3%, 100%, by comparision, those of qPCR with serum are 44.4%, 95.3%, 50%, 94.2%. In conclusion, qPCR with bronchoavleolar lavage fluid has a higher sensitivity than serum(Fisher test, p=0.029), but both have a similar specificity (Chi-square test, p=0.350), PPV(Fisher test, p=1) and NPV(Fisher test, p=0.06).

Poster Session

MEM-PTH3000 - Hyphae on real materials: the influence of water on the hyphal growthrate of Penicillium rubens on gypsum

Karel van Laarhoven[1], Mirjam Bekker[1], Henk Huinink[1], Jan Dijksterhuis[2], Olaf Adan[1]
[1]Eindhoven Technical University, Eindhoven, the Netherlands, [2]CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands

The primary cause for indoor mould growth is generally understood to be the presence of moisture. Specifically, it is the pore water in porous building materials that can be used by fungi. Research on growth on porous substrates has mainly focussed on the role of ambient relative humidity (RH). This is due to its coupling to the water activity (aw) of the pore water, known for its influence on growth. The role of a substrate's porous properties, which influence this coupling and other parameters such as moisture content (θ), has received less attention. Further, most studies approached the quantification of fugal growth macroscopically, as microscopy is difficult on these rough substrates. The aim of this research was to relate the extension rate of hyphae to the state of water in porous substrates. We constructed a video microscopy setup that monitors hyphae on gypsum substrates in a chamber with well-defined moisture conditions. Gypsum samples were equilibrated with a dynamically controlled RH and partially soaked with an aqueous glycerol solution. This way, the aw and θ of the substrate's pore water could be adjusted separately. Penicillium rubens, a typical indoor fungus formerly known as P. chrysogenum, was used as test organism. The first results suggest that hyphal growth rate decreases when aw or θ are decreased. Further, it was seen that the minimal aw required for growth depends on θ. One explanation for the influence of θ is its relation to the spatial distribution of water. A sparser network of water could limit a hypha's access to both water and nutrients. Another possible explanation could be the varying availability of osmolytes that allow the fungus to deal with osmotic stress. The research is ongoing and will subsequently focus further on the hyphal response to changing moisture conditions.

Poster Session

MEM-PTH3002 - Purple amaranth (amaranthus hypochondriacus l.), a new host for macrophomina phaseolina (tassi) goid

Mohsen Naderpour[1], Naser Safaie[1], Babak Pakdaman Sardrood[2], Ali Mokhtassi-Bidgoli[3]
[1]Department of Plant Pathology, Agricultural Faculty, Tarbiat Modares University, Tehran, Iran, [2]Department of Plant Protection, Agricultural Faculty, Ramin Agricultural and Natural Resources University, Khuzestan, Iran, [3]Department of Agronomy, Agricultural Faculty, Tarbiat Modares University, Tehran, Iran

In the summer 2012, the symptoms of charcoal rot including wilting, stem blackening were observed with purple amaranth plants grown in the field of Agricultural Faculty, Tarbiat Modares University, and exhibiting the presence of abundant tiny microsclerotia on their infected chlorotic foliar tissues. The pathogen was isolated and identified as Macrophomina phaseolina (Tassi) Goid. based on cultural and morphological characteristics such as mycelium color, colony shape, the formation of tiny, black, spherical to oblong or irregularly shaped microsclerotia immersed in agar. Molecular confirmation of the pathogen specification was made using species-specific primers MpKFI/MpKRI. Based on the present references, Amaranthus hypochondriacus L. is reported as a globally new host for the pathogenic fungus, M. phaseolina. It is expected that the pathogen can be considered as a serious threat against the production of purple amaranth not only in Iran but also throughout the world.

Poster Session

MEM-PTH3004 - Synthesis and anticandidal activity of new thiazole derivatives

Gülhan Turan[1], Mehlika Dilek Altintop[1], Ahmet Ozdemir[1], Zafer Asim Kaplancikli[1], Gökalp İşcan[2]
[1]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Eskişehir, Turkey, [2]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Turkey

Thiazoles have received considerable attention as important pharmacophores in drug design and development. Many studies have confirmed that thiazole derivatives exhibit a wide range of biological effects including antimicrobial activity.1 In the present study, new thiazole derivatives were synthesized via the ring closure reaction of 4-(1H-imidazol-1-yl)benzaldehyde thiosemicarbazone with phenacyl bromides. All compounds were tested in vitro against various Candida species. The microbiological results revealed that 2-(2-(4-(1H-imidazol-1-yl)benzylidene)hydrazinyl)-4-phenylthiazole was the most effective derivative against Candida utilis with a MIC value of 31.25 µg/mL when compared with ketoconazole (MIC= 3.12 µg/mL). [1] Kashyap SJ, Garg VK, Sharma PK, Kumar N, Dudhe R, Gupta JK. Thiazoles: having diverse biological activities. Med. Chem. Res. 21, 2123-2132, 2012.

Poster Session

MEM-PTH3006 - The ESCRT machinery interacts with Rim101 pathway and is implicated in iron utilization and capsule elaboration in *Cryptococcus neoformans*

Guanggan Hu[1], Melissa Caza[1], Brigitte Cadieux[1], Eunsoo Do[2], Wonhee Jung[2], Jim Kronstad[1]
[1]Michael Smith Laboratories, The University of British Columbia, Vancouver, Canada, [2]Department of Systems Biotechnology, Chung-Ang University, Anseong, Republic of Korea

Iron availability is a key regulator of the production of capsule and melanin, two major virulence factors in *C. neoformans*. We previously found that the ESCRT-I component Vps23 functions in iron utilization, capsule formation and virulence. A further characterization of the ESCRT machinery revealed that the additional ESCRT sub-complexes (-II and –III, but not -0 and -DS) are also involved in capsule formation and in iron utilization from heme. Deletion genes encoding components of ESCRT-I (*VPS23*), II (*VPS22*) and III (*VPS20* and *SNF7*) caused significantly reduced capsule formation due to the defective attachment of capsular material to the cell wall. ESCRT-I, -II and –III deletion mutants are unable to grow on heme as the sole iron source, and show increased resistance to non-iron metalloprotoporphyrins. Moreover, ESCRT –I, -II and -III mutants display increased sensitivity to alkaline pH, similar to a *rim101* mutant, indicating that ESCRT machinery plays a role in the response to pH. The ESCRT machinery directly interacts with the Rim101 pathway as revealed by the physical interaction of Snf7 (ESCRT-III) and Rim20 (a regulatory component of Rim101 pathway) in a yeast two-hybrid assay. Defects in ESCRT-I (*vps23*), II (*vps22*) and III (both *vps20* and *snf7*) led to the mislocalization of GFP-Rim101 from nuclei to cytoplasm in the low-iron condition, while deletion of either genes for ESCRT-0 (*VPS27*) or –DS (*VPS4* and *BRO1*) had no marked influence on GFP-Rim101 localization. Remarkably, overexpression of truncated versions of Rim101 in ESCRT –I, -II and –III mutants partially restored capsule formation to the mutants. However, in contrast to *rim101* mutant that was hypervirulent, deletion of genes for ESCRT –I, or –II, or–III caused significantly attenuated virulence in both macrophage survival assays and in a mouse inhalation model. In summary, the ESCRT machinery is involved in capsule formation, iron utilization from heme and virulence in *C. neoformans*. We hypothesize that the ESCRT complexes function via Rim101-dependent and independent pathways.

Poster Session

MEM-PTH3008 - Molecular genetic analysis of the Pumilio gene family in fission yeast

Darren Henry[1], Iain George[1], Gordon Chua[1]
[1]*University of Calgary, Calgary, Canada*

The Pumilio (Puf) protein family contains a highly-conserved group of posttranscriptional regulators in eukaryotes. Members of this protein family contain a RNA-binding Pumilio homology domain that interacts directly with regulatory sequences in the 3'-UTR of target mRNAs. Binding of the Puf protein to the 3'-UTR can cause decreased stability, translational repression and changes in cellular localization of its mRNA targets. In *Schizosaccharomyces pombe*, there are seven nonessential genes that encode proteins containing a Pumilio homology domain. The biological function and identity of the mRNA targets remain unknown for these Puf proteins. We have used overexpression analysis to functionally characterize these *puf*+ genes as the corresponding deletion strains do not show a mutant phenotype in rich media. Overexpression of all seven *puf*+ genes singly under the *nmt1* promoter results in a variety of mutant cell phenotypes including aberrant shapes, elongation, lysis, and flocculation. Transcriptome profiling of the deletion and overexpression strains of certain *puf*+ genes reveal putative targets regulated at the level of mRNA stability. We are currently validating these putative mRNA targets to enhance our understanding of these *puf*+ genes in *S. pombe*.

Poster Session

VIR-PTH2001 - Aerosol vaccination against Ebola Virus

Michelle Meyer[1,4], Ndongala Lubaki[1,4], Tania Garron[1,4], Gene Olinger[5], Chad Mire[2,4], Curtis Klages[4], Thomas Geisbert[2,4], Peter Collins[6], Alexander Bukreyev[1,2,3,4]

[1]Department of Pathology, The University of Texas Medical Branch, Galveston, USA, [2]Department of Microbiology & Immunology, The University of Texas Medical Branch, Galveston, USA, [3]Sealy Center for Vaccine Development, The University of Texas Medical Branch, Galveston, USA, [4]Galveston National Laboratory, The University of Texas Medical Branch, Galveston, USA, [5]Viral Pathogenesis and Immunology Branch, Virology Division, United States Army Institute for Infectious Diseases, Frederick, USA, [6]National Institute of Allergy and Infectious Diseases, Bethesda, NIH, USA

Aerosolized immunization via the respiratory mucosa represents an ideal and feasible means of delivery that has never been tested for hemorrhagic fever viruses. We used Rhesus macaques to test immunogenicity and protective efficacy of the mucosal human parainfluenza virus type 3-vectored vaccine expressing the glycoprotein (GP) of EBOV (HPIV3/EboGP) delivered to the respiratory tract in small particle (2.5-4.0μM) aerosol form. In study 1, we characterized the systemic and local immune responses to vaccination. Macaques were vaccinated with two doses of (i) aerosolized or (ii) liquid HPIV3/EboGP delivered to the respiratory tract and (iii) Venezuelan equine encephalitis virus (VEE) replicon-vectored vaccine expressing EBOV GP delivered intramuscularly. The EBOV-specific antibody and cell-mediated responses generated by aerosolized HPIV3/EboGP were equal to or exceeded that induced by liquid HPIV3/EboGP. High EBOV-specific IgG, IgA and neutralizing antibody titers were detected in serum and mucosal samples. Polyfunctional EBOV specific CD8+ T cells and their subset expressing CD103 (αE integrin) and predominantly Th1 CD4+ T cells expressing multiple cytokines were detected in HPIV3/EboGP vaccine recipients. The magnitude of the polyfunctional CD4+ T cell response was much greater in animals that received the aerosolized vaccine than those that received the liquid vaccine. The magnitude of the cell-mediated responses generated by HPIV3/EboGP was greatest in the lung tissues followed by the peripheral blood and spleens. Comparisons of the antibody and cell-mediated responses to the respiratory (HPIV3/EboGP) and systemic (VEE-replicon) vaccines in various tissues will be provided. In study 2, we compared protective efficacy of one or two doses of aerosolized HPIV3/EboGP against 1,000 PFU of EBOV delivered intramuscularly; both groups were fully protected against death and severe disease. Our findings show that a single dose of aerosolized HPIV3/EboGP induces abundant local and systemic responses and is protective against lethal EBOV challenge.

Poster Session

VIR-PTH2003 - Cell entry mediated by the glycoprotein of a novel European filovirus requires host endosomal cysteine proteases and the filovirus receptor NPC1

Melinda Ng[1], Esther Ndungo[1], Yingyun Cai[2], Sheli Radoshitzky[3], Elena Postnikova[2], Anabel Negredo Anton[4], John Dye[3], Gustavio Palacios[3], Jens Kuhn[2,5], Kartik Chandran[1]

[1]*Albert Einstein College of Medicine, Bronx, USA,* [2]*Integrated Research Facility at Fort Detrick, NIAID, National Institutes of Health, Frederick, USA,* [3]*United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, USA,* [4]*Instituto de Salud "Carlos III," Madrid, Spain,* [5]*Tunnell Consulting, Incorporated, King of Prussia, Pennsylvania, USA*

A novel filovirus, Lloviu virus (LLOV) was described in 2009 and is proposed to be the causative agent of massive bat die-offs in northern Spain. Viral nucleic acids were isolated from the bat carcasses and assembled into a near complete genome. However, cells programmed with this genome have failed to yield infectious virus to date. In order to study the mechanism by which LLOV enters host cells, we focused on its GP (glycoprotein) gene. We first investigated the properties of sGP and delta-peptide, two secreted glycoproteins predicted to be encoded by LLOV. Like their Ebola virus (EBOV) counterparts, purified LLOV sGP and delta-peptide inhibited infection by authentic filoviruses. We next used reverse genetics to generate the first recombinant vesicular stomatitis virus (rVSV) expressing the LLOV transmembrane glycoprotein in place of the VSV entry glycoprotein. rVSV-LLOV GP replicated to high titers in tissue culture, indicating that the viral genome detected in bats contains a functional glycoprotein. Computational and experimental analyses revealed that LLOV GP, like the GP proteins of all other known filoviruses, is synthesized as a precursor protein that is cleaved to GP1 and GP2 subunits by the proprotein convertase furin. We also found that LLOV GP undergoes extensive N– and O–linked glycosylation, and encodes a mucin-like region that is proposed to shield GP from host immune detection. Entry assays using chemical inhibitors and knockout cell lines showed that LLOV GP, like its other filovirus counterparts, requires endosomal acid pH and cleavage by host cysteine proteases to mediate viral entry. Finally, LLOV GP, like other filovirus GP proteins, requires Niemann-Pick C1 (NPC1) as an entry receptor in a broad range of primate and bat cell lines. Taken together, our studies provide evidence that the LLOV entry pathway closely resembles those of other filoviruses.

Poster Session

VIR-PTH2005 - Etiology and seasonality of lower respiratory tract infections in hospitalized young children in southern Taiwan

Yung-Feng Huang[1], Chih-An Chou[1], Yu-Shen Chen[1], Kai-Sheng Hsieh[1]
[1]The Department of Pediatrics of the Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

Background/Purpose: Lower respiratory tract infections (LRTI) play an important role in pediatric diseases. Both viruses and bacteria are common etiologies, and some pathogens have a seasonal trend according to latitude. However, there is limited epidemiological data about LRTI in southern Taiwan. This study aimed to investigate the clinical and epidemiological data of LRTI in southern Taiwan. Methods: Children aged under five years who were hospitalized at a medical center in southern Taiwan with acute LRTIs from July to October (summer) 2010 and March to May (spring) 2011 were prospectively enrolled. Nasopharyngeal aspirates were obtained and sent for viral cultures and two multiplex polymerase chain reactions (PCR). Quick antigen tests were arranged according to clinical need by the patient's primary physician. The clinical features, laboratory data and imaging findings were recorded and analyzed. Results: A total of 90 children were enrolled, 70 of whom had detectable pathogens. The positive rate of conventional viral and bacterial cultures was 25.6%, which increased to 77% after combining with the two multiplex PCR methods. The seasonal trend of viral infections in southern Taiwan was different from northern Taiwan. There were no differences in demographic data, severity of disease or hospital stay between single and mixed infections. A similar result was found between non-pneumococcal and pneumococcal infections, except for C-reactive protein level. Conclusion: Multiplex PCR methods are rapid assays that can increase the diagnostic yield rate. Mixed infections do not seem to affect the severity of disease. Early detection may aid clinicians in appropriate decision-making and treatment.

Poster Session

VIR-PTH2007 - Characterization of self-assembled virus-like particles of Ferret Hepatitis E virus generated by recombinant Baculoviruses

Tiancheng Li[1], Tingting Yang[2], Michiyo Kataoka[1], Noriko Kishida[1], Masayuki Shirakura[1], Hidemasa Asanuma[1], Yasushi Ami[1], Yoriko Suzaki[1], Naokazu Takeda[3], Takaji Wakita[1]
[1]National Institute of Infectious Diseases, Tokyo, Japan, [2]Affiliated Hospital of Qingdao University Medical College, Qingdao, P.R. China, [3]Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Ferret hepatitis E virus (HEV), a novel hepatitis E-like virus, has been identified in ferrets in the Netherlands. Due to the lack of a cell culture system for ferret HEV, the antigenicity, pathogenicity and epidemiology of this virus have remained unclear. In the present study, we used a recombinant baculovirus expression system to express the 112-N-terminus and 47-C-terminus amino-acid-truncated ferret HEV ORF2 protein in insect Tn5 cells, and found that a large amount of a 53 kDa protein (F-p53) was expressed and efficiently released into the supernatant. Electron microscopic analysis revealed that F-p53 was self-assembled into virus-like particles (ferret HEV-LPs). These ferret HEV-LPs were estimated to be 24 nm in diameter, which is similar to the size of G1, G3, G4 and rat HEV-LPs derived from both the N-terminus- and C-terminus-truncated constructs. Antigenic analysis demonstrated that ferret HEV-LPs were cross-reactive with G1, G3, G4 and rat HEVs, and rat HEV and ferret HEV showed a stronger cross reactivity to each other than either did to human HEV genotypes. However, the antibody against ferret HEV-LPs does not neutralize G3 HEV, suggesting that the serotypes of these two HEVs are different. An enzyme-linked immunosorbent assay (ELISA) for detection of anti-ferret HEV IgG and IgM antibodies was established using ferret HEV-LPs as antigen, and this assay system will be useful for monitoring ferret HEV infection in ferrets as well as other animals. In addition, analysis of ferret HEV RNA detected in ferret sera collected from a breeding colony in the USA revealed the genetic diversity of ferret HEV.

Poster Session

VIR-PTH2009 - Epidemiology and genetic analysis of 2009 pandemic Influenza A/ H1N1 viruses from 2009 to 2013 in Taiwan

Jen-Ren Wang[1,2], Su-Jhen Hung[1], Kun-Chan Tsai[1], Sheng-Wen Huang[1]
[1]National Cheng Kung University, Tainan, Taiwan, [2]National Health Research Institutes, Tainan, Taiwan

The outbreak of 2009 pandemic Influenza A/H1N1 virus (pH1N1) occurred in Mexico and soon spread worldwide. The pH1N1 virus caused a large peak from August to December in 2009 and another peak in January 2011 in Taiwan. To monitor genetic differences of pH1N1 virus that circulated in human, we analyzed the evolution of nucleotide and amino acid sequences of isolates randomly selected from 2009 to 2013. The phylogenetic trees of HA, NA and NS genes showed that our isolates belonged to cluster II, and further evolved into three clades with characteristic substitutions. By the FluSurver analysis, the clade II-1 substitutions were shown to be dominant among the 2009 pandemic H1N1 viruses worldwide and the clade II-2 and -3 substitutions were the second-highest mutations. Furthermore, we identified a semi-annual epidemic cycle accounting for the pH1N1 dynamics in Taiwan, in addition to the results of influenza activity surveillance, suggesting that the lineage diversification of pH1N1 in phylogenetic tree was associated with the precipitation in early summer season. In conclusions, increasing genetic variations of pH1N1 viruses were identified, while most will be selected out by purifying selection in the long term, but these genetic variations contributed to nonsynonymous mutations still may affect the virus properties and pathogenesis. Continuous surveillance of pH1N1 genetic variation should be monitored, though the pH1N1 showed significant decline after its debut since 2009.

Poster Session

VIR-PTH2011 - Human enteric virus in various environmental matrices in the Northwest of Morocco

Amar Laila[1], Hundesa Ayalkibet[2], Fresno Sandra[2], Manzano Jesús Rodriguez[2], Wakrim Lahcen[1], Girones Rosina[2], Lkhider Mustapha[3]

[1]Institut Pasteur, Casablanca, Morocco, [2]Department of Microbiology, University of Barcelona, Spain, [3]Faculty of Science and Technology, Mohammedia, Morocco

A total of 72 samples were collected between 2006 and 2008 in the Mediterranean coast of Morocco (Smir lagoon water= 30, connected seawater =30, sediments=6, mussels = 6). The water samples were concentrated by glass wool method then analyzed for human enterovirus infectious by integrated cell culture-PCR (ICC/RT-PCR) and by qRT-PCR for other enteric viruses. Human adenovirus, polyomavirus JC, Norovirus GII and rotavirus A were detected in 63, 57, 43 and 36% of 30 lagoon samples respectively. For Norovirus GI, in 10 tested samples only 10 % revealed positive. Human adenovirus was detected in 60% while polyomavirus JC and Norovirus GII were detected in 10% of ten seawater samples. All were negative for hepatitis virus E and hepatitis virus A. The infectious entérovirus were detected in 40 and 36% of lagoon and seawater samples respectively and sequencing analyses showed that sequences belong to coxsackievirus B1. Mussels samples were positive for Rotavirus A (83%) and none of Norovirus were detected. Sediments were positive for Rotavirus A (33%) and Norovirus GI/GII (17%). This study revealed a human viral pollution of the environment and suggested Human adénovirus as a suitable viral indicator of human fecal pollution for recreational beaches quality; Key words: Adenovirus, Enterovirus, Hepatitis virus A and E, Lagoon, Mussels, Norovirus, Seawater, Sediments

Poster Session

VIR-PTH2013 - Standarization of real time RT-PCR for the diagnosis of hazardous virus for the cattle industry in Mexico

Mario Palma Zepeda[1], Rogelio Alejandro Alonso Morales[1], Amanda Gayosso Vazquez[1], Juan Pablo Pintor Rios[1], Rosa Elena Sarmiento Silva[1]
[1]*Facultad de Medicina Veterinaria y Zootecnia-UNAM, Distrito Federal, Mexico*

Among the diseases that affect cattle production systems in the world are the virus associated with Bovine Respiratory Disease Complex (BRDC) in feed lots and bovine viral leukosis in dairy systems each with its own consequences of host, clinical signs and economic implications. These diseases are multifactorial and synergistic. The virus of bovine viral diarrhea virus (BVDV), the bovine herpesvirus type 1 (BHV1), bovine respiratory syncytial virus (BRSV) and bovine parainfluenza type 3 (BPIV3) are the major viral agents implicated in the BRDC, causing immunosuppression and damage to pulmonar epithelium, predisposing to oportunistic bacterial infections such as Manhemia haemolytica, Pasteurella multocida, H. somni and Mycoplasma. Cattle infected by Bovine leukemia virus (BLV) usually do not show clinical signs: 30-70% of infected animals develop persistent lymphocytosis and 0.1-10 % develop tumors. In Mexico serological studies indicate prevalence between 20 and 68 % of the BRDC-associated virus. There are no epidemiological data for the BLV in the country. The aim of this work was the standardization of a real time RT-PCR method for the diagnosis of the BVDV, BHV1, BRSV, BPIV3 and BLV. We developed DNA quantification standard controls from reference virus. The sensitivity of the test was determined by absolute quantification by serial log dilutions of DNA quantification standard controls as well by dilutions of titled virus. The sensitivity level detection was of 100 copies of viral genome for BHV1, 1000 for BLV, 1000 for BVDV, and 10 for BRSV.

Poster Session

VIR-PTH2017 - Presence of maternal HIV-1 broadly neutralizing antibodies In mother to child transmission

Takafira Mduluza[1], Sheron Dzoro[1,2], Keabetswe Bedi[2,3], Sununguko Wata Mpoloka[2]

[1]University of Zimbabwe, Harare, Zimbabwe, [2]University of Botswana, Gaborone, Botswana, [3]Botswana Harvard Partnership, Gaborone, Botswana

Neutralizing antibody assays are now being widely employed in different laboratories in search for correlates of protective immunity. There are strong arguments in favor of a beneficial role of some broadly neutralizing antibodies in prevention of HIV infection if these antibodies exist prior to exposure. Archived plasma samples at the Botswana-Harvard HIV Institute, were assayed for presence of neutralizing antibodies using TZM-bl cells with a tat regulated luciferase reporter gene. A panel of well characterized HIV-1 strains was used, and results were expressed as fifty percent or ninety percent inhibitory dose (ID50/ ID90), defined as the plasma dilution causing fifty percent or ninety percent reduction in relative luminescence (RLU) compared to virus control wells after subtraction of background RLU. Non transmitters had higher baseline mean titres for ninety percent neutralization of THA- subtype AE strain and IN93 –subtype C strain. At delivery, non transmitters had significantly higher mean antibody titres for ninety percent neutralization of all the panel viruses. Overall, there was moderate neutralization of the subtype C viruses: IN93, ZA and ZM, representative of the HIV-1 strain predominant in Southern Africa. The presence of maternal HIV-1 broadly neutralizing antibodies contributes to reduced transmission of HIV from infected mothers to their infants in utero and at delivery.

Poster Session

VIR-PTH2019 - The salinity effect on giant grouper innate immunity and betanodavirus replication

Tz-Shiang Chen[1], Shau-Chi Chi[1]
[1]*Department of Life Science, Taipei, Taiwan*

In Taiwan, giant grouper is one of the cultured marine fish species with high economic value. However, the industry of grouper aquaculture was severely damaged in the past 20 years by nervous necrosis virus (NNV). According to the experience of a few grouper farmers, the mortality of NNV-infected groupers could be down-regulated by decreasing the salinity of the sea water. Ambient salinity is an important factor to fish physiology, but it is still unclear if the change of salinity has impact on fish immunology. NNV is a double-stranded RNA virus, and will be sensed by host toll-like receptor 3 (TLR-3), and induce the expression of inflammatory precursor gene, interleukin-1 beta (IL-1 beta), and interferon-inducible gene, Mx gene. In this study, real time PCR was used to examine the impact of salinity change on the expression of the above innate immunity genes in the spleen and head kidney, including TLR-3, IL-1 beta and Mx genes, and the expression of NNV genes in the brain which is the target organ for NNV infection. The result indicated that fish acclimated under low salinity (15‰) showed higher gene expression of TLR-3 and IL-1 beta than that under high salinity (30‰); however, higher Mx gene expression was found in the fish acclimated at high salinity (30‰). After NNV challenge, lower mortality was observed in the fish acclimated at low salinity (15‰), and the gene expression of TLR-3 and IL-1 beta genes were higher in the dead and survived fish acclimated at high salinity (30‰) than that at low salinity (15‰). How the salinity influences the innate immunity gene expression and the mortality post NNV infection will be examined in our following works.

Poster Session

VIR-PTH2021 - Interaction with cellular CD4 exposes HIV-1 envelope epitopes targeted by antibody-dependent cell-mediated cytotoxicity

Maxime Veillette[1], Anik Désormeaux[2], Halima Medjahed[1], Nour-Elhouda Gharsallah[2], Mathieu Coutu[1], Joshua Baalwa[3], Yougjun Guan[4], George Lewis[4], Guido Ferrari[5], Beatrice H. Hahn[6], Barton F. Haynes[5], James Robinson[7], Daniel Kaufmann[1,2,10], Mattia Bonsignori[5], Joseph Sodroski[8,9,10], Andrés Finzi[1,2,11]

[1]Centre de recherche du CHUM, Université de Montréal, Montreal, Canada, [2]Centre de recherche du CHUM, Montreal, Canada, [3]Department of Pathology and Medicine, University of Alabama at Birmingham, Birmingham, USA, [4]Division of Basic Science and Vaccine Research, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, USA, [5]Duke Human Vaccine Institute, Duke University Medical Center, Durham, USA, [6]Departments of Medicine and Microbiology, University of Pennsylvania, Philadelphia, USA, [7]Department of Pediatrics, Tulane University School of Medicine, New Orleans, USA, [8]Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA, [9]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, USA, [10]Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard, Boston, USA, [11]Department of Microbiology and Immunology, McGill University, Montreal, Canada

With more than 34 million infected individuals worldwide, the human immunodeficiency virus type 1 (HIV-1) remains one of the most significant public health challenge. While there has been significant progress in clinical treatment of HIV-1 and in understanding its relation with the human immune system, there are still no effective vaccines or therapies that eradicate the virus. However, the recent anti-HIV-1 RV144 clinical trial resulted in approximately 30% protection of vaccinees. Subsequent analysis of protection correlates revealed that anti-HIV-1 envelope glycoprotein (Env) antibodies without broadly-neutralizing activity could play a role in the observed protection, stimulating interest in other protective mechanisms involving antibodies, such as antibody-dependent cell-mediated cytotoxicity (ADCC). Env epitopes targeted by many antibodies effective at mediating ADCC are poorly exposed on the unliganded Env trimer. Here we investigate the mechanism of exposure of ADCC epitopes on Env, and show that binding of Env and CD4 within the same HIV-1-infected cell effectively exposes these epitopes. Env capacity to transit to the CD4-bound conformation is required for ADCC epitope exposure. Importantly, cell-surface CD4 down-regulation by Nef and Vpu accessory proteins and Vpu-mediated BST-2 antagonism modulate exposure of ADCC-mediating epitopes and reduce the susceptibility of infected cells to this effector function. Altogether, our observations describe a highly-conserved mechanism required to expose ADCC epitopes that might help explain the evolutionary advantage of counteracting BST-2 by Vpu and downregulating cell-surface CD4 by the HIV-1 Vpu and Nef accessory proteins.

Poster Session

VIR-PTH2023 - Impact of TB co-infection on expression of T-cell inhibitory markers PD-1 and Tim-3, and its relationship to immune activation in HIV-1 patients

Corena de Beer[1], Richard Glashoff[1], Maxine Golden[1]
[1]*University of Stellenbosch Medical School, Cape Town, South Africa*

South Africa has one of the highest rates of HIV-1/TB co-infection and mortality in the world. HIV infection is associated with exhaustion marker up-regulation on CD8+ T cells (including PD-1 and Tim-3). TB infection has similarly been associated with inhibitory marker up-regulation. Inhibitory markers lead to poor responsiveness of T cells to antigenic stimulation, hence provide a target for immune intervention. This study addressed co-infection and how these markers link to immune activation and immune exhaustion. Fresh blood was collected at clinics Cape Town peri-urban region in EDTA tubes and transported to the laboratory for processing within 6 hours. A total of 75 blood samples were collected. The samples collected included 30 HIV positive, 15 HIV-1 and TB co-infected and, 30 uninfected control samples. Flow cytometric analysis was performed on both CD4+ and CD8+ T cells. PD-1 and Tim-3 expression was determined in conjunction with markers of immune activation. The HIV/TB co-infected group had a significantly higher viral load than the HIV mono-infected group (196 617.5 copies/ml vs 47 970 copies/ml; p <0.001). Conversely, the CD4 count was significantly lower in the co-infected group, and both infected groups were significantly lower than the control group (1014 cells/ml vs. 309.5 cell/ml vs 234.0 cells/ml; p <0.001). Tim-3 expression on CD8+T cells was almost 2 times higher in the HIV-1/TB group (11.7 %) than in the HIV-1 (5.9 %) group (p <0.001). Tim-3 expression was significantly higher on the CD8+ T cells than on CD4+ T cells. Tim-3 expression was not dependent on PD-1 expression. This study has shown that Tim-3 is significantly increased on CD8+ T cells in both HIV-1 mono infected and TB dual infection. Tim-3 expression is at a lower level than PD-1. Tim-3 may be an important potential therapeutic target in HIV-1/TB co-infection worthy of future studies.

Poster Session

VIR-PTH2025 - Negative regulation of cytokine expression by type one interferon signalling in VSV infection

Scott Walsh[1], Alexandra Rasiuk[1], Byram Bridle[1]
[1]*Department of Pathobiology, University of Guelph, Guelph, Canada*

Type I interferon (IFN) signalling is known to induce expression of cytokines in leukocytes, especially in the context of virus infection. However, the role of type I IFN signalling in controlling the level of cytokine expression after initial induction has not been explored. Mice lacking functional type I IFN signalling are extremely susceptible to virus infection. As expected, we have observed high mortality in mice lacking the IFN receptor (IFNAR-/-) infected with recombinant vesicular stomatitis virus (rVSV) but typical clinical signs associated with VSV pathogenesis, including neurotoxicity, were lacking. Observed clinical signs indicated that the immune response induced by infection rather than rVSV replication was responsible for pathology. Chimeric mice were generated to assess the role of IFN and cytokine toxicity in the pathology of rVSV infection. Lethally irradiated wildtype (WT) mice were reconstituted with bone marrow from IFNAR-/- mice. Chimeras could produce type I IFN but only non-hematopoietic cells could detect it. IFNAR-/- leukocyte mice infected with rVSV showed increased morbidity (reduced body temperatures and respiratory rates) compared to WT controls. All mice with IFNAR-/- leukocytes reached endpoint by 24 hours post-infection with no signs of neurotoxicity. Serum cytokine levels of 22 out of 23 cytokines analyzed were significantly elevated in rVSV infected mice with IFNAR-/- leukocytes compared to WT mice. Importantly, no rVSV titers could be detected in brain homogenates from IFNAR-/- chimeras. Infection was also performed with 20% or 50% IFNAR-/- leukocytes and the severity of morbidity and cytokine upregulation correlated with the proportion of leukocytes lacking IFNAR. This suggests that toxicity was not due to viral replication and that sensing of type I IFN is required to downregulate cytokine production in leukocytes responding to rVSV infection.

Poster Session

VIR-PTH2027 - CTB fused to porcine arterivirus M and GP5 structural proteins failed to enhance the GP5-specific antibody response in pigs immunized with adenovectors

Elodie Roques[1], Martin Lessard[2], Denis Archambault[1]
[1]University of Quebec at Montreal, Montreal, Canada, [2]Agriculture and Agri-Food Canada, Sherbrooke, Canada

The porcine reproductive and respiratory syndrome virus (PRRSV) is an arterivirus that belongs to the Nidovirales order. As the current commercial vaccines are incompletely effective to ensure protection against PRRSV infection, a vaccine strategy using replicating but nondisseminating adenovectors (rAdVs) expressing the PRRSV M matrix protein in fusion with the neutralizing major epitope-carrying GP5 envelope protein was developed by us (Veterinary Research 44:17, 2013). Although this strategy enabled the production of GP5-specific antibodies (Abs), no PRRSV-specific neutralizing Abs (NAbs) were induced with rAdVs expressing either M-GP5 or M-GP5m (which is a mutant form of GP5 that was designed to theoretically enhance the production of PRRSV-specific NAbs). In this study we wished to determine whether the fusion of the cholerae toxin B subunit (CTB, known for its adjuvant effect) to the C-terminus of M-GP5 or M-GP5m was able to enhance the Ab response to GP5 and to induce PRRSV-specific NAbs. Six rAdVs were generated and designated rAdV-GFP (expressing the green fluorescent protein), rAdV-GP5 (expressing wild-type GP5), rAdV-M-GP5, rAdV-M-GP5m, rAdV-M-GP5-CTB or rAdV-M-GP5m-CTB. Three weeks-old pigs (six groups) were immunized twice both intramuscularly and intranasally at 3-weeks intervals with the various rAdVs. GP5-specific Abs and PRRSV-specific NAbs were determined in sera of individual pigs prior to immunization, and at subsequent post-primary immunization (PPI) days by ELISA and a virus neutralization assay, respectively. Immunization of pigs with rAdV-M-GP5m generated a GP5-specific Ab response that was higher than that observed in pigs inoculated with rAdV-M-GP5- or rAdV-GP5 from 21 days PPI. Unexpectedly CTB fused to the virus proteins had a severe negative impact on GP5-specific Ab production when compared to the use of M-GP5 or M-GP5m alone. Moreover PRRSV-specific NAbs could not be detected in any pigs of all groups. Experiments are under way to delineate the mechanism associated to the negative effect of CTB.

Poster Session

VIR-PTH2029 - Establishment of a high-throughput screening assay to identify inhibitors targeting influenza A virus transcription and replication stages

Zhen Wang[1], Yongxin Zhang[1], Qian Gao[1], Zhenlong Liu[1], Xiaoyu Li[1], Yuhuan Li[1], Shan Cen[1]
[1]*Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Pekin Union Medical College, Beijing, China*

Influenza A virus (IAV) poses significant threats to public health because of recent emergence of highly pathogenic strains and wide spread of resistance to available anti-influenza drugs. Therefore, there is a need for new antiviral targets and new drugs against influenza virus infections. Although IAV RNA transcription / replication represents a promising target for antiviral drug development, no assay ideal for high-throughput screening (HTS) application is now available to identify inhibitors targeting these processes. In this work, we developed a novel HTS assay for screening inhibitors targeting IAV RNA transcription / replication using an A549 cell line stably expressing IAV RNA-dependent RNA polymerase (RdRp) complex, NP and a viral mini-genomic RNA. Both secreted Gaussia luciferase (Gluc) and blasticidin resistance gene (Bsd) were encoded in the viral minigenome and expressed under the control of IAV RdRp. Gluc serves as a reporter tomonitor the activity of IAV RdRp, and Bsd is used to maintain the expression of all of the foreign genes. Biochemical studies and statistical analysis presented herein demonstrates the high specificity, sensitivity and reproducibility of the assay. This work provides an ideal HTS assay for the identification of inhibitors targeting the function of IAV RdRp and a convenient report system for mechanism study of IAV RNA transcription / replication.

Poster Session

VIR-PTH2031 - **Species-specific genomic signatures of influenza A Viruses – An update after the emergence of H1N1pdm in the 21st Century**

Guang-Wu Chen[1,2], Yu-Nong Gong[1], Shin-Ru Shih[2,3]

[1]Dpt of Computer Sc and Info. Engineering, Chang Gung University, Taoyuan, Taiwan, [2]Research Center for Emerging Viral Infections, Taoyuan, Taiwan, [3]Department of Medical Biotechnology, Chang Gung University, Taoyuan, Taiwan

What genetic variations render an avian influenza virus becoming a pandemic strain in human has been an important question to address for years. In our 2006 paper (Emerging Infectious Disease) we computationally analyzed 306 human and 95 avian influenza A genomes to reveal 52 species-associated amino acid signatures that differ significantly between the two species. In 2009 when the new H1N1pdm firstly emerged, we re-computed these signatures based on nearly 3,000 human and avian genomes, respectively. The identified signatures based on this much larger dataset mostly remained the same. However, since H1N1pdm carries genes from ancestral avian viruses during its evolutionary course, we'd expect such species barrier to blur in these years. At the end of 2013 we collected over 10,000 genomes from each of the human and avian influenza A viruses to demonstrate how the previously identified signatures may become obscure. For example, PB2 E627K has long been known a molecular determinant for an avian virus to replicate efficiently in mammalian cells. In avian viruses, the amino acid composition at this position showed a dominant E in the mentioned two earlier studies (196Es and 19Ks in 2006, and 3,113Es and 228Ks in 2009). In human viruses PB2 627 was overwhelmingly K in both investigations. While it remains largely E in avian viruses through the end of 2013 (10,187Es and 510Ks), the familiar human signature K was no longer prevailing in human isolates. Rather, the once-called avian signature E appears 4,767 times (or 44.7%) in the analyzed 10,666 human viruses. In this study we present molecular markers to differentiate human and avian viruses based on an updated influenza virus sequence database in mid-2014, including new species-specific amino acid signatures as well as what gene segment(s) to best serve the purpose for molecular diagnosis of human vs avian influenza A viruses.

Poster Session

VIR-PTH2033 - Impact of screening influenza viruses for oseltamivir resistance in a routine diagnostic setting

Avram Levy[1,3], Aeron Hurt[2], Simone Tempone[1], David Smith[1,3]
[1]PathWest Laboratory Medicine WA, Perth, Australia, [2]WHO Collaborating Centre for Reference and Research on Influenza, North Melbourne, Australia, [3]School of Pathology and Laboratory Medicine University of Western Australia, Nedlands, Australia

Since the emergence of the pandemic influenza virus A(H1N1)pdm09 in 2009, we screened samples from hospitalised patients and from the broader community for the presence of the H275Y neuraminidase (NA) mutation which confers resistance to the influenza antiviral oseltamivir. Clinical samples were initially tested by a multiplex PCR, and a sample of those positive for influenza A(H1N1)2009 were then screened for the H275Y mutation by real-time RT-PCR. Positives were confirmed by DNA sequencing, and culture positive samples underwent phenotypic neuraminidase inhibitor susceptibility testing. 1449 of a total of 8485 A(H1N1)pdm09-positive samples (17.1%) were tested between May 2009 and December 2013. The H275Y NA mutation was identified in samples from 14 patients (1.2% of patients tested), 10 of whom were hospital inpatients at the time of sampling and another was sampled just prior to admission. Eight of these patients had received oseltamivir treatment. Three cases were epidemiologically linked and there was high sequence similarity between the viruses, suggesting limited human-to-human transmission, but there was no evidence of ongoing activity. None of these three patents had received oseltamivir and all had mild illnesses and recovered spontaneously. The yield of oseltamivir-resistant viruses from community samples was low (0.24%), even in 2013 when all available A(H1N1)pdm09-positive samples were tested. Ongoing monitoring of antiviral resistance in influenza viruses is important, however we found that routine testing of patients in the community had a low yield with little clinical or public health relevance. In contrast, testing of viruses from hospitalised patients, especially those receiving oseltamivir treatment was useful in guiding treatment and appropriate infection control measures.

Poster Session

VIR-PTH2035 - Avian influenza virus surveillance in duck farms and wet markets in Vietnam

Kosuke Soda[1], Kozue Hotta[2,3], Susumu Toyoumi[1], Yukiko Uno[1], Yasuko Nagai[1], Mai Q. Le[3], Lien S. Phuong[4], Hiroichi Ozaki[1], Tetsu Yamashiro[2,3], Toshihiro Ito[1]
[1]Tottori University, Tottori, Japan, [2]Nagasaki University, Nagasaki, Japan, [3]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, [4]Vietnam Veterinary Association, Hanoi, Vietnam

Since the emergence of H5N1 highly pathogenic avian influenza (HPAI) viruses in Asian countries, numerous efforts have been made worldwide to control outbreaks and eradicate the virus. The number of outbreaks has decreased, however, incidences of H5N1 virus infections among poultry have been reported sporadically in Vietnam. It has been suggested that HPAI viruses show moderate pathogenicity to domestic ducks while high pathogenicity to chickens. Therefore, domestic ducks are suggested as a potential source for HPAI viruses, playing a role in dissemination of viruses to environment. In the present study, virological surveillance has been carried out to elucidate constellation of avian influenza virus among domestic ducks in wet markets and duck farms in Vietnam. A total of 1,000 and 840 swab samples (throat and cloacal) were obtained from apparently healthy domestic ducks on farms in Nam Dinh province and on wet markets in Hanoi, Quang Ninh, Nha Trang and Long An provinces, and applied for virus isolation. As of January 2014, total 182 influenza A viruses had been isolated (20 H3N2, 5 H3N6, 43 H3N8, 5 H4N6, 1 H4N9, 29 H5N1, 7 H6N2, 44 H6N6, 20 H9N2, 3 H10N2 and 5 H11N9). Phylogenetic analyses revealed that HA genes of all the H5N1 virus isolates except one were classified into clade 2.3.2.1 which contained the recent HPAI viruses in Eastern Asian countries. The isolates were divided into three subclade A-C, and only subclade C viruses have been isolated since 2013. Cross neutralization test revealed that clade 2.3.2.1A and C viruses were not neutralized by the chicken anriserum against clade 2.3.2.1B strain. These results suggested that antigenic variation of H5N1 viruses is progressively occurring in domestic ducks in Vietnam and that the current applied clade 2.3.2.1B virus-based vaccine has little effect on protecting poultry industries from latest circulating clade 2.3.2.1C viruses.

Poster Session

VIR-PTH2037 - Predicting antigenic changes influenza viruses through data assimilation

Kimihito Ito[1]
[1]*Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan*

The hemagglutinin (HA) molecule of influenza A viruses is the prime target of antibodies that neutralize viral infectivity. The strong immune pressure against HA in the human population selects a new variant every 2–5 years. Thus, influenza A viruses undergo antigenic changes with gradual accumulation of amino acid substitutions on HA. This antigenic change is one of the primary reasons why vaccination is not a perfect measure to control seasonal influenza. Influenza vaccine often requires replacement to avoid antigenic mismatch between vaccine and epidemic strains. The decision of vaccine replacement must be made several months before a minor strain become dominant strain. Therefore, the prediction of antigenic change of influenza A virus has been one of the major public health goals. To establish a practical method enabling us to predict the future direction of the viral evolution, the timing when the dominant epidemic strains were replaced by other strains, and the magnitude of outbreaks of seasonal influenza, we developed a new prediction method based on a framework of data assimilation. Data assimilation is a statistical method by which actual observations are integrated into computer simulations. So far data assimilation techniques have been applied in many scientific fields, including geosciences, life science, and engineering. Significant improvements in prediction accuracy have been reported, especially in weather forecast and hydrology. In this presentation, I will introduce the newly developed data-assimilation-based system for the prediction of influenza virus evolution. We constructed a mathematical model of viral population, infection, and host immunity. Based on the developed model, actual viral evolution observed in past 42 years was analyzed by a sequential Bayesian inference method called particle filters. Currently available results showed the data assimilation could have better potential to predict future amino acid substitutions on HA than our previous method.

Poster Session

VIR-PTH2039 - Effect of neuraminidase on influenza B virus growth characteristics

Vladimir Lugovtsev[1], Darya Melnyk[1], Jerry Weir[1]
[1]*CBER/FDA, Bethesda, USA*

Functional balance of hemagglutinin (HA) and neuraminidase (NA), the major surface glycoproteins of influenza A and B viruses, is required to ensure efficient virus replication. In this study we investigated the effect of viral NA on growth properties of influenza B viruses. Using a reverse genetics technology a panel of 7:1 reassortants, differing only by the RNA segment 7, encoding NA/NB, was established. The RNA segments 7 were derived from influenza B virus strains, representing different phylogenetic groups. Growth characteristics of the rescued viruses were evaluated by yield of HA in chicken embryonated eggs, plaque size phenotype, and growth kinetics in MDCK cell line. The obtained results demonstrated that that NA/NB component may significantly affect the growth properties of the virus. Virus variants differed significantly by plaque size, however regardless of the plaque phenotype, the growth kinetics in MDCK was relatively similar in most cases. The significant differences between viruses were observed in yield in eggs, where the yield of HA (μg) varied between virus variants in a range of an order of magnitude. The results observed in this study suggest a novel approach for improvement of virus growth characteristics and increase of HA yield by selection of optimal NA/NB component.

Poster Session

VIR-PTH2041 - Multiple clades of highly pathogenic Avian Influenza H5N1 viruses circulated in Central Vietnam between 2013 and 2014

Huu Tinh Nguyen[1], Wonyong Kim[1]
[1]*Chung-ang University, Seoul, South Korea*

Highly pathogenic avian influenza (HPAI) H5N1 viruses are endemic to poultry in Vietnam. However, there are few reports on viruses circulating in Central Vietnam, especially in South Central region. In this study, 47 H5N1 positive samples were collected from outbreaks in poultry in Khanh Hoa and Phu Yen provinces between 2013 and 2014. Genetic analysis indicated that all the samples possessed common motif sequence for HPAI virus at cleavage sites. Phylogenetic analysis indicated that three clades of HPAI H5N1 exist including clade 1.1.2 (n = 1), clade 2.3.2.1a (n = 1) and clade 2.3.2.1c (n = 45). Recently, the clade 2.3.2.1 viruses were dominant in Northern Vietnam, while the clade 1 was circulated in Southern provinces. Therefore, multiple clades of HPAI H5N1 viruses with the prevalence of the clade 2.3.2.1c were co-circulated in Khanh Hoa province, which belong to South Central of Vietnam may raises a concern of introduction of the clade 2.3.2.1c viruses into Southern Vietnam.

Poster Session

VIR-PTH2043 - The molecular epidemiology of HIV in Alberta, Canada: 1998-2013, a retrospective 15 year analysis

Dena Adachi[1], Marlin Benedet[1], Ameeta Singh[2], Ron Read[3], Stephen Shafran[2], Geoffrey Taylor[2], Stanley Houston[2], Kimberley Simmonds[4], Christopher Sikora[5], Julian Tang[1,6]

[1]Alberta Provincial Laboratory for Public Health, Edmonton, Canada, [2]Department of Medicine, University of Alberta, Edmonton, Canada, [3]Department of Medicine, University of Calgary, Edmonton, Canada, [4]Infectious Disease Epidemiology, Government of Alberta, Edmonton, Canada, [5]Medical Officer of Health, Alberta Health Services, Edmonton, Canada, [6]Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada

BACKGROUND. The province of Alberta, Canada has a population of approximately 4 million, in which about 240 new cases of HIV are diagnosed per year. With the large, mobile immigrant population (for both work and study) in this province, some of whom come from HIV-endemic countries, it is useful to periodically survey the mixture and numbers of HIV subtypes for public health epidemiology and health resourcing purposes. METHODS. HIV genotype sequences (partial reverse-transcriptase and complete protease sequences from the pol gene) obtained during 1998-2013, for routine HIV genotyping as part of patient clinical management, were used for an epidemiological and phylogenetic analysis. A maximum likelihood tree was produced showing HIV subtype prevalence and clustering. In addition, the HIV subtype data was stratified by age and sex. RESULTS. The majority of HIV infections were diagnosed in the 20-40 year age groups. These were caused by HIV-1 Group M subtype B and C across both sexes. The majority of the younger population being diagnosed with HIV subtype B and C infections occurred during 2007-2012, peaking around 2008-2009. This coincided closely with the immigration of non-permanent residents into Alberta. This immigration rose steeply after 2004-2005, probably as a result of the discovery of oil sands and natural gas in Northern Alberta, which attracted many migrant workers. Other HIV subtypes are much rarer and continue to remain rare in this population, being less than 10% of the known HIV-infected individuals. These other subtypes include B/D, A/E, AG and the various cpx recombinant circulating forms. CONCLUSION. This analysis suggests that if current trends continue, the proportion of HIV subtype B infections may increase at the expense of subtype C and possibly A, in this population. Periodic population surveillance of circulating HIV types/ subtypes is useful for understanding epidemiological trends and appropriate health resource allocation.

Poster Session

VIR-PTH2045 - Galectin-3 is involved in HIV-1 expression in latently infected cells through NF-κB activation and the interaction with Tat.

Mika Okamoto[1], Akemi Hidaka[1], Masaaki Toyama[1], Masanori Baba[1]
[1]*Center for Chronic Viral DIseases, Kagoshima University, Kagoshima, Japan.*

Antiretroviral therapy (ART) has achieved successful reduction of plasma viral load in human immunodeficiency virus type 1 (HIV-1)-infected patients below the undetectable level. However, the current ART cannot eradicate the virus from patients because of latently infected cells in their bodies. The latently infected cells exist for a long period of time in patients as viral reservoirs, which lead to viral rebound in case of treatment interruption. The machinery of HIV-1 expression in latently infected cells, especially cellular factors involved in this process, has not been fully understood yet. Galectin-3, a member of the lectin family binding to β-galactoside, is widely expressed in various cells and plays an important role in cell proliferation and differentiation and modulating inflammation. In this study, we have demonstrated that Galectin-3 promotes HIV-1 expression in latently infected cells. When the latently infected cell line OM-10.1 was treated with tumor necrosis factor (TNF)-α, Galectin-3 expression, along with HIV-1 expression, was up-regulated at both mRNA and protein levels in a dose dependent manner. Knockdown of Galectin-3 expression by RNA interference resulted in the suppression of HIV-1 production in TNF-α-stimulated OM-10.1 cells. Furthermore, the suppression of Galectin-3 reduced the TNF-α-induced nuclear translocation of nuclear factor (NF)-κB in the cells. In addition, we found that the level of Galectin-3 correlated with strong expression of HIV-1 Tat, a viral trans-activation factor essential for efficient HIV-1 transcription, in OM-10.1 cells after stimulation with TNF-α, when determined by double immunochemical staining with antibodies specific to Tat and to Galectin-3. Furthermore, direct interaction between Galectin-3 and Tat was observed in TNF-α-stimulated OM-10.1 cells by co-immunoprecipitaion. These results suggest that Galectin-3 is involved in HIV-1 expression in latently infected cells through NF-κB activation and the interaction with Tat.

Poster Session

VIR-PTH2047 - Azasugar-containing phosphorothioate oligonucleotide (AZPSON) DBM-2198 inhibits the replication of HIV-1 variants by blocking HIV-1 gp120 without affecting V3 region

Jinjoo Lee[1], Yideul Jeong[1], Yong-Soo Bae[1]
[1]*Sungkyunkwan University, Suwon, Korea*

DBM-2198, a six-membered azasugar nucleotide (6-AZN)-containing phosphorothioate (P=S) oligonucleotide (AZPSON), was featured in our previous report,1 with regard to its antiviral activity against a broad spectrum of HIV-1 variants. This report describes the mechanisms underlying the anti-HIV-1 properties of DBM-2198. The LTR-mediated reporter assay revealed that the anti-HIV-1 activity of DBM-2198 is likely attributed to extracellular activity rather than intracellular sequence-specific antisense activity. Nevertheless, the antiviral properties of DBM-2198 and other AZPSONs were highly restricted to HIV-1. Unlike other P=S ONs, DBM-2198 caused no host cell activation during its administration into cultures. Once HIV-1 was pre-incubated with DBM-2198, virus did not show any infectivity to host cells, while host cells pre-incubated with DBM-2198 remained susceptible to HIV-1 infection, suggesting that DBM-2198 acts on the virus particle rather than cell surface molecules for the inhibition of HIV-1 infection. Affinity competition assays with anti-gp120 and anti-V3 antibodies revealed that DBM-2198 acts on the viral attachment site of the HIV-1 gp120, but not on the V3 region. This report provides a better understanding of the antiviral mechanism of DBM2198 and may contribute to further study of DBM-2198 for the development of a potential therapeutic drug against a broad spectrum of HIV-1 variants.

Poster Session

VIR-PTH2049 - Long-term longitudinal evaluation of the first-line ART in a cohort at Pointe-Noire, Republic of Congo

Raphael Taty-Taty[1], Miyuki Yasunaga[2], Aya Umehara[2], Jacob Barnor[3], Henri-Joseph Parra[4], Shoji Yamaoa[5], Eiji Ido[3,5]

[1]Centre de Pathologie Infectieuse, Pointe-Noire, Republic of Congo, [2]Institute for Virus Research, Kyoto University, Kyoto, Japan, [3]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [4]Laboratoire National de Sante Publique, Brazzaville, Republic of Congo, [5]Tokyo Medical and Dental University, Tokyo, Japan

Background: Since the first-line ART consisting triple drugs (two NRTIs and one NNRTI) was introduced in 2006 to the Republic of Congo (Brazzaville), one of the extremely resource-limiting countries in Africa, more than seven years have passed. However, any proper evaluation of the current regimen has not been conducted to date due to the lack of experimental facilities. Thus we attempted to evaluate the first-line ART by conducting a long-term longitudinal study using a cohort in Pointe-Noire, a coastal city in western Republic of Congo where a large variety of HIV genotypes are known to be co-circulating. Methods: Twenty patients under ART have been recruited for this study. A series of blood samples were collected from 2006 till 2013, serologically diagnosed, and viral loads in plasma were measured by in-house qRT-PCR methodology. DNAs extracted from PBMCs were analyzed in part of pol region for phylogenetical classification and in 5'-half of RT region for drug-resistant mutations. Results: The genotype distribution was highly diversified with the predominant subtype A (approximately 40 %) followed by G, CRF19, D, H, C, F, J, etc. The plasma viral loads were all below the detection limit (160 copies/ml) except one case with a very low value. No significant multi-drug-resistant mutations were found in the RT genomic region so far although typical drug-resistant mutations for each drug (e.g. M184V for 3TC, etc.) were observed extensively. Conclusions: A genetic profile examined in this study was greatly diversified. Nonetheless, the current ART seemed to be effective in most patients from a viewpoint of controlling the plasma viral loads even after its long-term usage. However, the spread of drug-resistant mutations suggest that an alternative regimen including protease inhibitors or other new drugs may be required very soon.

Poster Session

VIR-PTH2051 - Development and validation of TaqMan probe based real time PCR assays for the specific detection of genotype A and B small ruminant lentivirus strains

Darja Barlic - Maganja[1], Urska Kuhar[2], Joze Grom[2]
[1]University of Primorska, Faculty of Health Sciences, Izola, Slovenia, [2]University of Ljubljana, Veterinary Faculty, Virology Unit, Ljubljana, Slovenia

Caprine arthritis encephalitis virus (CAEV) and Maedi Visna virus (MVV) are small ruminant lentiviruses (SRLVs) within the Retroviridae family, which infect goats and sheep worldwide. The AGID and the ELISA are the most commonly used means of detecting specific antibodies and the most convenient way of diagnosing SRLV infections. The most frequent molecular method for detecting the provirus genome is PCR, using peripheral blood leucocytes as target cells. There have also been a few reports describing real time PCR for the detection of SRLV. The highly heterogeneous SRLV genome and low proviral load hinder the usefulness of PCR for diagnosing infection with SRLV, although the development of an assay based on viral strains circulating in a particular geographic area might solve the former problem. Phylogenetic analyses divides SRLV into five genotypes, A to E. Genotypes A and B are widely distributed throughout the world, whereas genotypes C, D and E are geographically restricted. Since the phylogenetic analysis of Slovenian SRLV strains revealed that ovine strains belong to genotype A and caprine strains to genotypes A and B, two new TaqMan probe based real time PCR assays for the specific detection of genotype A (MVV) and B (CAEV) SRLV strains and differentiation between them was developed and validated. Such a method has not to date been described. Primers and probes were designed in the gagMA gene region. Validation experiments followed the (MIQE) guidelines to ensure the integrity and experimental transparency of development of the new assays. The validation results showed that the assays are not only highly specific and sensitive but also repeatable and reproducible. The two new TaqMan probe based real time PCR assays can serve as an additional tool for confirming infection with SRLV and may also be useful for early detection of infected animals prior to seroconversion.

Poster Session

VIR-PTH2053 - Characterization of the bovine immunodeficiency virus Rev protein multimerization activity

Claude Marchand[1], Andrea Gomez Corredor[1], Denis Archambault[1]
[1]*University of Quebec at Montreal, Montreal, Canada*

Bovine immunodeficiency virus (BIV) is a lentivirus of the Retroviridae family which shares morphologic, genetic, antigenic and/or biologic properties with human immunodeficiency virus type 1 (HIV-1) and other animal lentiviruses. The BIV provirus DNA shows the typical retroviral 5'-3' gag, pol and env structural gene organization and the presence of flanking long terminal repeats (LTRs) at the 5' and 3' termini. The BIV genome also contains additional open reading frames that may encode nonstructural/regulatory proteins. Among the latter proteins is Rev [186 amino acids (aa) in BIV]. Rev is a shuttling protein that transports unspliced and partially spliced lentiviral RNAs from the nucleus to the cytoplasm. HIV-1 Rev protein contains at least three central functional domains: a basic arginine-rich domain that mediates RNA binding (RBD) and contains the nuclear/nucleolar localization signals (NLS/NoLS), a multimerization domain and a leucine-rich domain that is necessary for the nuclear exportation of Rev. We previously reported the presence of novel types of NLS and NoLS in BIV Rev protein (Journal of Virology 83:12842-12853, 2009). Here we report on the multimerization activity of BIV Rev. Protein cross-linking experiments using recombinant Rev showed that BIV Rev multimerizes in vitro. By using a series of Rev deletion mutants, two regions encompassing aa 1 to 30, and 90 to 110 of the BIV Rev sequence appeared to be necessary for the multimerization of the protein in vitro. The use of a mutant form of BIV Rev that does not localize to the nucleus together with wild-type Rev indirectly showed that the protein also multimerizes in living cells as determined by confocal microscopy analysis. This result was confirmed by using a bimolecular fluorescence complementation (BiFC) assay. Experiments are under way to identify more precisely the regions and aa that mediate the multimerization of BIV Rev by using BiFC analysis.

Poster Session

VIR-PTH2055 - Examining phylogenetic origins of endogenous retroviruses expressed during mammalian oogenesis and embryogenesis

Gilberto Jaimes[1]
[1]*University of South Florida College of Medicine, Tampa, USA*

Transposons impact on genome structure and function is driven, in part, through Long Terminal Repeats Transposable Elements (LTR TE) propagation. Oogenesis and preimplantation development was previously identified as a major window for LTR TE propagation within laboratory mice, *Mus musculus*. This LTR TEs activity provides a glimpse into the molecular machinery used to control gene expression. It has been shown that mouse embryo and eggs express TEs, notably the MuERV-L and MuLV endogenous retroviruses, as well as non-autonomous MTA and ORR1. In order to explore phylogenetic origins of of these highly expressed LTR TEs a series of sequence analyses aimed at describing homologous relationships were performed. Continuing the investigation of high expression levels of LTR TE during preimplantation development, we examined LTR TE expression patterns in two mammalian species, *Bos taurus* (cow) and *Oryctolagus cuniculus* (rabbit). Our preliminary studies similarly identified LTR TE with two ERV-like elements in cow and three ERV-like elements in rabbit as highly expressed. Unexpectedly, no homologs were identified when analyzed against close relatives of these mammals. To resolve missing homology, we used Basic Search Alignment Tool (BLAST) and MEGA5 to conduct a phylogenetic analysis of these highly expressed ERV-like sequences. The preliminary findings for cow and rabbit transposons expressed in preimplantation development indicate their polyphyletic origins. Furthermore, comparative sequence analysis of MuERV-L of *M. musculus* to other species revealed restricted homology to avian retrovirus, specifically *Anas platyrhynchos* (mallard duck). Together, these findings may imply LTR TE interactions with host genomes and patterns governing LTR TE evolution.

Poster Session

VIR-PTH2057 - Production of antiviral small interfering RNAs during dengue virus infection of high- and low-susceptibility strains of Aedes aegypti mosquitoes

William Johnson[1], Abhishek Prasad[2], David Price[1], Gregory Ebel[2], Immo Hansen[1], Kathryn Hanley[1]
[1]New Mexico State University, Las Cruces, USA, [2]Colorado State University, Fort Collins, USA

Mosquito populations are known to vary in vector competence for dengue virus (DENV), but determinants of vector competence are poorly understood. To investigate whether differences in RNA interference (RNAi) are associated with differences in susceptibility to DENV, we compared production of antiviral small interfering RNAs (viRNAs) of two strains of Ae. aegypti with high and low susceptibility to DENV: respectively the Rockefeller strain and a field-derived strain from Las Cruces, New Mexico (LC). Mosquitoes were fed a bloodmeal containing approximately 6.7 log10 PFU/ml DENV serotype 4 (DENV-4) and incubated 14 days. Midguts and carcasses were separated, pooled into 3 groups of 5 and homogenized; virus titer in carcass pools was determined by serial titration and immunostaining. Small RNAs between 19 and 28 nucleotides long were extracted from the midgut pools, sequenced, and aligned to the DENV-4 genome. Both strains produced predominantly 21-nucleotide viRNAs (83% and 85% of total); subsequent analyses focused only on 21-nucleotide viRNAs. Abundance of viRNAs normalized to total siRNA abundance was significantly greater in Rockefeller (mean ±1SE: 7070±746.9) than LC (124±77.5) (Mann-Whitney U, DF = 4, $P = 0.03$); approximately half of viRNAs targeted the positive-sense virus genome in both strains. Across both strains viRNA abundance showed a significant positive correlation with viral titer (Pairwise correlation, DF = 4, $P = 0.003$). A sliding-window analysis revealed that the distribution of viRNAs across both positive- and negative-sense genome was similar in the two mosquito strains (Spearman's ρ, $P < 0.01$ for both comparisons). However genome coverage of viRNAs differed significantly from random in both mosquito strains for both positive- and negative-sense virus genomes (Chi Squared test, $P < 0.001$ for all comparisons). These findings indicate that viRNA production at day 14 post infection is driven by virus replication and that viRNAs show hot- and cold-spots in genome coverage.

Poster Session

VIR-PTH2059 - Regulation of Epstein-Barr virus DNA replication by microRNA

Yueh-Lung Wu[1], Carol P. Wu[2], Yu-Chan Chao[2]
[1]*Department of Entomology, National Taiwan University, Taipei, Taiwan,* [2]*Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan*

MicroRNAs (miRNAs) are a class of small RNA molecules found in most eukaryotic cells and primarily known to function as post-transcriptional regulators in multiple cellular processes. Here we show that a human cellular miRNA, microRNA-155 (hsa-miR-155), can regulate the latent replication origin (oriP) of Epstein-Barr virus (EBV). This miRNA was found to compete with Epstein-Barr nuclear antigen 1 (EBNA1) for binding directly to the dyad symmetry (DS) sequence on the oriP, and hinder the latent DNA replication of this virus. When the binding of hsa-miR-155 to the DS sequence was abolished by introducing a mutation into the hsa-miR-155 or DS sequences, replication resumed. Furthermore, innate hsa-miR-155 was found to be capable of targeting specifically to the EBV genomic replication origin in EBV type I-latently infected cells and regulating the viral DNA replication. Our discovery represents a hitherto undiscovered important function of miRNA for the control of DNA replication, and demonstrates a probable mechanism of how this can be achieved using the latent replication origin of EBV.

Poster Session

VIR-PTH2061 - The control of pathogenesis by regulatory T (Treg) cells during CHIKV infection

Wendy WL Lee[1,2], Teck Hui Teo[1,2], Zhi Sheng Her[1], Fok Moon Lum[1,2], Olaf Rotzschke[1], Lisa FP Ng[1,2]
[1]Singapore Immunology Network, Singapore, [2]National University of Singapore, Singapore

Chikungunya virus (CHIKV) has re-emerged as an important arbovirus responsible for Chikungunya fever (CHIKF), a disease characterized by fever, muscle pain and the characteristic joint pain that could last up to years in patients. Human cohort studies have indicated that the pathogenesis of CHIKF is cytokines-driven, while studies in mouse models have implicated the involvement of CD4+ T cells in joint pathology. However, the role of Treg cells that are central to the maintenance of immune tolerance and homeostasis remains elusive in CHIKV-induced pathogenesis. In order to evaluate the role of Treg cells during CHIKV infection, anti-IL-2 (JES6-1A12) complexed with IL-2 (IL-2 Ab Cx) was administered to adult C57BL/6 mice before CHIKV infection to selectively enhance Treg population in vivo. Enhancement of Tregs markedly reduce disease severity at the joints but has no effect on virus clearance. Similarly, histological analysis of the joint footpad also displayed reduced oedema in mice treated with IL-2 Ab Cx. This reduction in pathology was found to be associated with the absence of CD4+ T cells infiltration into the infected joint footpad with no distinctive effects on the other immune populations. In agreement with the reduction of joint pathology, two-way cluster analyses on gene expression studies performed on extracted CHIKV-infected tissues also revealed a diminution of pro-inflammatory signals. In conclusion, through selective enhancement of the Treg population, collateral damages caused by activation of the immune system could be effectively subdued without influencing virus clearance. It leaves to be further established if this approach could be an alternative immune-based strategy to alleviate symptoms experienced in CHIKF patients while allowing natural clearance of the virus.

Poster Session

VIR-PTH2063 - Force-induced globule-coil transition in Laminin Binding Protein and its role for viral – cell membrane fusion

Boris Zaitsev[1], Fabrizio Benedetti[2], Andrey Mikhaylov[2], Denis Korneev[1], Sergey Sekatskii[2], Pavel Belavin[3], Elena Protopopova[1], Giovanni Dietler[2], Valery Loktev[1]

[1]State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk region, Russia, [2]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [3]Institute of Cytology and Cell Biology Russian Acad. Sci., Novosibirsk, Russia

We report the results of experiments where we exploit the existence of two very distinct receptor regions in the flaviviral protein E, and different dependencies of the specific interactions involved on pH value, to clarify still unclear details of the flaviviral – cell membrane fusion process. The specific interactions of the pairs laminin binding protein (LBP) – purified Tick-Born Encephalitis Viral surface protein E and certain recombinant fragments of this protein, as well as West Nile Viral surface protein E and certain recombinant fragments of that protein, are studied by combined methods of Single Molecule Dynamic Force Spectroscopy (SDFS), enzyme immunoassay and a biosensor based on optical surface waves measurements. The experiments were performed at neutral pH=7.4 and acid pH=5.3 conditions. The data obtained confirm the role of LBP as a cell receptor for two typical viral species of the flavivirus genus. The comparison of these data with similar data obtained for another cell receptor for this family, viz. human alphaVbeta3 integrin, reveals that both these receptors are very important. Studying the specific interaction between the cell receptors at question and specially prepared monoclonal antibodies against them, we could show that both interaction sites involved in the process of virus – cell interaction remain intact at pH=5.3. At the same time, for these acid conditions characteristic for an endosome during flavivirus – cell membrane fusion, SMDFS data reveal the existence of a force induced (effective already for forces as small as 30 – 70 pN) sharp globule – coil transition for LBP and LBP – fragments of protein E complexes. We argue that this conformational transformation, being an analogue of abrupt first order phase transition and having similarity with famous Rayleigh hydrodynamic instability, might be indispensable for the flavivirus – cell membrane fusion process and have other biological implications.

Poster Session

VIR-PTH2065 - Role of TSPAN9 in alphavirus entry

Katie Stiles[1], Yaw Shin Ooi[1], Catherine Liu[1], Margaret Kielian[1]
[1]*Albert Einstein College of Medicine, Bronx, USA*

Alphaviruses are spread by mosquitos and cause severe human disease such as arthritis, myocarditis, and encephalitis. For example, recent outbreaks of chikungunya virus (CHIKV) are responsible for millions of cases of acute illness and long term complications. Alphaviruses are small positive-sense RNA viruses. Their envelope contains an ordered lattice of the viral proteins E1 and E2. Alphaviruses enter cells by binding to cell surface receptors through E2 and are internalized by clathrin-mediated endocytosis. In the endosome, low pH triggers conformational changes in E1 leading to virus-membrane fusion. After replication, new virions bud from the plasma membrane. Despite many studies that have determined the general pathways of alphavirus entry and exit using Semliki Forest virus (SFV) and sindbis virus (SINV), we know little about host factors that influence virus infection. To identify cellular proteins involved in the alphavirus life cycle, we performed a genome-wide RNA interference screen using the prototype alphavirus SINV. From this screen, TSPAN9 was identified as a proviral host factor whose depletion inhibited infection by SINV as well as the alphaviruses SFV and CHIKV. However, infection by the flavivirus dengue virus was not inhibited. We found that the block for SFV infection in TSPAN9-depleted cells was at a late step after the acid-induced conformational change in E1. In the absence of TSPAN9, SFV membrane fusion was severely reduced. TSPAN9 is a multi-pass membrane protein and is a member of the tetraspanin superfamily. Using U-2 OS cells stably overexpressing TSPAN9, we showed that this protein is localized at the plasma membrane and on vesicular structures in the cytoplasm. Furthermore, SFV particles co-localized with TSPAN9 in vesicular structures early during infection. Results of the studies of the role of this novel host protein in alphavirus entry and the endocytic pathway will be presented.

Poster Session

VIR-PTH2067 - Encapsidation of host RNA by Brome mosaic bromovirus

Philipp Weber[1]
[1]*DeKalb, USA*

Philipp Heinrich Weber1, and Józef J. Bujarski1, 2 1Plant Molecular Biology Center and the Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, USA. 2Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland. Although RNA viruses evolved the mechanisms of specific encapsidation of their genomic RNAs, inefficient miss-packaging of host (cellular) RNAs has been described in several RNA virus systems, e.g. in Flock house virus, but also in DNA viruses. In order to find out if Bromoviridae, model multipartite RNA viruses can encapsidate cellular RNAs, we have used Brome mosaic bromovirus (BMV), the type virus. BMV was grown in three different hosts, i.e. barley, Nicotiana benthamiana and Chenopodium quinoa, and extracted by using a standard initial protocol. Thereafter, viral preparations were decontaminated by sucrose cushion ultracentrifugation, treated with ribonucleases and finally purified via centrifugal ultrafiltration. The presence of intact viral RNAs was confirmed reflecting the correctness of the used methodology. Currently, we are in the process of analysis of the RNA content by using next-generation sequencing. These studies are expected to reveal the range of non-viral RNAs encapsidated by BMV and its host dependence, allowing us to address if bromoviruses could function as vectors in the horizontal gene transfer.

Poster Session

VIR-PTH2069 - Zippering of the stem region in the alphavirus E1 post-fusion trimer during membrane fusion

Nina Grau[1], Karine Moncoq[1], Marie-Christine Vaney[1], Annalisa Meola[1], Thomas Krey[1], Claudia Sanchez- san Martin[2], Margaret Kielian[2], Felix Rey[1]

[1]Unité de Virologie Structurale, Institut Pasteur, Paris, France, [2]Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York, USA

Chikungunya virus (CHIKV) is a mosquito-borne *Alphavirus* that has caused important outbreaks of severe disease during the last decade. The virus particle exposes an icosahedral glycoprotein layer at its surface, made of 80 trimeric spikes composed of glycoproteins E2 and E1, which are responsible for receptor binding and membrane fusion, respectively. Each spike is formed by three E1/E2 heterodimers, in which the fusion loop of E1 is buried at the interface with E2. Binding of E2 to a cellular receptor results in particle uptake into an endosome, where the environment is acidic. This results in protonation of the E1/E2 proteins and dissociation of the heterodimer, with concomitant exposure of the E1 fusion loop, which inserts into the endosomal membrane. A subsequent conformational change of E1 leads to a homotrimeric form that induces fusion of viral and endosomal membranes, thereby releasing the viral nucleoprotein core into the cytoplasm of the cell, which becomes infected. Previous crystallographic studies on the Semliki Forest virus E1 post-fusion trimer showed an overall hairpin conformation, in which the fusion loop and the region near the C-terminal transmembrane (TM) segment are in close proximity. However, in those studies the protein lacked the stem region - immediately upstream of the TM segment. We have now produced and crystallized a version of CHIKV E1 that contains the intact ectodomain, and determined its crystal structure. The structure shows that the stem region zippers together the three subunits in the E1 trimer, such that the three fusion loops interact with each other at the membrane proximal end of the post-fusion trimer, which was not the case in the absence of the stem. These results therefore provide further insight into the fusogenic conformational change of E1, bringing evidence for intra-trimer zippering of the subunits to bring the two membranes into close apposition.

Poster Session

VIR-PTH2071 - Human heat shock protein 40 promotes Influenza A virus replication by assisting nuclear import of viral ribonucleoprotein

Jyoti Batra[1], Shashank Tripathi[1], Amrita Kumar[2], Jacqueline M. Katz[2], Nancy J. Cox[2], Renu B. Lal[2], Suryaprakash Sambhara[2], Sunil Lal[1]

[1]International Centre for Genetic Engineering & Biotechnology, New Delhi, India, [2]Centers for Disease Control and Prevention, Atlanta, USA

The nuclear import of Influenza A virus (IAV) genome is an indispensable step for establishing virus infection. The IAV nucleoprotein (NP) is known to mediate the entry of vRNPs into the nucleus via nuclear localization signal. It also functions as a key adaptor molecule between virus and cell processes through its ability to interact with various viral and host cellular proteins. Here, we identify a cellular heat shock protein 40 (Hsp40), which facilitates nuclear import of IAV vRNPs. Hsp40 interacts with NP component of influenza virus ribonucleoprotein and co-localizes with NP in the nucleus during early stages of the infection. The NP-Hsp40 interaction is mediated by J domain of Hsp40 and N-terminal region of NP as observed by GST pull down studies. Confocal microscopic analysis and cell fractionation studies indicated that over-expression of Hsp40 enhanced nuclear import of IAV RNP complex whereas down regulation of Hsp40 either by an inhibitor or specific siRNA reduced nuclear accumulation of nucleoprotein. Furthermore, knockdown of Hsp40 levels reduced viral transcription, translation and replication. These studies demonstrate an important role for cellular chaperone Hsp40 in influenza virus life cycle which assist nuclear trafficking of viral ribonucleoproteins.

Poster Session

VIR-PTH2073 - Are we heading towards the third epidemic in cotton: Past, present and future consequences? OR Cotton serving as a melting pot of geminiviruses

Muhammad Haider[1], Muhammad Shafiq[1], Muhammad Rehman[1], Mukhtar Ahamad[1], Muhammad Manzoor[1], Fakhra Shamim[1], Muhammad Sattar[1], Muhammad Ilyas[1]

[1]*University of the Punjab, Lahore, Pakistan*

Cotton leaf curl disease (CLCuD) is a main threat to cotton production worldwide. Most of the CLCuD-associated viruses belong to the genus Begomovirus (Family: Geminiviridae). The disease was first appeared in Pakistan near Moza Khokhran in 1966. But the first epidemic due to CLCuV (now called CLCuMuV and CLCuKoV) was observed in 1988 when it produced a significant loss to cotton crop in Pakistan. After a period of calm, in 2001 the second epidemic started when all CLCuD resistant cotton varieties became susceptible to the disease. It was due to a resistance breaking recombinant strain “Cotton leaf curl Burewala virus” (CLCuBuV) and associated recombinant betasatellite “Burewala strain” of Cotton leaf curl Multan betasatellite (CLCuMuB). Till then, cotton crop is harboring more than seven different species of begomoviruses globally, including Africa. Recently, we found Mesta yellow vein mosaic virus (MeYVMV) (Genus: Begomovirus; Family: Geminiviridae) and Chickpea chlorotic dwarf virus (CpCDV) (Genus: Mastrevirus; Family: Geminiviriade) from cotton. Presence of a new begomovirus and a mastrevirus in cultivated cotton crop points towards the risk of a new epidemic to cotton crop. Many of the CLCuD associated begomovirus species identified from Pakistan have been subsequently found from India. Recently, we have characterized Cotton leaf curl Alahbad virus (CLCuAlV) from cotton crop. This is the first report about identification of a CLCuD associated begomovirus from cultivated cotton crop in Pakistan. Thus, cotton crop may serve as a melting pot for so many geminiviruses in this region. This situation may give rise to the emergence of new species infecting cotton crop, which may start a third epidemic in this region.

Poster Session

VIR-PTH2000 - Characterization of the envelope glycoprotein of a novel filovirus, Lloviu virus

Junki Maruyama[1], Hiroko Miyamoto[1], Masahiro Kajihara[1], Hirohito Ogawa[2,3], Ken Maeda[4], Yoshihiro Sakoda[5], Reiko Yoshida[1], Ayato Takada[1,6]

[1]Div. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido Univ., Sapporo, Japan, [2]Hokudai Center for Zoonosis Control in Zambia, School of Vet. Med., The Univ. of Zambia, Lusaka, Zambia, [3]Hokkaido Univ., Research Center for Zoonosis Control, Sapporo, Japan, [4]Laboratory of Veterinary Microbiology, Faculty of Agriculture, Yamaguchi Univ., Yamaguchi, Japan, [5]Laboratory of Microbiology, Dept. of Disease Control, Graduate School of Vet. Med., Hokkaido Univ., Sapporo, Japan, [6]School of Vet. Med., the Univ. of Zambia, Lusaka, Zambia

Recently, a filovirus-like RNA genome was detected in bat (Miniopterus schreibersii) carcasses found in Spain. This novel filovirus, Lloviu virus (LLOV), is phylogenetically distinct from other filoviruses (viruses in the genus Ebolavirus and Marburgvirus) known to cause severe hemorrhagic fever in humans and/or nonhuman primates, and thus proposed to belong to the new genus Cuevavirus, species Lloviu cuevavirus in the family Filoviridae. However, the biological properties of this virus are uncharacterized since infectious LLOV has not been isolated yet. To provide information for estimation of the infectivity and potential pathogenicity of LLOV, we characterized its envelope glycoprotein (GP) which likely plays major role in the replication cycle and the pathogenicity of filoviruses. We first found that LLOV GP principally shares the primary structure with the other filovirus GPs and that, similarly to the other filoviruses, virus-like particles (VLPs) produced by transient expression of LLOV GP, matrix protein, and nucleoprotein in 293T cells had densely arrayed GP spikes on a filamentous particle. Mouse antiserum to LLOV VLP was little cross-reactive to viruses of the other genera, indicating that LLOV is a serologically distinct from the other known filoviruses. Using a replication-incompetent vesicular stomatitis virus (VSV) pseudotype system, we found that human C-type lectins act as attachment factors for LLOV entry into cells. We further found that LLOV GP has the potential to mediate viral entry into cells of various animal species including primates, while LLOV GP-pseudotyped VSV infected particular bat cell lines more efficiently than viruses bearing other filovirus GPs. These results suggest that LLOV GP mediates cellular entry in a manner similar to the other filoviruses while showing preferential tropism for some bat cells.

Poster Session

VIR-PTH2002 - Expression of IGF2 affects Borna disease virus production in infected cells

Akiko Makino[1], Kan Fujino[1], Takuji Daito[1], Tomoyuki Honda[1], Keizo Tomonaga[1]
[1]Kyoto University, Kyoto, Japan

Borna disease virus (BDV) is a non-segmented, negative-strand RNA virus that belongs to the Mononegavirales. BDV possesses several unique features distinguishing it from other RNA viruses. One of the most striking characteristics is that it readily establishes a long-lasting persistent infection in the cell nucleus. Furthermore, it has been demonstrated that BDV produces only a small number of infectious particles from infected cells. However, the mechanism of how the virion production of BDV is regulated has not yet been investigated. The aim of this study is to elucidate the regulation mechanism of BDV production in infected cells. To this end, we first performed screening experiment using an shRNA library with working hypothesis that certain host factor(s) repress the production of infectious particles in BDV-infected cells. Human oligodendroglia (OL) cells transduced with the shRNA library were infected with BDV, and the virus titers in the supernatants were monitored. As the result, we obtained several clonal cells, which produced high titers of BDV when compared with parental cells. By using next-generation sequencing analysis, we focused on 70 target genes of shRNAs enriched in the cells producing high titer BDV. Among them, human IGF2 (insulin-like growth factor 2) was identified as a host factor involved in the regulation of BDV production. IGF2 is involved in cell-growth and strongly associated with cell cycle progression. We demonstrated that siRNA knockdown of IGF2 significantly increased the BDV production. Furthermore, quantitative real-time PCR showed that the expression of IGF2 mRNA is markedly up-regulated in persistently BDV-infected cells. Moreover, the treatment of the reagents, which affect the cell cycle progression, enhanced the virus titer in the supernatant of BDV-infected cells. These data suggest that IGF2 has an important role in the regulation of BDV production via affecting the cell-growth.

Poster Session

VIR-PTH2004 - Incidence of co-infections with human norovirus and Clostridium difficile in hospitalized patients

Janelle Stokely[1], Vincent B. Young[1], Christiane Wobus[1]
[1]University of Michigan, Ann Arbor, United States

Human norovirus and Clostridium difficile are the two most common causes of infectious gastroenteritis in adults, as well as the two leading infectious causes of gastrointestinal morbidity in the elderly in the US. Detection of viral and bacterial co-infections is on the rise, but little is known about the relationship between these two pathogens. Published research has shown that human norovirus and Clostridium difficile co-infections occur, especially in long-term care facilities and children's hospitals. Our study aims to determine the prevalence of human norovirus in fecal samples that tested positive for the presence of Clostridium difficile and toxins A and B using the QuikChek Complete test in patients whose samples were tested at the University of Michigan Health System. In addition, we wished to determine whether specific symptoms, such as vomiting, are associated more frequently with co-infections. To date, 129 fecal samples of patients who reported vomiting have been analyzed for the presence of human norovirus genome by qRT-PCR. Of 91 Clostridium difficile-positive fecal samples from patients with vomiting, 28 tested positive for the presence of human norovirus, while none of the 39 samples from patients negative for Clostridium difficile who had vomiting symptoms tested positive for human norovirus. These data indicate a significant degree of co-infection with norovirus, in patients with documented Clostridium difficile infection who present with vomiting as part of their clinical picture. Ongoing work is investigating if this level of co-infection with norovirus is encountered in patients with in Clostridium difficile who present without vomiting. We are also determining whether a particular human norovirus genotype is associated more frequently with co-infections. Successful completion of the study will aid in improving knowledge about the impact of co-infections on symptoms. Collectively this information might help to improve infection control and clinical care through quarantine measures and treatments.

Poster Session

VIR-PTH2006 - Serologic evidence of hepatitis E virus infection in pigs from abattoirs and farms in Nigeria

Daniel Oluwayelu[1], Adebowale Adebiyi[1], Olusoji Abiola[1]
[1]University of Ibadan, Ibadan, Nigeria

Hepatitis E (HE) is considered endemic and a major public health concern in many developing countries where sanitation conditions are poor. However, despite the presence of poor sanitation conditions in many Nigerian communities, scanty information exists on the prevalence of this disease in the country. Since pigs and wild boars have been identified as its reservoirs, this study aims at investigating the occurrence of HE virus (HEV) infection in domestic pigs in Ibadan, Nigeria. Sera obtained from 176 apparently healthy pigs were used for this study. They comprised 103 and 73 pigs respectively from Bodija municipal abattoir and six pig farms located in different parts of Ibadan, Oyo state, Nigeria. The pigs were aged 0-6, 7-12 and > 12 months, and belonged to five different breeds. Sera were screened for anti-HEV antibodies with a commercial indirect ELISA kit (IDVet, Montpellier, France) that uses a recombinant antigen derived from the capsid of genotype 3 HEV. The test was performed according to manufacturer's instructions. Overall, 57.4% (101/176) of the pig sera were demonstrated to be reactive against genotype 3 HEV with 53.4% and 63.0% of abattoir and farm pigs respectively being seropositive. No significant differences in HEV seropositivity were observed between sexes, age groups and breeds. This study, which is the first evidence of swine HEV presence in Nigeria, demonstrates that domestic pigs in Ibadan had been exposed to HEV. Since some of the pigs slaughtered at the Bodija abattoir were obtained from some neighbouring states, it is likely that HEV infection is widespread in Nigeria. These findings suggest the need for more extensive studies to determine the actual prevalence of HEV infections in Nigerian pigs.

Poster Session

VIR-PTH2008 - Isolation of severe fever with Thrombocytopenia Syndrome Viruses (SFTSV) from patients in Korea, 2013

Sun-Whan Park[1], Seok-Min Yun[1], Myung-Guk Han[1], Chan Park[1], JungSang Ryou[1]
[1]Division of Arboviruses, National institute of Health, Korea Centers for Disease Control & Prevention, Korea

Background: Severe fever with thrombocytopenia syndrome (SFTS) is a new emerging infectious disease in China. It is caused by SFTS virus (SFTSV), in the genus of Phlebovirus (family Bunyaviridae). The major clinical symptoms and laboratory parameters of SFTS are fever, thrombocytopenia, leukopenia, and elevated serum hepatic enzymes, and SFTS patients usually die due to multiple organ failure. SFTSV was presumably transmitted by ticks, because it has been detected in Haemaphysalis longicornis ticks. Methods: Total RNA extracted from serum was amplified with one-step reverse-transcriptase polymerase chain reaction (RT-PCR), designed to detect a portion of the viral N and Gc protein gene using specific primers for S or M segment. After analyzing aligned nucleotide sequences, we constructed the phylogenic tree based on partial S or M segment sequences. We tried to isolate viruses from patient by infection VeroE6 cells with the sera. Results: We conducted RT-PCR with total RNA which is extracted from the patient sera. Among the 289 samples, thirty four samples are resulted in positive. The nucleotide sequences were assembled by the SeqMan program implemented in DNASTAR software (version 5.06; Madison, WI, USA) to determine the consensus sequences. Nucleotide sequence of the Korean strains showed 93 to 98 % homology to Chinese and Japanese strains. We also isolated 26 SFTSVs among the virus-detected 34 samples. Conclusion: We examined the clinical specimen from the suspected case of SFTS in Korea. We detected 34 SFTSVs of 289 patient sera by RT-PCR, and isolated 26 viruses among them. Nucleotide sequences of positive samples were not only included in SFTSV by the phylogenic analysis but also formed the Korean strain group.

Poster Session

VIR-PTH2012 - WITHDRAWN - High incidence of multiple viral infections identified in upper respiratory tract infected children under three years of age in Shanghai, China

Lu Zhang[1], Xiaoming Zhou[1], Hongping Wang[1]
[1]Shanghai Public Health Clinical Center, Shanghai, China

Background: Upper respiratory tract infection (URTI) is a major reason for hospitalization in childhood. More than 80% of URTIs are viral. Etiological diagnosis of URTIs is important to make correct clinical decisions on treatment methods. However, data for viral spectrum of URTIs are very limited in Shanghai children. Methods: Nasopharyngeal swabs were collected from a group of 164 children aged below 3 years who were hospitalized due to acute respiratory infection from May 2009 to July 2010 in Shanghai. A VRDAL multiplex PCR for 10 common respiratory viruses was performed on collected specimens compared with the Seeplex® RV15 ACE Detection kit for 15 respiratory viruses. Results: Viruses were detected in 84 (51.2%) patients by VRDAL multiplex PCR, and 8 (4.9%) of cases were mixed infections. Using the Seeplex® RV15 ACE Detection kit, viruses were detected in 129 (78.7%) patients, 49 (29.9%) were co-infected cases. Identified viruses included 37 of human rhinovirus (22.6% of cases), 32 of influenza A virus (19.5%), 30 of parainfluenzavirus-2 (18.3%), 23 of parainfluenzavirus-3 (14.0%), 15 of human enterovirus (9.1%), 14 each of parainfluenzavirus-1, respiratory syncytial virus B and adenovirus (8.5%), 8 of coronavirus 229E/NL63 (4.9%), 6 of human bocavirus (3.7%), 5 each of influenza B virus and respiratory syncytial virus A (3.0%), 3 of parainfluenzavirus-4 (1.8%), 2 of coronavirus OC43/HKU1 (1.2%), and 1 human metapneumovirus (0.6%). Conclusions: A high frequency of respiratory infections (78.7%) and co-infections (29.9%) was detected in children with acute respiratory infection symptoms in Shanghai. The Seeplex® RV15 ACE detection method was found to be a more reliable high throughput tool than VRDAL method to simultaneously detect multiple respiratory viruses.

Poster Session

VIR-PTH2014 - Serological survey for hantavirus in West Central, Goias, Brazil

Marcos Moreli[1], Vivaldo Costa[1]
[1]*Federal University of Goias, Jatai-GO, Brazil*

Emerging diseases are of great interest for public human and animal health systems, especially those associated with high mortality, such as hantaviruses detected in Americas since 1993. Hantavirus, family *Bunyaviridae*, is transmitted to humans through aerosols of excreta from infected wild rodents. Currently, Brazil present the 1573 with 40% of lethality. The state of Goiás has recorded cases of illness and is located between endemic states for hantavirus. Jataí county is the third in the state in number of cases of illness. However, no epidemiological studies related to disease in this region. Accordingly, with aim to know the levels of IgG antibodies agains hantavirus and to become individuals more aware about illness, this research was conducted in Jataí (Ethics Committee of Federal University of Goias n° 348/2010). The participants resided in peri-urban and rural areas and the samples were collected on filter paper, through use of disposable microknife in the fingertip. Additionally, it was applied a questionnaire. Subsequently, samples in the filter papers, diluted in PBS buffer, were processed by ELISA test, using N protein of Araraquara virus. 429 serum samples were collected and processed, of which 52% were males and 48% females. The positivity was 5% (12/238) for males and 2.6% (5/191) for female. The Age of participants ranged from 10 to 78 years and was observed seroprevalence IgG anti-hantavirus of 3.9%. There was no association between seroprevalence of hantavirus and gender of participants by Fisher test (p=0.123). There was also no statistical difference by Fisher test seropositive compared to the urban and rural areas (p=0.279) (p<5%). Based in our results the seroprevalence of IgG anti-hantavirus in population of Jatai was 3.9%. In conclusion, due to the majority of city population is unaware of disease is need public health policies aimed at awareness severity of hantaviruses.

Poster Session

VIR-PTH2016 - Hepatitis B virus DNA in patients with HBsAg in South Western Nigeria

Georgina N. Odaibo[1], Samuel O. Ola[2], David O. Olaleye[1]
[1]Department of Virology, University of Ibadan, Ibadan, Nigeria, [2]Department of Medicine, University of Ibadan, Ibadan, Nigeria

There are about 400 million people with chronic hepatitis B virus (HBV) infection worldwide with a potential of adverse sequelae including hepatocellular carcinoma. Recent data have shown that the level of HBV DNA in serum or plasma of an infected person probably reflects more accurately the replicative activity of the virus and therefore may serve as a better maker for management of the infection. This study was designed to determine the rate of detection of HBV DNA in blood samples of patients with HBsAg positive in Nigeria in comparison with the HBe and anti-HBe used widely as serological markers of infectivity. Plasma samples from 105 patients with HBsAg positive were tested for the presence of HBeAg and anti-HBe using a commercial enzyme-linked immunosorbent assay while plasma HBV DNA was quantified using the COBAS Amplicor HBV Monitor assay. Of the 105 HBsAg samples, 17 (16.2%) and 85 (81%) were positive for HBeAg and anti-HBe, respectively, while 8 (7.6%) were negative for both HBeAg and anti-HBe. HBV DNA was detected in 86 (81.9%) of the samples, out of which 15 (18.1%) and 67 (80.7%) were positive for HBeAg and anti-HBe, respectively. HBV DNA was detected in 78.4% of the HBeAg negative samples and in all the eight samples that were negative for both HBeAg and anti-HBe. The implication of these findings in the management of patients with HBV infection is compelling.

Poster Session

VIR-PTH2018 - Dissection of host-virus interactions in Chikungunya using expression proteomics analysis

Rachy Abraham[1], Abdul Jaleel[1], Easwaran Sreekumar[1]
[1]*Rajiv Gandhi Center for Biotechnology,Thiruvananthapuram,India.*

Chikungunya is an acute, febrile disease in the tropics leading to chronic debilitating arthralgia and occasional hepatic and neural complications. The disease, transmitted by the bite of Aedes mosquitoes, is caused by a positive-stranded RNA virus in the alpha virus genus of the Togaviridae family. In order to understand the molecular pathogenesis by elucidating the cellular pathways altered during infection, we performed high throughput protein profiling and expression analysis in virus infected cells using multi-dimensional liquid chromatography- tandem mass spectrometry. We used a label-free method for the relative protein quantification to compare the protein expression levels in infected and uninfected Human Embryo Kidney cells (HEK) 48h post-infection. We identified more than 1000 proteins that are differentially expressed with a fold-change higher than 30% (ratio of either <0.70 or >1.3]. 254 proteins which were consistently modulated in three biological replicates were short-listed and analysed using the information from Swiss-Prot/TrEMBL database. Half of the up-regulated proteins were cytoplasmic, mostly involved in transcription and translation machinery and stress response while most of the down-regulated proteins were mitochondrial, involved mainly in bioenergetics process. A detailed bioinformatics analysis using STRING and KEGG to identify the virus regulated host response networks and pathways revealed that chikungunya virus infection in these cells is coordinated primarily through the up-regulation of the ubiquitin–proteosome pathway, aminoacyl-tRNA biosynthesis, pentose phosphate pathway, ribosome machinery and also the regulation of actin cytoskeleton. The network analysis on the down-regulated proteins showed a drastic effect of infection on cells through the regulation of citric acid cycle and oxidative phosphorylation which are the converging and final cellular bioenergetic pathway. Further, comparison of our results with data from other similar studies has helped us in identifying a set of common genes involved, the study of which will be valuable in elucidating the molecular mechanisms of chikungunya pathogenesis.

Poster Session

VIR-PTH2020 - Subpopulations of lymphocyte and NK cells in adults with viral community- acquired pneumonia (CAP)

Vivian Luchsinger[1], Pablo Correa[1], Yara Prades[1], Mauricio Ruiz[2], Rolando Pizarro[3], Marcela Hermoso[4], Maria Luisa Garmendia[5], Carmen Larrañaga[1], Luis Avendaño[1]

[1]Programa de Virologia, Facultad de Medicina, Universidad de Chile, Santiago, Chile, [2]Hospital Clínico U.Chile, Santiago, Chile, [3]Hospital L.Córdova, Santiago, Chile, [4]Programa de Inmunología, Facultad de Medicina, U. de Chile, Santiago, Chile, [5]INTA, Universidad de Chile, Santiago, Chile

Introduction: Adult CAP is frequently caused by viruses, and often associated with death. CAP severity seems to be dependent on the host immune response rather than on etiological agent. Changes in T lymphocytes (LT) and natural killer (NK) cells could be associated with inflammatory response in severe CAP. Objective: To compare blood LT and NK subpopulations from adults with severe or mild community-acquired pneumonia by viruses. Methodology: Lymphocyte subpopulations were determined by flow cytometry (Beckon Dickinson®) in peripheral blood from patients ≥18 years old hospitalized with CAP and from 22 asymptomatic adults in Santiago, Chile, 2012-2014. Cases were classified according to pneumonia severity index (PSI). Viruses were detected in respiratory samples by multiplex real time reverse transcription polymerase chain reaction (Sacace®) and/or Luminex®. Statistical tests were performed with SigmaPlot® and $p<0.05$ was considered significant. Results: Viruses detected in 51 CAP patients were: rhinovirus in 24 cases, respiratory syncytial virus in 9, influenza in 16, adenovirus in 5, paraflu in 2, metapneumovirus and coronavirus with one each one. Agent was not detected in 10 cases. Cases were classified as mild (41%) and severe (59%). CAP adults had significantly less proportions of LT, activated (CD56+/NKG2D+) and inhibited (CD94+/NKG2A+) NK cells than asymptomatic adults (median: 14%; 1.9%; 0.8% vs 26%; 4,1%; 2.5% cells/ul, respectively). These parameters and regulator LT (Fox P3) were similar between adult viral CAP and cases without detected agent (median: 11%; 1.9%; 0.8%; 0.1% vs 14%; 1.4%; 1.0%; 0.1% cells/ul, respectively). Lymphocytes, CD56+/NKG2D+ and CD94+/NKG2A+ NK cells were significantly less in patients with severe than mild CAP (10.5%; 1.6%; 0.6% vs 19.7%; 2.5%; 1.3% cells/ul, respectively). Conclusions: In adults with viral CAP compared with asymptomatics, the cellular immune response is diminished independently of etiology, particularly in severe cases. Chilean project FONDECYT 1121025

Poster Session

VIR-PTH2022 - Ubiquitin-specific protease USP30 is involved in the antiviral activity of interferon α

Hom-Ming Yeh[1], Ho-Chun Yang[2], Yi-Ling Lin[1,2]

[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [2]Graduate Institute of Microbiology, National Taiwan University, Taipei, Taiwan

Viral infection induces a series of signaling events leading to production of interferons (IFNs), which then trigger antiviral responses through JAK-STAT signaling pathway to inhibit viral replication. IFN system is tightly controlled by cellular machinery of post-translation modification such as ubiquitination system. By using RNA interference screening of human deubiquitinating enzyme genes, we identified a mitochondrial outer membrane-located deubiquitinating enzyme USP30, whose knockdown decreased IFN signaling and enhanced dengue virus replication. The tyrosine phosphorylation of STAT3 triggered by IFNα was lower in cells with USP30 knockdown, while the total STAT3 protein level was not much changed. Furthermore, IFNα treatment induced STAT3 ubiquitination that can be reduced by USP30 and a protein-protein interaction of STAT3 with wild-type but not transmembrane domain-deleted USP30 was noted in cells with or without IFNα treatment. To better understand the detailed involvement of STAT3 in IFN-mediated antiviral response, we will further investigate the role of ubiquitinated STAT3 and USP30 in IFN signaling and in dengue virus infection. Overall, we identify USP30 as a positive regulator of the type I IFN antiviral signaling pathway.

Poster Session

VIR-PTH2024 - HIV-1 neutralizing factors in blood and vaginal samples of Beninese commercial sex workers

Laurie-Anne Batraville[1,2], Michel Alary[3], Annie-Claude Labbé[1,5], Pierre Fournier[2,4], Johanne Poudrier[1,2], Andrés Finzi[1,2], Michel Roger[1,2]

[1]Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montréal, Canada, [2]Centre de Recherche du CHUM, Montréal, Canda, [3]Centre de recherche du CHU de Québec, Département de médecine sociale et préventive, Université Laval, Québec, Canada., [4]École de santé publique de l'Université de Montréal, Montréal, Canada, [5]Hôpital Maisonneuve-Rosemont, Montréal, Canada

We are pursuing a study on a cohort of commercial sex workers (CSW) from Cotonou (Benin) that are highly exposed to HIV-1, but remain uninfected despite more than 7 years of active sex work. This absence of infection in spite of the high-risk exposure can be described as a “resistance” towards the virus. We believe that resistance may be associated with the ability of the host to produce neutralizing factors and/or the absence of factors that could facilitate the infection in individuals that are not resistant to the virus. GOAL: Our aim is to screen in an in vitro system, the serum and cervicovaginal fluids (CVL) of these women in order to evaluate their neutralization potential towards HIV-1. METHOD: Our study focuses on 3 groups of women: 1) untreated-HIV+ CSW with <3 years of sex work (n=23), 2) highly-Exposed-Persistently-Seronegative (HEPS) CSW with ≥7 years of sex work (n=20) and 3) HIV-uninfected women from the general population with no history of sex work (n=23). HIV-1 particles expressing envelope glycoproteins from different clades (A, B, C, G) were produced to evaluate the breath and neutralization of these factors. Detection of antibodies specifically targeting HIV-1 envelope glycoproteins were detected with a cell based ELISA assay expressing trimeric envelope. RESULTS: The sera and CVL of some untreated-HIV+ CSW presented a neutralizing activity against multiple HIV-1 clades. This activity seemed to be linked to the presence of IgGs specifically targeting the HIV-1 envelope, especially when the viral envelope was bound to its CD4 receptor. So far, 20% of HEPS seem to have a neutralization ability in their sera against a local (clade A) HIV-1 strain. CONCLUSION: While the anti-HIV-1 immune response observed in HEPS is against clade A (predominant in Benin) and not clade B virus, the response of untreated-HIV+ CSW seems to be cross-clades.

Poster Session

VIR-PTH2026 - Investigation of Bovine Herpesvirus Type 1 immune response in cattle herd

Megan Lee[1], Aimee Reed[1], Satoko Izume[1], Ling Jin[1]
[1]*Oregon State University, Corvallis, USA*

Bovine herpesvirus type 1 (BHV-1) is an important pathogen of dairy cattle as it is the causative agent behind reproductive disorders and abortions as well as respiratory disease. Although current protocol is to vaccinate the herd yearly against BHV-1, the duration of protective antibody coverage has not been investigated. This study investigated antibody titers against BHV-1 viral isolates with sera samples collected at random from a dairy herd in Corvallis, OR. Nineteen bovine serum samples were each challenged with four different BHV-1 viral strains by serum neutralization assay. Data collected was compared by age of the individual cow as well as the time since most recent vaccination. A significant difference was observed in serum titers against BHV-1 between cows of 3 years and 5 years of age. A significant difference in titers was also noted between cows that were vaccinated less than 3 months prior and cows that were vaccinated greater than 6 months prior to sera attainment and testing. A general trend of declining antibody titer was also found as the age of the cows increased that was independent of the number of times vaccinated or time since last vaccination. This investigation will be useful in assessing abortion events in vaccinated cows and will also contribute to the assessment of current vaccine protocols and how well they are providing protective coverage against BHV-1 infections.

Poster Session

VIR-PTH2028 - Genomic analysis reveals novel sites of virus-induced RNA transcription

Roli Mandhana[1], Jonathan Freaney[1], Curt Horvath[1]
[1]Northwestern University, Evanston, USA

Virus infection initiates host cell signaling cascades that lead to the activation of gene expression profiles that produce cell-autonomous antiviral responses and facilitate innate and adaptive immune responses. By combining ChIP-sequencing analysis of transcription factors interferon regulatory factor 3 (IRF3) and nuclear factor κB (NFκB), RNA polymerase II (Pol II) and associated co-regulators, we have comprehensively analyzed the human genomic landscape of the innate antiviral response network activated by Sendai virus infection. Results reveal extensive cooperation of IRF3 and NFκB with PolI II in virus-induced transcription. Additionally, IRF3 and NFκB were found to not only stimulate transcription at known messenger-RNA loci but also at many annotated intergenic and previously unannotated loci. Direct evaluation of these virus-inducible loci verifies that the ChIP data represent genuine RNA species transcribed in response to infection. The novel virus-induced RNAs, which we termed “nviRNAs”, are activated in response to diverse RNA virus infections including Sendai virus, influenza A virus, vesicular stomatitis virus and encephalomyocarditis virus as well as by transfection of poly (I:C). This widespread gene regulation defines a large amount of previously unrecognized virus-induced gene regulation that likely represents unappreciated aspects of the cellular antiviral response. The nviRNAs and their roles in regulating innate antiviral responses are being investigated with molecular biochemical and genomic techniques.

Poster Session

VIR-PTH2030 - Molecular and serological characterization of influenza A isolated from wild birds in the state of São Paulo, Brazil

Adelia Hiroko Nagamori Kawamoto[1], Luciano Matsumiya Thomazelli[2], Danielle Bruna Leal Oliveira[3], Edison Luiz Durigon[3]

[1]Laboratory Virology, Butantan Institute, São Paulo, Brazil, [2]Laboratory Especialist, São Paulo University, São Paulo, Brazil, [3]Molecular Clinic Laboratory of Virology, São Paulo University, Brazil

Avian Influenza virus belongs to Orthomyxoviridae family. The last years several low pathogenic avian influenza subtypes have caused outbreaks and epidemic in human and poultry. The wild and migrating birds may be participating of maintenance and interspecies transmission of the 16 subtypes of the Hemagglutinin and 9 Neuraminidase in nature. Our study aimed subtyping samples positive by serological test haemagglutination inhibition (HI) technique and Molecular Biology. The samples from species Elaenia mesoleuca (2), Sporophila lineola (1) Sporophila caerulescens (1), Vireo olivaceus (3), Columbina talpacoti (3), Paroaria dominicana (2), were collected in reserves and experimental field stations located in the São Paulo State - Brazil, during the years 1997 and 1998. The samples were identified by HI test (according WHO) using the 20 antibody patterns anti-influenza A type and one for the influenza type B and RT-PCR and Sequence analysis of Hemaglutinina and Neuraminidase gene. The HI test demonstrated that 12 samples presented an antigenic close relationship with A/HongKong/1/68 (H3N2), A/ Equine/Miami /63 (H3N8) and A/Duck/ Ukraine/ 63 (H3N8) antiserum.The sequencing analyses of Hemaglutinin and Neuraminidase gene of these 12 isolates revealed a high homology with H3N2. Phylogenetic analysis and genetic variabilities compared with GenBank sequences representing several countries have shown that our samples showed a close homology with the virus subtypes circulated in Siena (1991) Victoria (1990) and Beijim (1989). Amino acid analysis indicated that there are non-synonymous mutations in the gene of hemagglutinin (Y153F, K172A, K175A, T2641) and neuramidase (T55S), exclusive of our samples compared to samples AIV other countries. Our samples when analyzed protein NA, showed no mutations in amino acids and E119V N274Y conferring resistance to inhibitors Neuminidase subtype N2. Keywords: Influenza Virus A, Migratory and Wild birds, Influenza vírus A H3N2 subtypes.

Poster Session

VIR-PTH2032 - Antiviral activities of Tetherin/BST-2 against influenza virus in vitro and in vivo

Saori Sakabe[1], Jiro Yasuda[1]
[1]*Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan*

Tetherin, also known as BST-2, is an interferon-inducible membrane protein that contributes to the restriction of virus release from the cells. Recently, tetherin has been shown to have broad antiviral activities against various enveloped viruses such as retroviruses, filoviruses, and arenaviruses, whereas the potency of Tetherin against influenza virus is still unclear. Here, we generated the MDCK cells and transgenic mice stably expressing human Tetherin (MDCK-Tetherin cells and human Tetherin-Tg mice, respectively), and evaluated the antiviral activities of human Tetherin against influenza virus infection in vitro and in vivo. Influenza virus did not grow efficiently in MDCK-Tetherin cells compared to the control MDCK cells. In addition, human Tetherin expression also inhibited the influenza virus-like particle release. We found that human Tetherin has antiviral activities against influenza virus in a dose-dependent manner in vitro. However, there were no significant differences in survivals, body weight changes, and virus growth in mouse lungs between human Tetherin-Tg and wild type mice infected with a lethal dose of influenza virus. These results suggest that a certain level of human Tetherin is needed for its antiviral activities against influenza virus.

Poster Session

VIR-PTH2034 - Immune responses that protect pigs from influenza infection after intranasal vaccination of bivalent LAIV in pigs

Hyun Mi Pyo[1], Yan Zhou[1]
[1]*VIDO-Intervac, University of Saskatchewan, Saskatoon, Canada*

Due to the significance of pig producing reassortant influenza virus, an effective SIV vaccine is much in need. Current swine influenza virus (SIV) vaccine used in swine herd is killed vaccine with limited protection from variant SIVs. To deliver the better efficacy with broad protection, we generated an eight segment SIV harbouring two different SIV hemagglutinins (H1 and H3) as a potential live attenuated influenza vaccine (LAIV). Propagation of this H1H3 chimeric SIV depended on exogenously provided neuraminidase and the virus was attenuated in pigs. Intratracheal vaccination of the LAIV effectively protected pigs from H1N1 and H3N2 infection. Then, we carried out intranasal vaccination, which was considered more suitable for live vaccine. Pigs vaccinated twice with the LAIV developed high IgG titers against both H1N1 and H3N2 SIVs. All vaccinated pigs showed reliable virus neutralizing antibody titers against H1N1 SIV but not H3N2 SIV. When pigs were challenged with H1N1 and H3N2 SIVs, vaccinated pigs showed reduction of lung lesion and viral titer in lung tissue. It was revealed after the viral challenge that vaccinated pigs had more IFN-γ secreting cells in local lymph nodes. Also, robust induction of serum IgG and IgA in lung lavage of H3N2 viral challenged vaccine group. In conclusion, intranasal vaccination of this novel LAIV protected pigs from H1N1 and H3N2 SIVs although our pre and post challenge analyses implied the protection may attribute to different immune mechanisms.

Poster Session

VIR-PTH2036 - Regulation of influenza virus RNP complex formation by phosphorylation

Lauren Turrell[1], Frank. T Vreede[1], Ervin Fodor[1]
[1]*Oxford University, Oxford, UK*

The RNA genome of influenza A virus consists of eight single stranded negative sense vRNA segments. The vRNA segments are bound by viral RNA polymerase at the 5’ and 3’ ends and the remaining viral RNA is associated with nucleoprotein (NP) to form a viral ribonucleoprotein (vRNP) complex. These vRNP complexes carry out both viral transcription and replication. NP, in the RNP complex, forms oligomers through an interaction between the tail-loop and groove of neighbouring monomers and binds to the viral RNA in a non-sequence specific manner. However, NP is likely to exist in a monomeric state in the infected cell prior to being recruited into the vRNP. Phosphorylation sites within the groove of NP have been identified in both influenza A and influenza B viruses (S165 and S223, respectively) and we hypothesised that phosphorylation at these sites may regulate oligomerisation and RNA binding. Therefore, we mutated S165 to alanine or a phosphomimetic amino acid, i.e. glutamic or aspartic acid, and analysed these mutants for their ability to oligomerise, bind RNA and support transcription and replication in ribonucleoprotein reconstitution assays. In addition, the effect of these mutations on viral growth was evaluated using recombinant viruses. The data show that the presence of phosphomimetic amino acids within the groove of NP inhibits oligomerisation while a mutation to alanine has no effect. However, the growth of both alanine and phosphomimetic mutant viruses was severely inhibited suggesting a role for both the phosphorylated and unphosphorylated forms during the viral replication cycle. Taken together, these data suggest that NP oligomerisation and RNA binding in influenza A virus are regulated by phosphorylation and a similar mechanism might exist in influenza B virus.

Poster Session

VIR-PTH2038 - Molecular characterization of Influenza A(H1N1) pdm09 virus neuraminidase (NA) gene

Anitha Jagadesh[1], Govindakarnavar Arunkumar[1], Abdul Ajees[2]
[1]Manipal Centre for Virus Research, Regional Reference Laboratory for Influenza Virus, Manipal University, Manipal, India,
[2]Centre for Atomic and Molecular Physics, Manipal University, Manipal, India

Background: Influenza is one of the major threats to human and animal health due to its high transmissibility and, severe morbidity and mortality among susceptible hosts. The antigenic variability of HA protein has been studied extensively, but reports related to antigenic variability of NA protein are sparse. Such studies are important for vaccine as well as drug designing as; NA protein is an important target for drugs used to treat influenza. Materials and Methods: Influenza A (H1N1) positive samples archived at Manipal Centre for Virus Research from 2009 to 2013 were included in the study. In order to check the rate of nucleotide/aminoacid changes, 6 samples were selected per year (i.e. one sample for every 2 months). Madin Darby Canine Kidney (MDCK) cell line was used to isolate and propagate the virus. Nucleic acid was extracted from the culture supernatants, and polymerase chain reaction (PCR) was carried out for full length amplification of Influenza A virus neuraminidase (N1) gene. The amplicons were cloned and sequenced using M13 forward and reverse sequencing primers. The sequence of NA was then compared with the aminoacid sequence of 2012-13 vaccine strain Influenza A virus (A/California/7/2009 (H1N1)) obtained from NCBI Results: A total of 30 isolates were identified for NA cloning. Cloning protocol was standardized and sequencing was completed for one isolate. Analysis for the sequence identity between the Indian isolate and the vaccine strain showed 99.2% similarity. The catalytic site was conserved, but a single mutation was observed at I106V in antigenic site. Conclusion: The preliminary results indicate that NA is quite conserved among the clinical isolate of Influenza A(H1N1)pdm09 from India and the current influenza A vaccine strain. Outcome of this prospective study will significantly enrich the knowledge on molecular evolution of NA gene of Influenza A(H1N1)pdm09 virus in India.

Poster Session

VIR-PTH2040 - The relationship between H6N1 and H5N2 viruses in Taiwan and public health implications

Chwan-Chuen King[1], Chang-Chun David Lee[1], Huachen Zhu[2,3], Yun-Cheng Chang[1], Pei-Yu Huang[2], Chung-Lam Cheung[2], Min-Hsien Toby Chiang[1], Tommy Tsan-Yuk Lam[2], Yao-Tsun Li[1], Yi Guan[2]

[1]*Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan,* [2]*State Key Laboratory of Emerging Infectious Diseases & Centre of Influenza Research, School of Public Health, The University of Hong Kong, Hong Kong SAR,* [3]*Joint Influenza Research Centre (SUMC-HKU), Shantou University Medical College, Shantou, China*

Taiwan had the world's first human H6N1 pneumonia case, after the viral endemicity in chickens for 40 years. This study had three aims: (1) to investigate the genesis of Taiwan chicken H5N2 viruses circulated since 2003, (2) to elucidate the evolutionary relationship between H5N2 and H6N1 viruses isolated from chickens and ducks in different years/areas in Taiwan, and (3) to assess possible human risk. The full-length sequences of H5N2 and H6N1 viruses isolated from chickens/ducks through surveillance during Oct., 2005 –Feb. 2014 were compared. Phylogenetic analyses of each gene segment with Maximum-likelihood method and evolutionary rates of HA/NA with Bayesian MCMC were conducted. Furthermore, serological surveillance on chicken sera was initially screened for anti-influenza-NP by ELISA and followed by HI tests for H5N2, H6N1, H3N8 and H9N2 viruses. Phylogenetic analyses showed that all Taiwan chicken H5N2 viruses had two sources – (1) the HA and NA genes were closely related with the 1994 Mexican H5N2-like vaccine strain but different from the Taiwan local duck H5N2 viruses, and (2) the internal genes were derived from Taiwan enzootic chicken H6N1 viruses, rather than the 1994-Mexican H5N2 viruses. In addition, both the surface and the five internal genes (except PB1) involved two groups. Group A and B viruses were clustered with the 2012-13 and 2004-05 Taiwan H6N1 viruses, respectively. Moreover, the amino acids at the connecting peptide of HA demonstrated three patterns (REKR, RKKR, RRKR). Serological results showed low-middle levels of seropositivity rates of H5N2 and H6N1 viruses but unexpectedly high (>80%) seroprevalnce of H9N2 viruses. Additionally, nucleotide sequence identities of NS1, NP, PB1 and HA genes between Taiwan human and chicken H5N2 viruses ranged 97.6-99.5%. In conclusion, Taiwan chicken H5N2 viruses had multiple introductions, at least twice in the field. Misusage of vaccine in chickens and H6N1 viruses' reassortment capability need further investigation.

Poster Session

VIR-PTH2042 - Contribution of heparan sulfate on the cell surface to murine leukemia virus infection

Yohei Seki[1], Atsushi Tanaka[2], Yasuhiko Hayashi[1], Yuta Yunomae[1], Sayaka Takase-Yoden[1]
[1]Department of Bioinformatics, Faculty of Engineering, Soka University, Tokyo, Japan, [2]Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

There is increasing evidence that soluble glycosaminoglycans such as heparin can interfere with the infectivity of various viruses. We previously reported that heparin bound to Env of murine leukemia virus (MLV) and inhibited infection of MLV into cells (Virology, 424, 56-66, 2012). Although the interaction between Env and cationic amino acid transporter 1, the receptor molecule for MLV, appears to be required for adsorption of MLV on cells, contribution of heparan sulfate (HS) on the cell surface to infection of MLV is not well understood. In the present study, to examine whether or not HS on the cell surface contributes to infection of MLV, we used K4R/SDC1 cells. The amount of HS on the cell surface of K4R/SDC1 cells, which was transduced with the human syndecan 1 genes, was lager compared to parent K4R cells. When Friend MLV (F-MLV) and the neuropathogenic F-MLV clone A8 (A8-MLV) were infected to these cells, the viral production of A8- and F-MLV in these cells were significantly higher than that in K4R cells, respectively. MLV/EGFP-A8Env, which is replication competent and has EGFP-Env fusion protein, was also infected to K4R/SDC1 cells. The number of EGFP positive cells in K4R/SDC1 cells was larger than that in K4R cells. These results indicated that HS on the cell surface promoted MLV infection. Furthermore, to analyze the effects of HS on the early steps of viral replication, vesicular stomatitis virus-based pseudotyped viruses carrying the A8-Env was infected to the cells. The number of EGFP positive cells in K4R/SDC1 cells was significantly larger than that in K4R cells, indicating that HS promoted the viral replication process from the attachment to gene expression steps. Taken together, it was suggested that HS on the cell surface contributed to infection of MLV through mechanisms involving promotion of the viral attachment and entry steps.

Poster Session

VIR-PTH2044 - Effects of splicing of friend murine leukemia virus env-mRNA on its polysome structure formation and 3' end processing

Akihito Machinaga[1], Shuhei Ishihara[1], Sayak Takase-Yoden[1]
[1]Department of Bioinformatics, Faculty of Engineering, Soka University, Tokyo, Japan

Friend murine leukemia virus (MLV) is a member of the simple retroviruses in the Retroviridae family, with a genome that contains a 5' LTR, 5' leader sequence, gag, pol, env, and 3' LTR. The gag gene encodes the structural proteins of the virion and the pol gene encodes a protease, reverse transcriptase and integrase. The env gene encodes the Env protein, which has a surface domain and a transmembrane domain. There is a 5' splice site in the 5' leader sequence and a 3' splice site in the 3' end of the pol gene. Gag and Pol proteins are translated from the unspliced full-length viral mRNA, and the Env protein is translated from singly-spliced env-mRNA. The MLV Env protein plays important roles both in viral adsorption to host cells and in induction of neuropathogenic disease in MLV-infected mouse and rat hosts. Therefore, definition of the regulatory mechanism of Env expression is important for understanding the functions of the Env protein. We have previously shown that splicing increases env-mRNA stability and translation. In this study, we investigated detailed mechanisms for up-regulation of Env expression due to splicing of env-mRNA, using env expression vectors that produced spliced and unspliced env-mRNA. The results showed that more spliced than unspliced env-mRNA formed polysome structures. In addition, there was no significant difference in the length of the poly(A) tail between spliced and unspliced env-mRNA, although incomplete processing of the 3' end of mRNA was detected in a small fraction of unspliced env-mRNA. Taken together, these studies suggested that splicing up-regulated Env protein expression through promotion of env-mRNA polysome structure formation rather than through 3' end processing of env-mRNA. These splicing effects were env-mRNA specific, since they were not observed with expression vectors in which the env gene was replaced by the luciferase gene.

Poster Session

VIR-PTH2046 - Structural dynamics and correlated motions of HIV-1 gp120 revealed by molecular dynamics simulation

Masaru Yokoyama[1], Hironori Sato[1]
[1]*National Institute of Infectious Diseases, Musashimurayama, Japan*

HIV-1 has evolved to avoid neutralization by human antibodies. V3 loop of the HIV-1 gp120 participates in determination of viral infection coreceptor tropism and host humoral immune responses. The mechanism of the epitope masking of V3 loop by V1/V2 region remains unclear. Here, we investigated the structural dynamics of full-length gp120 monomer with glycans by using the molecular dynamics (MD) simulation, and showed the correlated motions with V3 loop by an analysis of atomic fluctuations in molecular dynamics. The JR-FL gp120 is resistance to neutralization by anti-V3 antibodies. Thus, we used JR-FL strain as a target sequence to construct the model of gp120 by the homology modeling, and obtained the equilibrium structure of the gp120 by the MD simulation for 50 ns. The equilibrium structure showed that V1/V2 regions were located on the outer domain, and V3 loop was located near V1/V2 stem, V2 loop, and β20-β21 loop, which is almost consistent with the cryo-EM structure (Lyumkis D et al. Science 342:1484-1490, 2013) and the crystal structure (Julien JP et al. Science 342:1477-1483, 2013). We performed the MD simulation for 150 ns of the equilibrium structure to investigate the structural dynamics of the gp120 and the correlated motions with V3 loop. The V1/V2 region stabilized the gp120 core by interacting with the glycans on outer surface. The V3 base exhibited correlated motions with the V2 loop, β17 and loop F of gp120. These results suggest that the conformation and motion of V1/V2 region can regulate the stability of gp120 and the position and motion of V3 loop to evade the neutralizing antibodies.

Poster Session

VIR-PTH2048 - Development of an ante-mortem diagnostic test for enzootic nasal tumor virus (ENTV-1)

Scott R. Walsh[1], Kevin J. Stinson[1], Paula I. Menzies[2], Sarah K. Wootton[1]
[1]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada, [2]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Canada

Enzootic nasal adenocarcinoma (ENA) is a contagious neoplasm of the nasal mucosa of sheep and goats and is associated with enzootic nasal tumor virus (ENTV). Since ENA is a common disease in North America and there are no vaccines against ENTV-1, diagnostic tests that can identify infected animals and assist with eradication and disease surveillance efforts are greatly needed. In this study, we endeavoured to develop a novel, non-invasive diagnostic tool that could be used to not only validate clinical signs of ENA, but also to detect ENTV-1 infection prior to the onset of disease signs (i.e. preclinical diagnosis). Cytology, serology and RT-PCR-based diagnostic methods were investigated. Although the cytology-based assay was able to detect ENTV-1 infection in some animals, it had poor sensitivity and specificity and thus was not a reliable ante-mortem diagnostic method. Three different assays, including ELISA, western blot and virus neutralization were developed to detect the presence of ENTV-1 specific antibodies in sheep serum. While a surprisingly large number of sheep mounted an antibody-mediated immune response against ENTV-1, and in some cases neutralizing, correlation with disease status was poor. In contrast, RT-PCR on RNA samples extracted from nasal swabs reliably detected exogenous ENTV-1 sequences, did not amplify endogenous ovine betaretroviral sequences, demonstrated high concordance with immunohistochemical staining for ENTV-1 envelope protein and had perfect sensitivity and specificity. This report describes a practical and highly specific RT-PCR technique for the detection of clinical and preclinical ENA that may prove beneficial in future control or eradication programs.

Poster Session

VIR-PTH2050 - Indigenizing HIV/AIDS campaign increases case detection

Marcus Bello[1], Ramota Ademola-Raheem[2], Festus Olajubu[1]
[1]Adekunle Ajasin University, Akungba-Akoko, Nigeria, [2]Olabisi Onabanjo University, Sagamu, Nigeria

ABSTRACT HIV/AIDS remains a global health challenge that still kills in developing countries of the world. Early detection can increase survival rate by 50%. Because of low turn-outs at government's Voluntary Counseling and Testing (VCT) centers, this study was therefore designed to serve as an alternate case detection method. The Supervisory Counselors for health in the two Local Government areas (Sagamu and Akoko South) used for the study were the anchor persons who spoke to their people together with trained HIV/AIDS counselors. In Sagamu Local government area of Ogun State, Nigeria, a total of 172 people comprising of 126 females and 46 males with the age range of 18-88years volunteered themselves for voluntary counseling and testing on a single day campaign. Blood samples of the volunteers were collected through vene-puncture and screened according to the national algorithm after proper counseling. Eight seropositive cases were discovered. One hundred and forty three (143) people in Akoko South Local government area of Ondo State, Nigeria responded. This was made up of 41 males and 102 females with age range between 21-75 years. Six seropositive cases were found in this one day campaign. All seropositive cases were referred to nearby tertiary health facility for follow up. The VCT in the two local government areas recorded 61 visits with 2 seropositive cases and 48 visits with no positive case respectively for a period of one month. It appears the trust and reliance the volunteers have in their representatives contributed to their coming out for the counselling and testing. The result achieved in one-day campaign surpasses that achieved by the established VCT centers, hence this method could be used in the developing countries of the world to increase case detection.

Poster Session

VIR-PTH2052 - Pattern of HIV-1 drug resistance among adults on ART in Nigeria

Georgina N. Odaibo[1], Prosper Okonkwo[2], Isaac F Adewole[3], David O Olaleye[1]
[1]Dept of Virology, University of Ibadan, Ibadan, Nigeria, [2]APIN, Abuja, Nigeria, [3]Dept of O&G, University of Ibadan, Ibadan, Nigeria

Development of anitiretroviral drug resistance may limit the benefit of antiretroviral therapy. There-fore it needs to closely monitor these mutations, especially as the use of ART increasing. This study was therefore de-signed to determine the ARV drug resistance pattern among ART naïve and exposed individuals attending a PEPFAR supported antiretroviral clinic in Nigeria. The study participants included patients attending the PEPFAR supported University College Hospital (UCH), Ibadan ART clinic who have been on HIV treatment for at least one year with consecutive viral load of over 2000 copies/ml as well some ART Naïve individuals with high (>50,000 copies/ml) baseline viral attending the hospital for pre-ART assessment. Blood sample was collected from each individual for CD4 enumeration, viral load level determination and DNA sequencing for genotypic typing. Antiretroviral drug resistance mutations (DRM) were determined using the Viroseq software and drug mutations generated using a combination of Viroseq and Stanford algorithm. DRM were classified as major or minor based on the June 2013 Stanford DR database. The most common major NRTI, NNRTI and PI mutation were D67N (33.3%), Y181C (16.7%) and M46L/I (55.6%) respectively. Lamivudine (3TC) and Emtricitabine (FTC); Nevirapin (NVP) and Nelfinavir (NFV) were the most common NRTI, NNRTI, and PI drugs to which the virus in the infected individuals developed resistance. Isolates from e 4 patients were resistant to triple drug class, including at least one NRTI, NNRTI and a PI. Only one (4.8%) of the isolates from drug Naïve individuals had major DRM that conferred resistance to any drug. Demonstration of high rates of antiretroviral DRM among patients on 1st and 2nd line ART and the presence of DRM in drug Naïve individuals in this study shows the importance of surveillance for resistance to ARV in line with the magnitude of scaling up of treatment program in the country.

Poster Session

VIR-PTH2054 - Involvement of dynein motor complexes and microtubules in HIV-1 uncoating

Paulina Pawlica[1], Lionel Berthoux[1]
[1]*Laboratory of Retrovirology, University of Québec at Trois-Rivières, Trois-Rivières, Canada*

The post-entry trafficking of HIV-1 towards the nucleus is thought to involve the microtubule network and the microtubule-associated dynein molecular motors. During this transport and/or after reaching the nuclear membrane, the HIV-1 capsid core undergoes uncoating. The mechanism of this process remains poorly understood, but proper uncoating is crucial for HIV-1 productive infection. Uncoating has also been linked to reverse transcription (RT), since RT inhibition results in slower core disassembly. In this study, we investigated the role of the microtubule network and dynein-dependent transport in the post-entry disassembly of HIV-1 cores. We used the pharmacological agents nocodazole and paclitaxel to inhibit microtubule dynamics, while dynein-mediated transport was perturbed either by an siRNA targeting dynein heavy chain (DHC) or by p50/dynamitin over-expression. By using immunofluorescence microscopy, we observed an accumulation of capsid foci in HIV-1 infected cells following DHC depletion or microtubule perturbation. Next, we employed the fate-of-capsid (FOC) assay that enables isolation of post-entry capsid cores from infected cells. Following DHC depletion, we observed a transient increase in the amounts of pelletable HIV-1 capsid cores, likely reflecting their stabilization in the cytoplasm. We also observed a stabilization of capsid cores isolated from cells transfected with p50/dynamitin. Additionally, DHC depletion transiently decreased the amounts of RT products at the same time point as which we observed core stabilization. Treatments with nocodazole and paclitaxel resulted in more durable stabilization of HIV-1 capsid cores in the cytoplasm. These treatments were thus expected to decrease HIV 1 infectivity, but instead we observed a mild stimulating effect on RT and overall HIV-1 transduction. This was explained by the cell cycle arrest caused by microtubule perturbation, which is also known to increase HIV-1 transduction. In conclusion, the microtubule-dependent transport of HIV-1 capsid cores towards the nucleus is likely coupled with their proper uncoating.

Poster Session

VIR-PTH2056 - Efficacy of double-stranded RNA against four white spot syndrome virus (WSSV) genes in the Pacific white shrimp Litopenaeus vannamei

Cesar Marcial Escobedo-Bonilla[1], Sergio Vega-Peña[2], Humberto Mejia-Ruiz[2]
[1]CIIDIR-IPN Unidad Sinaloa, Guasave, Mexico, [2]Centro de Investigaciones Biologicas del Noroeste, Baja, Mexico

White spot syndrome virus (WSSV) is the most damaging pathogen in shrimp aquaculture. RNA interference (RNAi) is a new, promising tool against viral infections. Previous works showed that not all RNAi sequences have equal antiviral efficacy. In this work, double-stranded RNA against two regulatory (ORF89 and wsv191) and two structural (vp26 and vp28) WSSV genes were evaluated to inhibit an experimental WSSV infection. ORF89 encodes a putative regulatory protein; wsv191 encodes a nonspecific nuclease whereas genes vp26 and vp28 encode a nucleocapsid and an envelope structural protein, respectively. Each dsRNA (4 µg per shrimp) was intramuscularly injected into a group of shrimp 48 h before challenged with a high dose of WSSV. Results showed that dsRNA against ORF89, vp28 and vp26 had the highest antiviral activity (cumulative mortality 10%, 13% and 21%, respectively). In contrast, dsRNA against wsv191 was the least effective (cumulative mortality 83%). All dead animals were WSSV-positive by one-step PCR whereas reverse-transcription PCR of surviving animals from all dsRNA treatments confirmed inhibition of virus replication. These results indicated that dsRNA against WSSV genes ORF89, vp28 and vp26 were highly effective to inhibit virus replication and suggest an essential role of these genes in WSSV infection.

Poster Session

VIR-PTH2058 - Temperature-dependent symptom recovery in Nicotiana benthamiana plants infected with tomato ringspot virus is associated with reduced translation of viral RNA2 and requires ARGONAUTE 1

Basudev Ghoshal[1], Hélène Sanfaçon[2]
[1]Department of Botany, The University of British Columbia, Vancouver, Canada, [2]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, Canada

Herbaceous plants infected with nepoviruses typically recover from systemic symptomatic infections and new leaves emerge that are asymptomatic. This phenomenon is known as symptom recovery and has been attributed to the induction of RNA silencing resulting in viral RNA clearance. However, recovery of tomato ringspot virus (ToRSV)-infected Nicotiana benthamiana plants is not accompanied by viral RNA clearance in spite of active RNA silencing triggered against the viral genome. In this study, we show that symptom recovery is associated with a reduction of the steady-state levels of RNA2-encoded coat protein (CP) and movement protein but not of RNA2. Markers of the hypersensitive response, such as PR1a, are induced in symptomatic leaves but are not detected in recovered leaves. This correlated with the accumulation of viral proteins, suggesting that one or several viral proteins elicit the hypersensitive-like response in a dose-dependent manner. In vivo labelling experiments revealed efficient synthesis of the CP early in infection, but reduced RNA2 translation later in infection. In contrast, we did not observe translation shut-off of host mRNAs in recovered leaves. Growing the plants at lower temperature (21°C rather than 27°C) alleviated the recovery and the RNA2 translation repression. Furthermore, shifting ToRSV-infected plants from 27°C to 21°C after the establishment of symptom recovery resulted in a partial reversal of the translation repression and the reinduction of a mild hypersensitive-like response, indicating that translation repression is a responsive mechanism regulating symptom maintenance. Silencing of Argonaute1-like (Ago1) genes prevented both symptom recovery and RNA2 translation repression. Taken together, our results suggest that recovery of ToRSV-infected plants is associated with an Ago1-dependent mechanism that represses the translation of viral RNA2. We conclude that several distinct mechanisms can cause the onset of symptom recovery in nepovirus-infected plants, including viral RNA clearance and viral RNA translation repression.

Poster Session

VIR-PTH2060 - Regulation of leukocytes apoptosis during dengue infection

Daniel Limonta[1], Amanda Torrentes-Carvalho[2], Cintia Ferreira Marinho[2], Elzinandes Leal de Azeredo[2], Luiz Jose de Souza[3], Ana Rita C. Motta-Castro[4], Rivaldo Venancio da Cunha[4], Claire Fernandes Kubelka[2], Rita Maria Ribeiro Nogueira[5], Luzia Maria de-Oliveira-Pinto[2]

[1]Pedro Kouri Institute of Tropical Medicine (IPK), Havana, Cuba, [2]Laboratório de Imunologia Viral, Instituto Oswaldo Cruz (IOC), FIOCRUZ, Rio de Janeiro, Brazil, [3]Centro Regional de Referência em Dengue, Campos dos Goytacazes, Rio de Janeiro, Brazil, [4]Setor Hemonúcleo, Universidade Federal do Mato Grosso do Sul, Mato Grosso do Sul, Brazil, [5]Laboratório de Flavivírus, Instituto Oswaldo Cruz (IOC), FIOCRUZ, Rio de Janeiro, Brazil

Background: Despite 390 million infections of dengue are estimated worldwide annually, dengue has no vaccine, antiviral treatment or reliable severity predictors. It has been shown that apoptotic cells from blood and tissues may be involved in the complex pathogenesis of dengue. However, very little is known about the interplay between blood proapoptotic and antiapoptotic regulators in this viral hemorrhagic fever. Methods: Plasma levels of the three proapoptotic mediators Fas ligand (FasL), tumor necrosis factor-α (TNF-α), and TNF-related apoptosis-inducing ligand (TRAIL) were measured by ELISA in Brazilian dengue patients. Patients were classified according to the World Health Organization classification of dengue revised in 2009. Additionally, inhibitors of apoptosis protein (IAPs) were determined in plasma (Survivin) by ELISA and peripheral blood mononuclear cells (PBMCs) lysates (cIAP-1, cIAP-2, XIAP) by an apoptosis array kit. Levels of apoptotic proteins in plasma were correlated with counts of PBMCs obtained by flow cytometry. Results: FasL and TRAIL levels were elevated in dengue patients without warning signs when compared to patients with severe dengue and controls. The highest level of FasL was measured in a fatal case. Dengue patients with warning signs showed decreased levels of Survivin compared to patients with severe dengue and controls. Survivin was positively correlated with leukocyte counts. In contrast, TRAIL was inversely correlated with counts of lymphocyte subsets. There was a trend of elevated IAPs levels in PBMCs of patients with severe dengue. No significant differences were found in TNF-α levels among the dengue patients studied. Conclusions: Our findings support the likely antiviral effect of TRAIL in dengue patients. It appears that TRAIL might be involved with apoptosis induction of lymphocytes, whereas IAPs might participate in protecting leukocytes from apoptosis. Further research is needed to explore the interactions between pro and antiapoptotic molecules and their implications in dengue pathogenesis.

Poster Session

VIR-PTH2062 - Japanese encephalitis virus nonstructural NS5 protein is involved in long-chain fatty acid metabolism impairment and cytokine production by interacting with mitochondrial trifunctional protein

Yu-Ting Kao[1,2], Ren-Jye Lin[2,3], Bi-Lan Chang[2], Hang-Jen Tsai[2], Yi-Ling Lin[1,2]
[1]Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan, [2]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [3]Department of Primary Care Medicine, Taipei Medical University Hospital, Taipei, Taiwan

Japanese encephalitis virus (JEV), a flavivirus causing acute human encephalitis with high mortality, can trigger pro-inflammatory cytokines induction and disturbance of metabolism such as hypoglycemia, a biochemical hallmark of fatty acid β-oxidation disorders. Since lipid biosynthesis is required for efficient replication of several viruses, we study whether JEV might modulate fatty acid metabolism during infection. Here, we find an impairment of long-chain fatty acid (LCFA) β-oxidation, leading to IL-6 and TNFα cytokines induction in JEV-infected cells. Furthermore, JEV nonstructural NS5 protein was found to interact with two LCFA β-oxidation enzymes, hydroxyacyl-CoA dehydrogenase α and β subunits (HADHα and HADHβ), which form mitochondrial trifunctional protein (MTP). Besides the well-known cytoplasmic location, some NS5 proteins could also be detected in mitochondria and colocalized with HADHα and HADHβ. Deletion/mutation study of NS5 suggests that the N-terminal methyltransferase domain of NS5 is involved in this interaction, whereas the enzyme activity is not required. Furthermore, a single point mutation of NS5 residue 19 from methionine to alanine (M19A) reduced its association with MTP, and the level of cytokine production was also lower in cells infected with this NS5-M19A-mutated JEV. Thus, we reveal a novel role of JEV NS5 in virus mediated LCFA β-oxidation impairment and cytokine production through interaction with MTP.

Poster Session

VIR-PTH2064 - Serotonin impairs kinetics of reovirus cell entry

Bernardo Mainou[1], Alison Ashbrook[2], Everett Smith[1], Daniel Dorset[3], Mark Denison[1,2], Terence Dermody[1,2]
[1]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, USA, [2]Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, USA, [3]VANTAGE, Vanderbilt University, Nashville, USA

Mammalian orthoreoviruses (reoviruses) are nonenveloped dsRNA viruses that infect most mammalian species. Although most humans are exposed during childhood, infection seldom results in disease. Due to its capacity to infect and kill transformed cells, reovirus is currently being tested in clinical trials as an oncolytic agent. Reovirus binds to cell-surface glycans and junctional adhesion molecule-A (JAM-A) and enters cells by receptor-mediated endocytosis in a process dependent on β1 integrin. Within the endocytic compartment, reovirus undergoes stepwise disassembly, which is followed by release of the transcriptionally active viral core into the cytoplasm. To identify cellular mediators of reovirus infectivity, we screened a library of small-molecule inhibitors for the capacity to block virus-induced cytotoxicity. In this screen, reovirus-induced cell killing was dampened by drugs that activate serotonin receptors. Concordantly, treatment of cells with the prototype serotonin receptor agonist, serotonin (5-HT), diminished reovirus infectivity. 5-HT did not alter reovirus binding but instead impaired reovirus disassembly following cell entry. Consistent with an uncoating block, 5-HT treatment did not dampen infection with in vitro-generated disassembly intermediates, which require attachment to JAM-A but bypass a requirement for endocytic sorting to productively infect cells. The effect of 5-HT on reovirus extended to other viruses, as treatment of cells with 5-HT decreased infectivity of alphavirus Chikungunya virus and coronavirus murine hepatitis virus. These data suggest that serotonin receptor signaling influences cellular activities that regulate viral cell entry and provide a new, potentially broad-spectrum target for antiviral drug development.

Poster Session

VIR-PTH2066 - Mapping the origin of assembly sequence (OAS) on Cucumber necrosis virus (CNV) RNA

Kankana Ghoshal[1], D'Ann Rochon[2]
[1]*Faculty of Land and Food system, The University of British Columbia, Vancouver, Canadaffiliation, City, Country,*
[2]*Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, Canada*

CNV is an icosahedral virus that encapsidates a 4.7 kb (+)ssRNA genome. During assembly CNV predominantly packages viral RNA over cellular RNA. Studies have shown that selective packaging of viruses involves specific sequences and/or secondary structures (OAS) on viral RNA. Three approaches were used to identify the OAS. First we have inserted different regions of the complete CNV genome into a heterologous viral RNA genome lacking its coat protein (CP) ORF. Chimeric RNA was then co-inoculated with CNV on Nicotiana benthamiana plants. Virion RNAs, analyzed by Northern blot analysis, showed that all heterologous chimeric virion RNAs are encapsidated. However chimeric RNA having an approximate 550 nt segment corresponding to the 5' terminal region of the CP ORF was encapsidated most efficiently suggesting that this region of the genome could have an essential element for encapsidation. In the second and third approaches for OAS identification, CNV mutants lacking the coding sequence for the CP and/or movement protein (MP) region were either inoculated on plants that are first agroinfiltrated with a binary plasmid that expresses the CNV CP (RaSP) or co-inoculated with CNV. In both approaches the deletion mutants were found to be less efficiently encapsidated than a CNV mutant deficient in CP production but which contains the complete CP coding sequence. A CNV deletion mutant that lacks most of the coding region of the MP was found to be encapsidated following inoculation on RCNMV MP transgenic plants. Taken together, these results suggest that the 5' terminal region of the CP ORF may contain an OAS. However, WT CNV and agroinfiltrated CNV CP were also found to encapsidate host RNA as well as other non-related viral RNAs indicating that other factors may contribute to encapsidation specificity.

Poster Session

VIR-PTH2068 - A screen of mutant NPC1 proteins from Niemann-Pick disease patients identifies sequences crucial for its activity as an Ebola virus entry receptor

Rohan Biswas[1], Eva-Maria Mittler[1], Kanagaraj Subramanian[2], Rohit Jangra[1], William Balch[2], Kartik Chandran[1]
[1]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, USA, [2]Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, USA

Niemann-Pick C1 (NPC1), a multispanning membrane protein localized to the limiting membrane of late endosomes, plays a crucial role in redistribution of cellular cholesterol from the endosomal compartment to the endoplasmic reticulum (ER) and other cellular sites. A wide array of point mutations distributed throughout NPC1 cause Niemann-Pick disease, a rare but devastating disorder associated with lysosomal storage of cholesterol and sphingolipids in the brain and other tissues. We recently uncovered a second, unanticipated, biological role for NPC1–it serves as an essential entry receptor for the Ebola and Marburg filoviruses. Using panels of truncation mutants and chimeras, we showed that the filovirus surface glycoprotein GP binds to NPC1 with high affinity and directly contacts NPC1's second luminal domain. However, these studies also raised the possibility that other regions of NPC1, including the third luminal domain, contribute to filovirus entry. To identify additional sequences in NPC1 that control its viral receptor activity, we screened a large panel of NPC1 point-mutant proteins isolated from NPC disease patients for their capacity to mediate Ebola virus entry in an NPC1-deficient Chinese hamster ovary cell line. Because many NPC disease mutations cause NPC1 to misfold and become retained in the ER (where it would presumably be inaccessible to entering virions), each mutant was also counter-screened for endosomal localization by fluorescence microscopy. Our analysis highlighted NPC1 disease mutations in both the second and third luminal domains, and unexpectedly, within a cytoplasmic loop, that reduce viral entry without abrogating NPC1 localization to LAMP1-positive late endosomes. These findings corroborate the hypothesis that regions of NPC1 distinct from the second luminal domain can influence filovirus receptor activity. Our ongoing studies are aimed at defining the precise molecular contributions of these newly identified sequences to the NPC1-dependent filovirus entry mechanism.

Poster Session

VIR-PTH2070 - Actin-mediated transport of alphavirus glycoproteins to the cell surface is dependent on Rac1, Arp3, and PIP5K1-α

Sheli Radoshitzky[1], Gianluca Pegoraro[1], Xiǎolì Chī[1], Lián Dǒng[1], Chih-Yuan Chiang[1], Jeremiah Clester[1], Christopher Cooper[1], Krishna Kota[1], Jens Kuhn[2], Sina Bavari[1]

[1]United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Frederick, USA, [2]Integrated Research Facility at Fort Detrick (IRF-Frederick), Frederick, USA

Transport of integral membrane proteins to the cell surface requires transit through the ER and Golgi apparatus. Typically, post-Golgi carrier-mediated transport to the plasma membrane is dependent on microtubules, rather than actin filaments. However, using positive-strand RNA alphaviruses, we reveal an essential role of actin-rearrangements for transport of viral glycoproteins (E2) to the cell surface. Nanoscale microscopy studies show that these virus-induced rearrangements involve generation of numerous actin comet-like tails with virus E2 at their termini. These rearrangements are also dependent on host Rac1 and Arp3 and occur concomitantly with E2 expression. E2 associates with actin and colocalizes with Rac1–PIP5K1-α at the tip of actin tails. Actin polymerization, Rac1, and Arp3 are also essential for active trafficking of E2 from the trans-Golgi to the cell surface. Our results extend the paradigm of actin-mediated transport to include subcellular viral membrane-protein trafficking and provide a unique therapeutic target against alphavirus diseases.

Poster Session

VIR-PTH2072 - The paucity of naive Th17 precursors contributes to Th17 deficiency in HIV-infected subjects

Sandrina Da Fonseca[1], Julia Niessl[1], Sylvia Pouvreau[1], Vanessa Sue Wacleche[1], Annie Gosselin[1], Nicole Bernard[2], Cecile Tremblay[1], Mohammad-Ali Jenabian[2], Jean-Pierre Routy[2], Petronela Ancuta[1]

[1]CHUM-Research Centre, Montreal, Canada, [2]Chronic Viral Illness Service, McGill University Health Centre, Montreal, Canada

Mechanisms contributing to Th17 depletion in HIV-infected subjects are not fully elucidated. Here, we investigated alterations in the ability of naive CD4+ T-cells to undergo Th17 polarization during HIV-1 infection. We demonstrate that naive T-cells from chronically HIV-infected aviremic subjects receiving antiretroviral therapy (CI on ART) compared to uninfected controls were impaired in their Th17 polarization potential in vitro. In uninfected subjects, IL-17-producing cells originated from naive (CD45RA+CCR7+) CD4+ T-cells with a regulatory phenotype (nTregs; CD25+CD127-FoxP3high) but also from CD25+CD127+ T-cells lacking FoxP3 (DP, double positive). Consistently, the Th17 polarization deficit in HIV-infected subjects coincided with the depletion of naive Th17 precursors with nTreg and/or DP phenotype. The paucity of Th17 precursors was associated with a decreased proportion of memory Th17 cells (CD45RA-CCR6+CD26+CD161+) in CI on ART versus uninfected controls. Compared to conventional naive CD25- T-cells, nTregs and DP cells harbored superior levels of integrated/non-integrated HIV-DNA in CI on ART subjects, suggesting that permissiveness to integrative/abortive infection likely impairs the survival of Th17 precursors and/or their polarization potential. Finally, a cross-sectional study in CI on ART subjects and a longitudinal analysis in a HIV primary infection cohort demonstrated a trend for increased nTreg, DP cell, and memory Th17 counts with early ART initiation. Together, these results support a model in which the paucity of naive Th17 precursors contributes to Th17 deficiency in HIV-infected subjects and emphasize the beneficial role of early ART interventions for the preservation of the naive Th17 precursor pool.

Workshop Sessions

BAM-WK119.01 - Mimicking environmental conditions in experimental ecology to assess microbial hydrocarbon degradation processes

Cristiana Cravo-Laureau[1], Robert Duran[1]
[1]Equipe Environnement et Microbiologie, Pau University, Pau, France

Muddy areas and coastal anoxic zones play a key role for maintaining the integrity of estuarine and coastal ecosystems. By their location, such ecosystems are particularly exposed to human activities, as oil spills or accidental events leading to the accumulation of the pollutants constituting “pollutant reservoirs” that are threats for the ecosystem and the human health as well. The recent catastrophe of Deepwater Horizon revealed the importance to better understand the microbial processes involved on hydrocarbon degradation in marine sediments raising strong interests of the scientific community. During the last decade, several studies have shown the key role played by microorganisms in determining the fate of hydrocarbons in oil-polluted sediments but only few have taken into consideration the whole sediment’s complexity. Marine coastal sediment ecosystems are characterized by remarkable heterogeneity, owning high biodiversity and are subjected to fluctuations in environmental conditions, especially to important oxygen oscillations due to tides. Thus, for understanding the fate of hydrocarbons in such environments, it is crucial to study microbial activities, taking into account sediment characteristics, physical-chemical factors (electron acceptors, temperature), nutrients, co-metabolites availability as well as sediment’s reworking due to bioturbation activities. Key information could be collected from in situ studies, which provide an overview of microbial processes, but it is difficult to integrate all parameters involved. Microcosm experiments allow to dissect in-depth some mechanisms involved in hydrocarbon degradation but exclude environmental complexity. To overcome these lacks, strategies have been developed, by creating experiments as close as possible to environmental conditions, for studying natural microbial communities subjected to oil pollution. A summary of these approaches will be presented, as well as the main conclusions of our studies in this field.

Workshop Sessions

BAM-WK119.02 - Characterization of a novel family of catechol 2,3-dioxygenases from a halophilic bacterial consortium

Hui Wang[1], Guang Guo[1], Fen He[1], Yong Huang[1], Qijia Cui[1]
[1]School of Environment, Tsinghua University, Beijing, China

Salt-affected soils represent about 40% of the world's lands, and polycyclic aromatic hydrocarbons (PAHs) have been found to be ubiquitous in ecosystems due to the intensive anthropic activities. As many PAHs have been identified as high toxic and carcinogenic organic pollutants at very low level, a great number of researches have been conducted to eliminate PAHs from the environment over the past decade. However, few effective methods have been developed for PAHs elimination in saline environment. Catechol 2,3-dioxygenases(C23O) has been identified as one of the two key PAH-degrading enzymes, controlling rate-limiting steps for PAHs decomposition. In the present study, a bacterial consortium was enriched from a saline soil contaminated by crude oil using phenanthrene as sole carbon and energy source at 10% salinity. The bacterial consortium can degrade 100 mg/L of phenanthrene within 4 days, and was dominated by haolophilic bacteria including Thalassospira, Rhodobium, Mariprofundus, Psychroflexus, Alcanivorax and Nitratireductor et al. Two enzymes named as C23O1 and C23O2 were cloned from the consortium, and biochemically characterized. The phylogenetic analysis show that the two C23Os can be classified into a novel family with their similar proteins, representing a novel halotolerant member of catechol 2,3-dioxygenase family. Both C23O1, C23O2 can be induced by phenanthrene, showing high C23O activity in range of 0-30% salinity and highest activity at 40oC and 60oC(the optimal temperature), respectively. Both C23O enzymes could effectively degrade 3-methylcatechol, 4-methylcatechol and 4-chlorocatechol with different degrading activity. The difference in DNA sequence and physiochemical properties shown by the two C23Os indicated that they may come from different bacterial source as result of gene transfer. This is the first report describing novel C23O encoding genes from halophiles capable of degrading phenanthrene. These C23O enzymes show high activity under wide range of salinity, thus have huge potential application in bioremediation of PAHs.

Workshop Sessions

BAM-WK119.04 - How redox oscillations influence microbial communities from polluted coastal marine sediments

Fanny Terrisse[1], Cristiana Cravo-Laureau[1], Alex J. Dumbrell[3], Terry J. McGenity[3], Marisol Goni-Urriza[1], Mathilde Gondard[1], Christine Cagnon[1], Karine Duboscq[2], Ronan Jezequel[2], Robert Duran[1]
[1]Equipe Environnement et Microbiologie, Université de Pau et des Pays de l'Adour, Pau, France, [2]Cedre, Brest, France, [3]University of Essex, School of Biological Sciences, Colchester, UK

Coastal sediments house abundant and diverse microbial communities that drive many ecosystems processes. Their dynamic and their roles in the biotransformation of organic compounds as petroleum constituents are widely impacted by changes in environmental properties. Anoxic/oxic oscillations due to tidal cycles and macrofauna activity constitute a major parameter governing the organization of microbial communities. However, little attention has been dedicated to this phenomenon. Therefore the knowledge on how the anoxic/oxic oscillations may affect the microbial communities and in turn their roles in determining the fate of hydrocarbons are still scarce. From a marine coastal sediment three hydrocarbonoclastic microbial communities were obtained in mesocosms[1] applying: Oil addition (O), Oil addition + Harrowing (OH) and Oil addition + Worms addition (OW). In order to characterize the effect of anoxic/oxic oscillations, the three microbial communities were incubated in bioreactors. Their responses to the oscillating conditions (15 days of incubation in anoxic condition with 2 aerated periods of 1 day at times 7 and 10 days) were followed. The oxygen addition induced a modification of the metabolically active bacterial community structure depending on the bacterial composition of the initial community. The expression rate of the 16S rRNA gene (copies numbers cDNA/DNA) revealed that the anoxic/oxic oscillations stimulated the O community, while it remained stable in OH and OW communities. Focusing on sulfate-reducing communities, the anoxic/oxic oscillations had a positive effect on expression rate of the dsrAB gene in O community, while the effect was negative in OH and OW communities. The observed response of the three microbial communities had a consequence on their biodegradation capacities (O=OH>OW), revealing different adaptation potentials. In depth characterization of these communities by high-throughput sequencing gave the opportunity to describe the microbial networks in oil polluted marine coastal ecosystems submitted to environmental fluctuations. [1]Stauffert et al, 2013. PlosOne. 8(6): e65347.

Workshop Sessions

BAM-WK119.05 - Freshwater sediment pesticide biodegradation potential as an ecological indicator of microbial recovery following a decrease in chronic pesticide exposure

Stéphane Pesce[1], Christelle Margoum[1], Nadine Rouard[2], Arnaud Foulquier[1], Fabrice Martin-Laurent[2]
[1]Irstea UR MALY, Villeurbanne Cedex, France, [2]INRA, UMR, Dijon Cedex, France

Water resources and aquatic ecosystems are an essential concern of any policy for sustainable development. In the context of the European Water Framework Directive, which aims to achieve good chemical and ecological status of waters, the herbicide diuron was banned in France in December 2008. In lotic ecosystems, benthic microbial assemblages are considered as useful potential indicators of ecological status because they integrate the effects of multiple anthropogenic disturbances and have strong capacities to adapt to novel environmental conditions. Field studies have thus revealed that in situ diuron exposure can induce microbial adaptation leading to an increase in diuron biodegradation potential of sediment microbial communities. It suggests that microbial biodegradation capacities can represent a powerful ecological indicator for monitoring spatio-temporal variations in pesticide contamination and evaluating associated ecological effects. The aim of this study was thus to evaluate the use of freshwater sediment biodegradation potential as an ecological indicator for monitoring microbial recovery following a decrease in chronic pesticide exposure. For this purpose, a four-year case study (2008–2011) was conducted in a small stream (Morcille river) long exposed to high diuron concentrations, increasing from upstream to downstream. Our results show that the ban on diuron resulted in a progressive decrease in its concentrations in the Morcille river over the survey period. The decrease in the level of chronic diuron exposure in the river caused a fall in sediment diuron-mineralizing capacities, assessed by radiorespirometry using [ring-U-14C] diuron. It thus revealed a corresponding recovery of microbial communities, showing that the use of freshwater sediment biodegradation potential may be useful for assessing microbial recovery after a decrease in chronic exposure to pollutants, opening prospects for developing a new class of ecological indicator to monitor the recovery of biological quality of water resources.

Thursday, 31 July 2014 **16:45 - 17:00 Room 524 B**

Workshop Sessions

BAM-WK119.06 - Biodegradation of Polychlorinated biphenyls (PCB) in polluted soil using different plants and bacterial inoculum

Nancy Pino Rodriguez[1], Gustavo Peñuela Mesa[2]
[1]Universidad de Antioquia, Medellín, Colombia

Polychlorinated biphenyls (PCB) are organic compounds, used extensively until the mild 1970's. PCBs representing a serious environmental problem due to their low degradability, high toxicity, and strong bioaccumulation. The use of the symbiosis between plant and rhizosphere called rhizoremediation, plays an important role in the removal of PCB from contaminated soil, for this reason currently there is a special attention on bacteria and plants that can act together for biodegradation of pollutants from the environment. The aim of this study was to evaluate the effect of bioaugmentation by bacterial inoculum able to degrade PCB, on the removal of PCB contaminated soil using Medicago sativa, Brassica nigra, Avena Sativa, and Brachiaria decumbens plants. Microcosm experiments were conducted using soil spiked with different degrees of chlorination PCB congeners: 44, 66, 118, 153, 138, 180, y 170. The assays were carried out in greenhouse pot experiments. The bacterial inoculum was previously isolated and identified from rizhosphere of plants growing in PCB polluted soil. Treatments included soil with plants and bacterial inoculum, soil with plants, soil without plants, and soil with bacterial inoculum. Plants were grown in pot with 200 gr of soil and inoculated with 50 ml of microbial culture. After four weeks of incubation, the effect of microbial inoculation on PCB removal was analyzed using GC with μECD. Plant growth, enzyme activity, presence gene bphA, were also analyzed. The bacterial abundance, was measured by colony counting and 16S rDNA quantification by real-time PCR. The bacterial inoculation significantly enhanced the growth and PCB removal of all test plant ($p < 0.05$). The copy numbers of the bphA genes, total bacteria counts, and enzyme activity were highest in planted and inoculated soil. It is concluded that the presence of inoculum significantly promoted the removal of PCBs and growth of plants used in the experiment.

Workshop Sessions

BAM-WK120.01 - Development of an innovative high throughput assay to functionally assess type III secretion in pathogenic Escherichia coli

Thangadurai Ramu[1], Jason Leblanc[1], Nikhil Thomas[1]
[1]*Dalhousie University, Halifax, Canada*

The virulence trait of type III secretion (T3S) is used by many significant pathogens to subvert host cells during infection. While T3S as a biological process is widely studied, the detailed molecular mechanisms that underlie T3S functionality are modestly understood. For any given pathogen with a type III secretion system (T3SS), at least 20 proteins are involved in forming the secretion system. To address this complexity, we have developed a genetically tractable high throughput assay that serves to functionally assess type III secretion in enteropathogenic E. coli (EPEC). The approach takes advantage of known mutagenesis techniques combined with genetic library construction in specific strain backgrounds. Defined growth conditions were determined that allowed for quantitative spectrophotometric detection of secreted proteins produced within in vitro culture supernatants. To test our approach, the newly developed assay was used to functionally dissect two genes, cesT and sepD, both of which are involved in T3SS in EPEC. Many EPEC cesT and sepD mutant alleles were identified in the functional screen. Sequencing of the mutant alleles identified critical amino acid residues that contribute to protein function. Mutant alleles that stably expressed CesT or SepD variants of interest were further validated using established infection assays and biochemical protein interaction studies. Therefore, this newly developed assay can be used to functionally dissect any gene that distinctly contributes to type III secretion. The assay is genetically tractable, quantitative, and amenable to high throughput screening. This approach further opens avenues into the discovery of type III secretion inhibitors that may exist in small compound drug libraries.

Workshop Sessions

BAM-WK120.02 - E. coli cell surface display for selection of nanobodies and the design of robust bacterial synthetic adhesins

Carlos Piñero-Lambea[1], Gustavo Bodelón[1], Valencio Salema[1], Elvira Marín[1], Luis Ángel Fernández[1]
[1]*Department of Microbial Biotechnology, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain*

Single domain antibodies (sdAbs) are small antibody fragments (ca. 12 kDa) with full antigen-binding capacity based on a single variable (V) immunoglobulin domain. The most common type of sdAbs, known as VHHs or nanobodies, are generated from natural heavy-chain-only antibodies (HCAbs) found in camelids (i.e. dromedaries, llamas). We have recently developed a novel selection method of nanobodies of high-affinity and specificity against an antigen of interest from immune libraries cloned in E. coli displaying the VHH domains on the bacterial cell surface with an outer membrane b-domain derived from intimin of enterohemorrhagic E. coli (EHEC). Here we report the use of this E. coli surface display system for the design of synthetic adhesins that allow an effective and controlled adhesion of E. coli to antigenic surfaces and specific cells in vitro and in vivo. We have generated synthetic adhesins against different antigen targets (e.g. GFP, TirM), expressed them stably and constitutively from the chromosome of E. coli, and demonstrate that bacteria carrying them show specific adhesion to abiotic surfaces coated with the cognate antigen and to mammaliam cells expressing the corresponding antigen on their surface. We have shown the potential of the synthetic adhesins for E. coli targeting tumor cells using in vitro and in vivo models. Using xenotransplants of human tumor cell lines in nude mice, we demostrated that E. coli bacteria colonize these tumors more efficiently when carrying a synthetic adhesin against an antigen expressed on the surface of the tumor cell. Given our ability to select VHH domains against most antigens, these synthetic adhesins have the potential to program a controlled adhesion of E. coli against any desired target surface or cell.

Workshop Sessions

BAM-WK120.03 - Mad bacteria: The RepA-WH1 prionoid causes a synthetic amyloid proteinopathy in Escherichia coli

Rafael Giraldo[1]
[1]*CIB-CSIC, Madrid, Spain*

Protein amyloids arise from the conformational conversion and templated assembly of a soluble protein into fibrillar aggregates with a crossed β-sheet backbone, leading to human neurodegenerative and systemic proteinopathies [1]. Besides epigenetic determinants, such as prions in yeast, functional amyloids are assembled in bacteria as extracellular scaffolds but, until recently, no proteinopathic amyloidosis had been found in microorganisms [2]. In some bacterial plasmids, RepA protein initiates DNA replication [3] and then, by physically coupling plasmid molecules through its origin-bound WH1 domain, RepA assembles as an amyloid to inhibit further replication rounds [4]. In vitro, RepA-WH1 can assemble into amyloid fibers upon binding to short, plasmid-specific DNA sequences [5,6]. RepA-WH1 causes an amyloid proteinopathy in E. coli, hampering cell proliferation [7]. RepA-WH1 amyloidosis is vertically transmissible from mother to daughter cells, but not infectious, enabling conformational templating by cross-seeding in vitro [7] and in vivo [4], thus it qualifies as a prionoid [2]. Through microfluidics, we have directly assessed the dynamics of the RepA-WH1 prionoid in E. coli cells [8]. Bacterial lineages maintain two mutually exclusive types (strains) of RepA-WH1 amyloids: either multiple globular particles that inhibit cell division, or a single elongated aggregate, mildly detrimental to growth. DnaK, the Hsp70 chaperone in E. coli, contributes to RepA-WH1 amyloidogenesis in vivo, but the Hsp104 chaperone ClpB does not have a major effect on the vertical spread of the RepA-WH1 amyloid aggregates. We have engineered repeats of the RepA-WH1 amyloid stretch to replace the Q/N-rich oligopeptide repeats in Saccharomyces cerevisiae Sup35p/[PSI+], thus building chimeric [REP-PSI+] prions. These are functional as epigenetic determinants, and become independent on Hsp104p for propagation [4]. REFERENCES: 1. ChemBioChem 11:2247-57 (2010). 2. Prion 5:60-4 (2011). 3. Nat.Struct.Biol. 10:565-71 (2003). 4. Submitted 5. Proc.Natl.Acad.Sci.USA 104:17388-93 (2007). 6. Nucl.AcidsRes. 36:2249-56 (2008). 7. Mol.Microbiol. 77:1456-69 (2010). 8. Mol.Microbiol. doi:10.1111/mmi.12518 (2014).

Workshop Sessions

BAM-WK120.04 - Discovery of two novel type III secretion effector proteins in Pseudomonas aeruginosa

Shirley Satanower[1], David Burstein[2], Michal Simovitch[1], Meital Zehavi[1], Yana Belnik[1], Gal Yerushalmi[1], Tal Pupko[2], Ehud Banin[1]

[1]The Mina and Everard Goodman Faculty of Life Sciences Bar-Ilan University, Ramat Gan, Israel, [2]Department of Human Genetics and Molecular Medicine Sackler Faculty of Medicine Tel-Aviv University, Ramat Aviv, Israel

Pseudomonas aeruginosa is a gram negative, opportunistic human pathogen that can infect an assortment of organisms. The virulence of P. aeruginosa is often associated with the activation of a type III secretion system (T3SS) that injects harmful effector proteins directly into the host cell's cytoplasm. Until today, despite strenuous efforts, only four T3SS effector proteins (ExoU, ExoS, ExoY and ExoT) have been identified in P. aeruginosa. The P. aeruginosa T3SS seems to have fewer effector proteins than any other well-characterized T3SS. This is especially intriguing in light of P. aeruginosa's ability to infect a wide range of hosts. In this study, we used a machine learning algorithm to predict novel P. aeruginosa T3SS effector proteins. Since the positive learning set from P. aeruginosa (i.e. the known T3SS associated proteins) is too small to support the learning process, we combined it with the 36 known T3SS effector proteins from Pseudomonas syringae, which is closely related to P. aeruginosa. Over 100 parameters were used for the machine learning cycle, providing a list that ranks all ORFs by their potential to be an effector protein. The top putative effector candidates were then screened for type III dependent secretion from the bacterial cell into the host cell. Using this methodology, we were able to identify two new P. aeruginosa proteins that are translocated via the T3SS. Identification of such new effector proteins can substantially increase our understanding of the virulence mechanisms utilized by this orgranism especially since the last T3SS effector protein, ExoY, was identified more than a decade ago.

Workshop Sessions

BAM-WK120.05 - Assembly of colonization factor CS6 of enterotoxigenic Escherichia coli: Role of specific residues

Anusuya Debnath[1], Takashi Hamabata[2], Nabendu Chatterjee[1]
[1]National Institute of Cholera and Enteric Diseases, Kolkata, India, [2]National Center for Global Health and Medicine, Tokyo, Japan

Enterotoxigenic Escherichia coli (ETEC) infection is the leading cause of infantile diarrhea and an important etiologic agent for traveler's diarrhea in developing countries. Along with virulence factors, the pathogenicity of ETEC involves several colonization factors which help bacteria to adhere to host cell successfully. CS6 is a prevalent colonization factor present on approximately 30% of ETEC worldwide. Thus this has become an important vaccine candidate. CS6 is a two-subunit protein consisting of CssA and CssB in the stoichiometry of 1:1. It is assembled via the classical chaperone-usher pathway into an afimbrial, oligomeric colonization factor on the bacterial surface. In order to investigate the functionally important amino acids in CssA and CssB subunits during CS6 assembly, we analyzed first twenty amino acids from N- and C-terminal of each subunit by alanine scanning with help of site-directed mutagenesis. We used pCS6 (pSTV28-cssABCD) as template, expressed in E. coli BL21 DE3 and tested for the expression of CssA and CssB in heat saline extract by western blotting. Some of the mutations did not affect the expression of functional CS6, whereas some did. We further analyzed the periplasmic fractions of these defective mutants to locate the step in the assembly pathway that was hindered. As a result, we found that residues I22, V29 and I33 in the N-terminal of CssA and residues G154, Y156, L160, V162, F164 and Y165 in the C-terminal of CssB are involved in the inter-subunit interaction during CS6 assembly. We also observed that in absence of chaperone, CssA is undetectable in the periplasm whereas CssB is always present. This suggested that the stable existence of CssA depends on the presence of chaperone. We further predict that T20, K25, F27, S36, Y143 and V147 amino acids in CssA is involved in the interaction with the chaperone for its stable expression.

Workshop Sessions

BAM-WK121.01 - The host as a growth medium: mining bacterial central metabolism for new drug target

Kevin Pethe[1]
[1]*Institut Pasteur Korea, Seongnam, South Korea*

The rapid emergence and spread of multi-drug resistant Mycobacterium tuberculosis and other pathogenic bacteria is a serious concern worldwide that advocates for the development of new classes of antibacterials with a novel mode of action. Current antibiotics derived mainly from natural sources and inhibit a narrow spectrum of cellular processes such as DNA replication, protein synthesis and cell wall biosynthesis. With the explosion of drug resistance, there is a renewed interest in the investigation of alternate essential cellular processes, including central metabolic pathways, as a drug target space for the next generation of antibiotics. However, the validation of targets in central metabolism is more complex, as essentiality of such targets can be conditional and/or contextual. In addition, interest in targeting central metabolism has been muted because of a concern about selectivity with human orthologs. Nonetheless, we and others have shown that selective inhibition can be achieved even for enzymes that are conserved between bacteria and humans. Bearing in mind our enhanced understanding of prokaryotic central metabolism, a key question arises: can central metabolism be the bacteria's Achilles heel and a therapeutic target for the development of new classes of antibiotics?

Workshop Sessions

BAM-WK121.02 - Two components, two systems: signal transduction in *Leptospira* and *Bacillus*, a way to approach pathogenesis through protein science

Alejandro Buschiazzo[1]
[1]*Unit of Protein Crystallography, Institut Pasteur de Montevideo, Montevideo, Uruguay*

Two-component systems (TCSs) are key players in bacterial signaling. Nevertheless, sensing and signal-transduction mechanisms are still poorly understood at the molecular level. This knowledge has not only fundamental importance for Biology, but also extends to a better understanding of virulence and pathogenesis in bacteria that cause disease. I will present results on two different bacterial species. The TCS DesK/DesR controls fatty acid desaturation in *Bacillus subtilis* in response to cold shock. Through a combined approach using crystallography, biochemistry and microbiology, we have gathered experimental evidence supporting a model of signal-dependent allosteric modulation of the sensor histidine kinase (HK) catalytic activity. Focusing on detailed structural/functional traits of these key protein constituents, we have also discovered a novel and non-canonical activation pathway for the cognate response regulator (RR). By solving the crystal structures of DesR in both, active and inactive configurations, molecular details of the activation switch have been unraveled. The α1α5 surface is shown to be essential for a non-canonical, phosphorylation-dependent dimerization and activation mechanism. We show that this surface is further involved in cognate HK binding, disclosing a novel view of the HK/RR interaction, ensuring signaling pathway specificity. I shall also present recent work on signaling systems in *Leptospira spp*., the etiologic agent of leptospirosis. This disease is a major zoonosis, with high impact in animal health in Uruguay, being one of the main causes of abortions in cattle. Protein science has been instrumental in deciphering heme metabolism regulation controlled by the TCS HemK/HemR. We show that HemR simultaneously acts as a transcriptional activator and repressor of key heme metabolism-related genes. A systematic effort to isolate and type the prevalent *Leptospira* serovars in Uruguay has been launched at our Institute. Genome- and proteome-based information thereof, will allow us to pursue vaccine optimization goals, linking protein science with field applications.

Workshop Sessions

BAM-WK121.03 - Characterization of antisense RNAs in bacteria using comparative transcriptomics

Rotem Sorek[1]
[1]*Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel*

Antisense RNAs, overlapping protein-coding genes in cis, have been abundantly found in multiple transcriptomes of bacteria and archaea, but their functional roles are currently unclear. Specifically, it was suggested that much of the antisense transcription observed in bacteria represents transcriptional noise. Within the talk I will present a comparative transcriptomics approaches that uncover conserved antisense RNAs more likely to be functional. Application of these approaches to the Listeria and Sulfolobus lineages, and resulting discoveries, will be discussed.

Workshop Sessions

BAM-WK121.04 - Genomic epidemiology of emerging multidrug resistant bacterial clonal groups: application to Klebsiella pneumoniae

Suzanne Bialek-Davenet[1], Alexis Criscuolo[1], Sylvain Brisse[1]
[1]*Institut Pasteur, Paris, France*

High-throughput sequencing has revolutionized our abilities to track novel bacterial strains as they emerge and disseminate globally. Multidrug resistant strains are emerging continuously in pathogenic bacterial species. We applied a population genomics approach to define Klebsiella pneumoniae clonal groups and developed a web-accessible genome database, BIGSdb-Kp, to allow easy extraction from genome sequences of genotype, virulence and resistance data in real time. Known sequences corresponding to resistance genes against beta-lactams, aminoglycosides, quinolones and other agents were searched in nearly 200 genomes. Detection of such genes was consistent with phenotypic antimicrobial susceptibility. High-risk isolates with combined virulence and resistance characteristics were uncovered. The BIGSdb-Kp database represents a novel tool for international collaboration on population biology, molecular surveillance and outbreak investigation of K. pneumoniae.

Workshop Sessions

BAM-WK121.05 - Detection of host immune responses against tuberculosis by using Mycobacterium tuberculosis Des Protein and different mycobacterial antigens for IGRA

Niaina Rakotosamimanana[1], Mark Doherty[2], Alimuddin Zumla[3], Brigitte Gicquel[4], Voahangy Rasolofo[5]
[1]Institut Pasteur de Madagascar, Antananarivo, Madagascar, [2]GlaxoSmithKline, Copenhagen, Denmark, [3]University College London, London, UK, [4]Institut Pasteur, Paris, France, [5]Institut Pasteur de Madagascar, Antananarivo, Madagascar

An estimated one third of the world's population is latently infected with Mycobacterium tuberculosis (Mtb), the world's deadliest single bacterial infectious disease, of whom 10% will likely progress to active tuberculosis (TB) disease at some time in their lives. Identifying the individuals most at risk of progressing to TB remains a key goal of TB control efforts worldwide. We evaluated the use of DES, a Mtb exported desaturase, with the most used Mtb antigens for stimulating the human host immune responses by interferon-gamma release assays (IGRA) that could be useful for screening Mtb infected host or with active tuberculosis. ESAT-6, CFP-10, Ag85A, PPD and recombinant Des Protein were used to stimulate PBMC from 384 BCG-vaccinated donors: 55 pulmonary TB-patients, 176 of their household contacts, 152 controls. ELISPOT was performed for IGRA and additional tuberculin skin tests (TST) were done for the contacts and the controls. A significant decreased ELISPOT responses in the TB patients after treatment and healing ($p<0.01$) with an elevated response after 3 months in the contacts ($p<0.01$), suggested that ELISPOT response after stimulation with ESAT6, PPD and CFP7 were associated with an active infection, probably with active bacterial replication in the host. An elevated IGRA response after DES protein stimulation in the TB contacts versus the control individuals ($p<0.001$) plus a stable host response after TB patients treatment suggested that DES protein stimulation response was associated with chronic infection. Combination of the different antigens suggested that the use of Des protein with TST gave the best diagnosis score to detect TB clinical cases. Further evaluations of using such bacterial products for tuberculosis diagnostics and case detection are required in low income and high TB burden areas.

Workshop Sessions

BAM-WK122.01 - Emergence of blaNDM-1 harboring Vibrio fluvialis and Salmonella enterica Senftenberg isolated from acute diarrheal patients

Goutam Chowdhury[1], Anirban Sarkar[1], Gururaja Perumal Pazhani[1], Sumio Shinoda[1], Thandavarayan Ramamurthy[1]
[1]*National Institute of Cholera and Enteric Diseases, Kolkata, India*

The increasing risk of carbapenemase-producing bacteria is now a global concern. New Delhi metallo-β-lactamase (NDM) is a newly identified carbapenemase not only in the Enterobacteriaceae but also in non-fermenters. Here we report the characterization of NDM-1-producing Vibrio fluvialis (NDM-VF) and Salmonella strains isolated from diarrheal patients admitted at the Infectious Diseases Hospital and BC Roy Memorial Hospital for children, Kolkata, India. Of the 100 V. fluvialis isolated from the stool specimens, 22 (20%) were positive for blaNDM-1. One strain out of 62 Salmonella spp was positive for blaNDM-1, which was identified as Salmonella enterica Senftenberg. This Salmonella strain was isolated from a child who had undergone treatment in the BC Roy Memorial Hospital for children. NDM-1-producing V. fluvialis and S. Senftenberg strains have been resistant to ampicillin, ceftriaxone, cefuroxime, cefotaxime, nalidixic acid, fluoroquinolones and streptomycin. E. coli transconjugants obtained from V. fluvialis and S. Senftenberg had plasmids about 80-90 kb and 200 kb, respectively. The transconjugants having blaNDM-1 have been resistant to ampicillin, streptomycin, ceftriaxone, and cefotaxime. The plasmid replicon was untypable in V. fluvialis, whereas S. Senftenberg had Inc A/C type. In the PCR assay, all the NDM-producers were positive for blaOXA-9, aadA, aadB, aac(6')Ib-cr, and sul1. The flanking sequence of the blaNDM-1 of V. fluvialis and S. Senftenberg was identical to Escherichia coli pNDM-HK (HQ451074) and pGUE-NDM (JQ364967), respectively. The gene bleMBL was commonly found in downstream of blaNDM-1 in both the pathogens. However, the upstream of blaNDM-1 in V. fluvialis had ISAba125 whereas the S. Senftenberg had delTnpA. Among the NDM-VF strains, 12 pulsed-field gel electrophoresis patterns were identified in two different clusters. Most of the strains in cluster A and B had about 92% and 85% similarities, respectively. To our knowledge, this is the first report on blaNDM-1 harboring V. fluvialis from diarrheal patients.

Workshop Sessions

BAM-WK122.02 - Fate of antibiotic resistance genes in soil following a commercial application of swine or dairy manure or municipal sewage sludge

Romain Marti[1], Yuan-Ching Tien[1], Roger Murray[1], Andrew Scott[1], Lyne Sabourin[1], Teddie Rahube[1], Peter Duenk[2], David Lapen[3], Edward Topp[1,2]

[1]Agriculture and Agri-Food Canada, London, Canada, [2]University of Western Ontario, London, Canada, [3]Agriculture and Agri-Food Canada, Ottawa, Canada

Animal manures and human waste recycled into crop-production land carry antibiotic-resistant bacteria. By means of PCR and qPCR, the present study evaluated the fate in soil of selected genes associated with antibiotic resistance or genetic mobility in field plots cropped to vegetables, fertilized with agronomic rates of manure or sewage sludge. Referenced to unmanured soil, fertilization with swine or dairy manure increased the abundance of the gene targets sul1, erm(B), str(B), int1 and IncW. A number of genes were detected in soil fertilized with sewage sludge. Gene persistence in soil was followed in plots receiving animal manures. Following manure application in the spring of 2012 gene copy number decayed exponentially, reaching background levels by the fall of 2012. In contrast, gene copy number following manure application in the fall of 2012, or spring of 2013 increased significantly in the weeks following application, and then declined. In both latter cases the abundance of gene copy numbers had not returned to background levels by the fall of 2013. Overall, these results suggest that under conditions characteristic of agriculture in a humid continental climate, a one year period following a commercial application of raw manure is sufficient to ensure that the additional soil burden of antibiotic resistance genes approaches background. Gene abundance exceeded background during the growing season following a spring application, or an application done the previous fall, and therefore this practice results in an increased risk of crop exposure to these genes. Results from the present study reinforce the advisability of treating manure or sewage sludge prior to use in crop production systems.

Workshop Sessions

BAM-WK122.03 - Prevalence of antimicrobial drug resistance of Klebsiella pneumoniae in rural Eastern Cape, South Africa

Sandeep Vasaikar[1], Larry Obi[2], Issac Morobe[3], Charles Mabotja[4], Teke Apalata[4]
[1]Department of Medical Microbiology, FHS, Walter Sisulu University, Mthatha, South Africa, [2]University of Fort Hare, Alice, South Africa, [3]University of Botswana, Botswana, [4]Walter Sisulu University, Mthatha, South Africa

Introduction: Infections caused by ESBL, multidrug resistant and emergence of carbapenem resistant isolates of Klebsiella pneumonia have rapidly increased all over the world including South Africa. Objective of this study to investigate the epidemiological status and laboratory characteristics of Klebsiella species and the drug resistance profiles. Methods: This prospective, descriptive study was carried out on non-repetitive 155 isolates of Klebsiella pneumonia from the Nelson Mandela Academic Hospital (NMAH), Mthatha, from Aug- Oct 2012. K. pneumoniae species were isolated and identified from clinical samples using standard microbiological techniques (bioMérieux API and Siemens autoSCAN-4 System Negative ID and Negative MIC panels). ESBL producers were detected by DDST, PCDDT and carbapenem resistance confirmation by Modified Hodge test. Demographic data of patients was collected. Results: Of 155 isolates K. pneumoniae subsp. pneumoniae were 129(83.2%), followed by K. pneumoniae subsp. oxytoca 25(16.1%) and subsp. mobilis 1(0.7%). Specimen distribution: Pus 34.8%, Sputum & throat swabs 24.6%, Blood culture 14.8%, Urine 9%, Fluids 8.4%, Eye & ear swabs 6.5% and 4.6% High vaginal swabs. Mean age 25.5 years (pediatric 38.1%), and 55.5% were females. Most of patients were from tertiary level 81(52.3%) and 63(40.6%) secondary level while few 11(7.1%) were from Primary health care. 88(57.4%) were ESBL and 67(43.2%) non-ESBLs. Antibiotic sensitivity: In ESBL producing Klebsiella species, highest resistance was seen in ampicillin 88(100%) followed by trimeth/sulfa 87(98.9%), amp/sulbactam 81(92.1%), chloramphenicol 38(46.9%), amox/K Clav 35(39.8%), ciprofloxacin 33(37.5%). In comparison non-ESBL showed highest resistance in ampicillin 62 (92.5%) followed by moxifloxacin 30(37%), trimeth/sulfa18 (26.9%), chloramphenicol and fosfomycin 9(13.5%). Two isolates were found to be carbapenem resistant. Conclusions: Our results showed a high prevalence of ESBL-producing Klebsiella in Mthatha. Antibiotics with good sensitivity for ESBL producing Klebsiella in our setting are carbapenems followed by amikacin, tigecycline, cefoxitin respectively while for non-ESBLs carbapenems followed by amikacin, cefotaxime, ceftazidime.

Workshop Sessions

BAM-WK122.04 - Influence of waste water treatment plants on spread of resistance to antimicrobial drugs in Guadeloupe

Stéphanie Guyomard-Rabenirina[1], Célia Dartron[2], Mélanie Falord[1], Syndia Sadikalay[1], Pierre Couespel du Mesnil[1], Olivier Gros[2], Antoine Talarmin[1]

[1]Institut Pasteur de Guadeloupe, Les Abymes, Guadeloupe, [2]Université des Antilles et de la Guyane, Pointe à Pitre, Guadeloupe

Antimicrobial resistance remains a serious and growing human health challenge. Antibiotic-resistant bacteria (ARB) and antibiotic residues can be released into the environment through human or animal waste. Waste water treatment plants (WWTP) are potential hot spots for exchanges between bacteria and are recognized as reservoirs for ARB. In Guadeloupe, few data are available concerning the resistance of bacteria to antimicrobial drugs in humans and animals. The objective of this study was to estimate the influence of guadeloupean WWTPs, which are often old, on the spread of antibiotic resistance in Guadeloupe. River and sea waters were collected downstream and upstream three WWTPs, and from a control area. Shells were collected near the outfalls. Raw and treated waste water samples of two WWTPs (one receiving hospitals sewages) were also analyzed. Enumeration of E. coli and Enterococci by the method of the Most Probable Number (MPN) and isolation of ARB on specific media added with antibiotics shown that the rivers near WWTPs were highly contaminated unlike water sea . During the study, 279 strains resistant to antibiotics were isolated, most of them resulting from the raw and treated waters of the hospital WWTP. Among these strains, 83 (19 %) were characterized as extended-spectrum beta- lactamase (ESBL) producers, most of them carrying out a bla CTX-M gene. Resistance to fluoroquinolone was also frequently observed among isolates (49%), and 61% of strains were resistant to more than one family of antibiotics. These results show an important contamination of the rivers of Guadeloupe by WWTPs effluents and outline the role of these releases in the spread of ARB in the environment. Poor efficiency of WWTPs contributes to this contamination particularly when effluents are discharged in the rivers near WWTPs. This study emphasizes the importance of promoting the release of WWTPs in the sea when possible.

Poster Session

BAM-WK122.05 - Evolution of antibiotic resistance in the host microbiota

Rebecca S. Shapiro[1,2], Nadia R. Cohen[1,2], B. Jesse Shapiro[3], James J. Collins[1,2]
[1]*Howard Hughes Medical Institute, Boston University, Boston, USA,* [2]*Wyss Institute of Harvard University, Boston, USA,* [3]*Université de Montréal, Montréal, Canada*

Microbial species, ranging from symbionts to pathogens, are a ubiquitous and integral component of life on Earth, and inhabit countless environmental niches. For humans and other mammals, life is inextricably linked with the microbial species that colonize them. These microbes, collectively known as the microbiome, or microbiota, inhabit the skin, mouth, gastro-intestinal tract, and other niches in mammalian species. Although these bacteria are typically not pathogenic, they are constantly exposed to antimicrobial agents, through the use or misuse of antibiotic therapeutics, or through prophylactic use of antimicrobials in agricultural animals. Experiment research in vitro, has demonstrated that exposure to antimicrobial agents generates resistance, and can expedite the evolution of cross-resistance to additional antimicrobial agents, however little is understood about the evolution of antimicrobial resistance amongst symbiotic microbial species inhabiting a mammalian host. Using a gnobiotic mouse model colonized with isogenic, antibiotic-susceptible Escherichia coli, we are studying the evolutionary and population-level dynamics of antibiotic resistance of microbial symbionts in vivo. We show that exposing healthy mice to antibiotic drugs causes increased antibiotic resistance in the E. coli that colonizes these mice. Antibiotic resistance develops not only to the antibiotic that the animals were exposed to, but also to different classes of antibiotic drugs. Genome sequencing analysis of these E. coli populations revealed signatures of selection, including convergent mutations, and is helping to elucidate the mechanisms by which these bacteria develop multi-drug resistance. Future experiments will assess whether these symbiotic E. coli bacteria are able to impart antibiotic resistance to pathogenic bacteria via horizontal gene transfer of genetic material. Together, this work will describe how symbiotic bacteria inhabiting a mammalian host can evolve resistance to antimicrobial agents, and provide insight into how the host microbiota may serve as a reservoir for antibiotic resistance.

Workshop Sessions

BAM-WK122.06 - Low-dose antibiotic therapy increases the risk of persistent urinary tract infection

Lee Goneau[1], Thomas Hannan[2], Scott Hultgren[2], Jeremy Burton[3], Hassan Razvi[3], Gregor Reid[1]
[1]Department of Microbiology and Immunology, Western University, London, Canada, [2]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, United States, [3]Department of Surgery, Division of Urology, Western University, London, Canada

Low-dose antibiotic management for recurrent urinary tract infections (RUTI) remains a challenge due to pathogen re-emergence, resistance and the risk of sepsis. Understanding a patient's risk of recurrence is critical in decreasing healthcare expenditure and improving quality of life. In murine UTI, ~50% of mice spontaneously resolve with the remainders developing chronic infection. These outcomes are determined within the first 24 hours and governed by pathogen virulence and host immune function, with modulation in either shifting the infection equilibrium towards resolution or chronicity. Previously we have shown that subinhibitory antibiotics are capable of increasing uropathogen adherence and internalization in vitro, questioning their potential to contribute to infection in vivo. Thus, we hypothesized that low-dose therapy will increase the risk of chronic infection in mice. Female, C3H/HeN mice inoculated with ciprofloxacin-primed (1/4th the sub-minimal inhibitory ciprofloxacin dose) Staphylococcus saprophyticus, or uropathogenic Escherichia coli (UPEC) showed significantly increased bacterial burden in both bladder and kidneys at 1 and 14 days post infection (d.p.i.). All mice infected with ciprofloxacin-primed UPEC presented with clinically significant (>104 cfu/mL) cystitis 14 d.p.i., compared to 60% in control mice. Primed uropathogens had upregulated adhesin expression, which resulted in larger intracellular bladder reservoirs. Sub-therapeutic (1/50th the empirical therapeutic dose) ciprofloxacin supplemented in the drinking water of chronically infected mice resulted in significantly increased bacterial urine load. In addition, mice whose infections had resolved then developed clinically significant infection following sub-therapeutic dosing. Prophylaxis therapy showed no significant effect on UPEC urine clearance, or bladder and kidney loads. However, therapy did significantly increase intracellular bladder reservoirs of bacteria, raising concerns over the clinical efficacy of this management strategy and risks of promoting persistent infection. It is hoped that this research will lead to a re-examination of how antibiotics are administered for management of patients suffering from persistent diseases.

Workshop Sessions

BAM-WK123.01 - Engineering of Lactobacillus panis PM1 for efficient conversion of biofuel glycerol

Tae Sun Kang[1], Darren R. Korber[1], Takuji Tanaka[1]
[1]*University of Saskatchewan, Saskatoon, Canada*

Lactobacillus panis PM1 is a novel microorganism isolated from thin stillage (TS) of bioethanol production and its glycerol reductive pathway converts glycerol into 1,3-propanediol (1,3-PDO). Our studies of its metabolic mechanism indicated: 1) the strain belongs to the group III lactobacilli that metabolize glucose using the 6-phosphogluconate/phosphoketolase pathway; 2) it does not metabolize glycerol for energy production; and 3) the glycerol reductive pathway is regulated through the supply of NADH, the transcriptional repressor (PocR), and substrate inhibition (by 3-hydroxypropionaldehyde; 3-HPA). In the present study, we genetically manipulated this strain in order to increase its productivity and efficiency of TS utilization. Since we previously showed both NADH limitation and substrate inhibition restrict the conversion of 3-HPA to 1,3-PDO, an E. coli NADPH-dependent aldehyde reductase gene was inserted to provide a bypass pathway. This engineered strain produced 1,3-PDO at 213.6 mmol/L in TS supplemented with 167 mmol/L (or 30 g/L) glucose and 179 mmol/L (of 16.5 g/L) glycerol. Further, we introduced an artificial glycerol oxidative pathway into the engineered strain to allow strain PM1 to use glycerol for energy metabolism, a feature that should eliminate requirements for exogenous fermentable sugars (e.g., glucose). The metabolically-engineered PM1 strain was cultured in TS without addition of any nutrients (i.e., exogenous nitrogen source) or sugars. A pH 6.5 culture converted glycerol (160 mmol/L) to lactic acid (85.4 mmol/L); whereas, pH 7.5 conditions allowed the strain to produce 1,3-PDO at 60.0 mmol/L. Under both conditions, a considerable amount of ethanol was produced (82.2 ~ 83.2 mmol/L) which indicated the artificial glycerol oxidative pathway consumed glycerol and split it into lactic acid/ethanol or 1,3-PDO/ethanol according to pH conditions. These results showed the potential of these engineered L. panis PM1 strains for use in industrial applications to utilize oversupply of glycerol from biofuel production without providing additional inputs.

Workshop Sessions

BAM-WK123.02 - Enzymatic synthesis of tumor-associated carbohydrate antigens using bacterial glycosyltransferases

Michel Gilbert[1], Anna Cunningham[1], Marie-France Goneau[1]
[1]National Research Council Canada, Ottawa, Canada

Aberrant surface glycosylation is observed for many types of human cancers, where several carbohydrate antigens have been identified as candidates for therapeutic vaccine development. Chemical synthesis of complex carbohydrates is very challenging, while the use of enzymatic methods has been limited by the availability of glycosyltransferases with appropriate specificities. Bacterial mucosal pathogens often display mimics of the glycan portion of human glycolipids in their outer layer, and their respective glycosyltransferases have now become excellent glyco-engineering tools through high-yield recombinant production in Escherichia coli. We report here the enzymatic synthesis of two tumor-associated carbohydrate antigens: fucosyl-GM1a (Fucα1-2Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glc1-1Cer; a small cell lung cancer marker) and 3' iso-LM1 (NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc1-1Cer; a glioblastoma marker) using four bacterial glycosyltransferases. The WbsJ α-1,2-fucosyltransferase from E. coli O128 was used to add an α-1,2-Fuc residue to the GM1a glycolipid. Following purification by thin layer chromatography and solid phase extraction, we obtained 11 mg of Fuc-GM1a. The enzymatic synthesis of 3' iso-LM1 started with the precursor Galβ-1,4-Glcβ-PEG3-N3 (Lac-PEG-N3). JHP1032 and JHP0563 from Helicobacter pylori were used to add a β-1,3-linked GlcNAc residue and a β-1,3-Gal-residue, respectively, to Lac-PEG3-N3 resulting in the production of Galβ-1,3-GlcNAcβ-1,3-Galβ-1,4-Glcβ-PEG3-N3 (LNT-PEG3-N3). Finally, the CstI α-2,3-sialyltransferase from Campylobacter jejuni was used to add a terminal α-2,3-linked NeuAc residue to LNT-PEG3-N3, resulting in 3'iso-LM1-PEG3-N3. The material was purified by solid phase extraction yielding 185 mg of 3'iso-LM1-PEG3-N3. This compound is suitable for the synthesis of conjugate vaccines since the azido group at the reducing end can be used for linking the 3'iso-LM1 glycan to carrier proteins.

Workshop Sessions

BAM-WK123.03 - Construction of a light-driven nonphotosynthetic photophosphorylation pathway in Saccharomyces cerevisiae and its application for bioproduction

Xiaoting Ye[1], Kiyotaka Hara[1], Akihiko Kondo[1]
[1]*Kobe University, Kobe, Japan*

Light as economic and clean energy has gained increased attention because of the energy and environment concerns. Considerable research efforts have been paid to increase the efficiency of light capture in plants or microalgae to produce sustainable renewable fuels and chemicals. However, their slow growth rate and relatively unknown genetic system often present difficulties in altering photosynthesis efficiency for optimal production of bioenergy. Recently, a unique nonphotosynthetic photophosphorylation pathway in the extreme halophiles which converts light energy into chemical energy through a light-driven pump, namely bacteriorhodopsin (bR), has gained interest. In this work, we introduced bR to the inner membrane of yeast mitochondria by fusing it with a mitochondria localizing protein. Under the condition of light and when carbon source is cut off, light energy can be converted into a proton gradient across the mitochondria inner membrane and subsequently, the photo-induced proton gradient from the bR is used by the F0F1-ATP synthetase to produce ATP. Through this artificial photosynthesis system, the ATP-consuming bioproduction (e.g., glutathione) in S. cerevisiae was increased by 200%. Our approach would be widely applicable to the membrane-associated microorganisms and would be applicable to replacement of current dependency on the feed stock and the enhancement of bioenergy production.

Workshop Sessions

BAM-WK123.04 - Hyper expression systems in actinomycetes

Michihiko Kobayashi[1]
[1]*University of Tsukuba, Grad. School of Life & Environ. Sciences, Tsukuba, Japan*

An inducible enzyme is a part of the operon model, which illustrates a way for genes to turn “on” and “off”. Although in general it is inductively formed by the enzyme substrate, other compounds exhibiting hyper induction of the enzyme can be obtained by mass-screening, where each of various compounds is added into the culture media of a microorganism. We have investigated microbial metabolism of nitrile compounds through the following two enzymatic pathways: nitrilase (NitA) hydrolyzing nitrile to acid and ammonia; and nitrile hydratase (NHase) hydrating nitrile to amide. We have discovered that an actinomycete, Rhodococcus rhodochrous strain produces the both enzymes, depending on the corresponding inducer. NHase and NitA are inductively overexpressed (more than 35% of all soluble proteins) on the addition of amide and nitrile, respectively, to the culture media. Particularly NHase has a unusual regulation mechanism; it is induced by the reaction product (amide), not by the reaction substrate (nitrile). Using each of the transcriptional regulation mechanisms and the strong gene promoters of the both enzymes, we succeeded in the construction of two hyper expression systems in actinomycetes. We also looked for signal sequences involved in secretion of proteins in Streptomyces. Using some of them, we constructed new expression systems that direct for the secretion of proteins, whose genes are inserted into the corresponding vector. Because Streptomyces has widely been for the production of biologically active compounds such as antibiotics, the above systems would be powerful tools for improving the productivity of various useful products.

Workshop Sessions

BAM-WK123.05 - A Bacillus subtilis cell factory efficiently converting myo-inositol into scyllo-inositol, a potential therapeutic agent for Alzheimer's disease

Ken-ichi Yoshida[1], Yoshihiro Toya[2], Shinji Takenaka[1], Hiroshi Shimizu[2], Kosei Tanaka[1]
[1]Kobe University, Kobe, Japan, [2]Osaka University, Osaka, Japan

Inositol (1,2,3,4,5,6-cyclohexanehexol) has nine possible stereoisomers. One of the stereoisomers, myo-inositol (MI), is most abundant in nature and supplied cheap from rice bran. On the other hand, another stereoisomer, scyllo-inositol (SI), is rare in nature, and precious because of being expected as a disease-modifying therapeutic agent for Alzheimer's disease, which is one of the most common and problematic forms of dementia, and thus SI has received a fast-track designation from the US Food and Drug Administration for treatment of mild to moderate Alzheimer's disease. We demonstrated a cell factory, which enables bio-conversion from MI to SI, made of Bacillus subtilis with the modified inositol metabolic pathway. In the B. subtilis cell factory, all "useless" genes involved in MI and SI metabolism were deleted, and in addition the two key enzymes responsible for the conversion, namely IolG and IolW, were overproduced under the control of one of the strongest promoters. All of 1% (w/v) MI contained in the medium was converted into SI at the rate of 10 g/ L/ 48h at least. The efficient conversion was achieved only in the presence of enriched nutrition in the form of 2% (w/v) Bacto soytone, which may be due to the increasing demand for regeneration of cofactors including NADPH. The results from our transcriptomic and fluxomic analyses suggested that the regeneration of NADPH might be enabled by generic alternation in central carbon metabolism including enhancement in hexose monophosphate shunt and gluconeogenesis.

Workshop Sessions

BAM-WK123.06 - Engineering a photosynthetic bacterium Rhodobacter sphaeroides for improved expression of heterologous membrane proteins by supplementation of rare tRNAs

Danhui Cheng[1], Kristala Prather[2], King-Lau Chow[1], I-Ming Hsing[1]
[1]The Hong Kong Univerisity of Science and Technology, Hong Kong, Hong Kong, [2]Massachusetts Institute of Technology, Cambridge, US

The photosynthetic bacteria, Rhodobacter species, have emerged as a promising expression host for heterologous membrane proteins. The highly diversified metabolism and incredible efficiency in solar harnessing have drawn increasing interests in this nonconventional host microorganism. Moreover, a light/oxygen tension induced intracytoplasmic membrane (ICM) allows the accommodation and enrichment of membrane proteins in functional state, making Rhodobacter an attractive candidate in production of membrane protein for structural biology studies over traditional expression host,e.g.,Escherichia.coli. One hurdle in utilizing Rhodobacter for protein expression is its codon preference. With a genome rich in GC content (68.8% for R. sphaeroides 2.4.1 as compared to 50.79% for E. coli K-12), Rhodobacter preferentially uses codons with G/C in the third position, resulting in a highly biased tRNA pool which impedes the expression of certain exogenous target genes with lower GC content according to our observations. In this study, Rhodobacter sphaeroides strain 2.4.1 is engineered for improved expression of heterologous membrane protein by supplementing rare tRNAs, through which a universal strategy is implemented to eliminate the incompatibility in codon usages between foreign genes and R.sphaeroides. Based on amino acid composition statistics obtained from UniProtKB/Swiss-Prot databank, and the codon frequencies in genome of R.sphaeroides 2.4.1, eight underrepresented codons are identified and the respective tRNAs are selectively expressed in R.sphaeroides. qRT-PCR indicates that the tRNA genes are successfully transcribed under their native promoters with a significant increase in the intracellular levels of each rare tRNAs. The tRNA fragments are then integrated into the genome following the oxygen/light responsive puc promoter. Influence of upstream puc promoter upon induction on the transcription of tRNA genes is investigated to pursue tunable overexpression of tRNA. Finally, a substrate-specific component of riboflavin ECF transporter from Lactobacillus Acidophilus La-14(GC content 34%) is expressed to evaluate the overall strategy.

Workshop Sessions

BAM-WK124.01 - The comparative pathogenicity of strains of six serotypes of Mannheimia haemolytica in experimental pneumonia of sheep

Moses Odugbo[1], Lillian Odama[2], Jarlath Umoh[3]
[1]National Veterinary Research Institute, Vom, Nigeria, [2]Microbiology Department, Kogi State University, Nigeria, [3]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria

Unanswered questions on the aetiology and relative pathogenicity of the many serotypes of Mannheimia haemolytica (Mh) associated with pneumonic pasteurellosis (mannheimiasis) of ruminants have allowed this disease to remain one of the most costly to the farmed ruminant industry. In this study, the experimental induction of mannheimiasis by six serotypes (A1, A2, A6, A7, A8 and A9) of Mh in groups of conventionally reared lambs were examined and compared. The groups of lambs were inoculated intratracheally with 8Log10 (10^8) colony-forming units of the Mh serotypes in the 6-hour log phase of growth. The variables measured as indicators of disease severity were clinical score, percentage lung consolidation and microbiological re-isolation. The clinical parameters for each group were computed daily for 6 days post infection and the lambs which died were necropsied while the remaining lambs were killed on day 7 post inoculation and the extent of lung consolidation was measured. Clinically, the mean scores for the Mh serotypes were A1 (6.1), A2 (18.8), A6 (0.5), A7 (17.4) and A9 (8.5). The mean percent lung lesion scores for Mh serotypes were A1 (12.5), A2 (66.3), A6 (5.0), A7 (51.3) and A9 (33.8). The percent mean pneumonic lung lesions recorded for groups inoculated with Mh serotypes A2, A7 and A9 were significantly ($P<0.05$) higher than the extent of lung lesions in the other groups. A statistically significant correlation was observed between clinical scores and the severity of the lung lesions ($r = 0.96$, $P < 0.01$). High titres of Mh (10 to 100 times the number of organisms inoculated) were recovered from lung lesions. It is concluded that Mannheimia haemolytica serotypes A2, A7 and A9 are to be regarded as highly virulent strains that have a greater predilection than the other serotypes for causing pneumonia in lambs.

Workshop Sessions

BAM-WK124.02 - Unexpected genetic diversity of Mycoplasma arginini isolates from Victoria, Australia

Olusola Olaogun[1], Anna Kanci[1], Stuart Barber[1], Kelly Tivendale[1], Philip Markham[1], Marc Marenda[1], Glenn Browning[1]
[1]Asia-Pacific Centre for Animal Health, Faculty of Veterinary Science, The University of Melbourne, Parkville, Australia

Background The contribution of Mycoplasma arginini to ruminant mycoplasmosis remains unclear because it is recovered from both healthy and diseased animals. In this study, we used multilocous sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) as molecular typing tools to assess the genetic diversity among M. arginini isolates. Methods M. arginini isolates were randomly selected from a collection of isolates made over two years in an epidemiological survey of small ruminant herds in Victoria, Australia (2011–2013). These isolates were subjected to PFGE typing using the restriction endonuclease BglI and MLST typing using six housekeeping genes. The genomic profiles and descriptive epidemiological data were analysed. Results There was consistency in the genomic profiles generated by the two typing tools, with MLST providing a more rapid assessment and a higher discriminatory power. The profiles indicated that M. arginini isolates are highly diverse both within and between herds. However, there was an association between genomic profile and clinical status of herds, with isolates from diseased herds clustering together and possessing a distinct profile suggesting that these strains may be pathogenic. Conclusion The unexpected genetic diversity among M. arginini isolates observed in this study contrasts with the assumption of homogeneity of M. arginini isolates. Both MSLT and PFGE offer effective typing tools for assessing the diversity of M. arginini isolates and their potential contribution to disease.

Workshop Sessions

BAM-WK124.03 - Identifying potential avian pathogenic Escherichia coli (APEC) for the development of a bacteriophage therapy treatment for colibacillosis

Wouter van der Westhuizen[1], Robert Bragg[1]
[1]University of the Free State, Bloemfontein, South Africa

Avian pathogenic Escherichia coli (APEC) cause the disease colibacillosis in poultry which leads to economic losses in the poultry industry globally. The pathogenicity of APEC is encoded by various virulence genes occurring in different combinations and on both plasmids and in the E. coli genome. APEC is generally treated and infections prevented through antibiotic treatment of the poultry. The use of fluoroquinolones in the poultry industry of the USA has been banned, as resistance genes became prevalent among APEC and can be horizontally transferred to the human pathogen Campylobacter. Alternative treatments to antibiotic therapy are required of which bacteriophage therapy is one such option. A bacteriophage therapy would have the advantage of being a very specific therapy against APEC while leaving the normal E. coli microbiota intact to reduce incidences of secondary infections. For the specific treatment of APEC with bacteriophages to be successful, APEC strains need to be successfully identified. This study investigated the use of multiplex PCR techniques to molecularly characterize various Southern African E. coli isolates and to screen bacteriophages for specificity to potential APEC. A higher prevalence of the 18 virulence genes were observed in the E. coli isolated from chickens with colibacillosis symptoms and various bacteriophages which can lyse APEC strains have been identified. Future research will include in vivo testing of the selected bacteriophages as a potential bacteriophage therapy for use in the poultry industry.

Workshop Sessions

BAM-WK124.04 - Escherichia coli STb enterotoxin dislodge claudin-1 from epithelial tight junctions

Hassan Nassour[1], J. Daniel Dubreuil[1]
[1]*Université de Montréal, Saint-Hyacinthe, Canada*

Enterotoxigenic Escherichia coli produce various heat-labile and heat-stable enterotoxins. STb is a low molecular weight heat-resistant toxin responsible for diarrhea in farm animals. A previous study demonstrated that cells having internalized STb toxin induce epithelial barrier dysfunction through changes in tight junction (TJ) proteins. These modifications contribute probably to the diarrhea observed. To gain insight into the mechanism of increased intestinal permeability we treated human colon cells (T84) with purified STb toxin after which cells were harvested and proteins extracted. Using a 1% Nonidet P-40 (a non-ionic, non-denaturing detergent)-containing solution we investigated the distribution of claudin-1, a major TJ protein responsible for the epithelium impermeability, between membrane (NP40-insoluble) and the cytoplasmic (NP-40 soluble) location. Using immunoblot and confocal microscopy, we observed that treatment of T84 cell monolayers with STb induced redistribution of claudin-1. After 24h, cells grown in low Ca++-containing medium (5 µM) treated with STb, showed about 40% more claudin-1 in the cytoplasm compare to the control. Switching from low to physiological Ca++-containing medium (1, 8 mM) increased the dislodgement rate of claudin-1, as comparable delocalization was observed after 6h. Medium supplemented with the same concentration of Mg++ or Zn++ did not affect the dislodgement rate compare to the low Ca++-containing medium. Using anti-phosphoserine and anti-phosphothreonine antibodies we observed that the loss of membrane claudin-1 was accompanied by dephosphorylation of this TJ protein. Overall, our findings showed an important redistribution of claudin-1 in cells treated with STb toxin. The loss of phosphorylated TJ membrane claudin-1 is likely to be involved in the increased permeability observed. The mechanisms by which these changes are brought about remain to be elucidated.

Workshop Sessions

BAM-WK124.05 - In vivo virulence due to production of salmochelins by extraintestinal pathogenic E. coli is highly dependent on the presence of cytoplasmic salmochelin esterase IroD

Amelie Garenaux[1], Melissa Caza[2], Sylvain Milot[1], Francois Lepine[1], Charles M. Dozois[1]
[1]INRS-IAF, Laval, Canada, [2]MSL, Vancouver, Canada

The battle for iron represents a major part of host-pathogen interactions. Previous work from our lab already demonstrated that pathogen-specific siderophores salmochelins and aerobactin played key roles in virulence of avian pathogenic Escherichia coli (APEC). Salmochelins are glycosylated forms of enterobactin. It has already been demonstrated that enterobactin was not involved in virulence, as it is sequestered by a host innate immune protein called siderocalin. Glycosylation of enterobactin by IroB glycosyltransferase to form salmochelins allows pathogenic bacteria to overcome this innate immune defense system. In order to release iron in the cytoplasm for further use by pathogens, enterobactin and salmochelins have to be degraded by specific esterases: Fes, IroE and IroD. Single deletion mutants of the genes coding for these three esterases were generated in an O78 APEC strain and tested in vivo in a three-week old chicken infection model. If fes and iroE mutants did not seem to be affected in their virulence, an important attenuation of virulence was observed for the iroD mutant. Mass spectrometry analyses revealed that this attenuation of the iroD mutant was correlated with a significant drop in salmochelins production. However, qRT-PCR analyses and western blots showed that this drop could not be attributed to a decrease in IroB expression. Furthermore, complementation with an esterase containing a point mutation in the active site failed to restore the ability to synthesize salmochelins, whereas complementation with the exogeneous C. jejuni Cee esterase allowed salmochelins production at the same levels as those obtained in the presence of IroD. These results show that IroD esterase could constitute a target for the development of therapies against ExPEC infections. It constitutes the first demonstration of the involvement of esterases in salmochelins production.

Workshop Sessions

BAM-WK124.06 - Metagenomic approach to study the taxonomic diversity of bacterial community in clinical and subclinical bovine mastitis

Reena Patel[1], Ramesh Pandit[1], Vaibhav Bhatt[2], Prakash Koringa[3], Prashant Kunjadia[4], Chaitanya Joshi[3], Anju Kunjadia[1]

[1]Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences, New V V Nagar, India, [2]DST inspire faculty, Dept. of Pharmaceutical Sciences, Saurashtra University, Rajkot, India, [3]Dept. of Animal Biotechnology, College of Veterinary Science and A. H., Anand Agricultural University, Anand, India, [4]B. N. Patel institute of paramedical and Sciences, Sardar Patel University, Anand, India

Bovine mastitis resulting from a bacterial infection is an economically important disease in the dairy industry throughout the world. Mastitis is generally classified as clinical or subclinical depending on the degree of inflammation in the mammary gland. It is estimated that 10% of dairy animals suffer from clinical mastitis and that up to 50% are subclinically infected. Applying the next generation sequencing method is necessary to surmount the limitations of classical culturing techniques in order to obtain an accurate description of the microbiota composition. In the present study we performed a 16S rRNA gene diversity profiling to characterize the microbiota associated with milk from clinical and sudclinically affected mastitis cows using 454 Roche, titanium chemistry and the MG-RAST and Qiime analysis pipeline. Statistical analysis was performed using PAST. Moreover, it lends to the growing understanding of the remarkable dynamic nature of our metagenome and its role in etiology of the diseases. Subclinical samples are more diverse in terms of bacterial species than the clinical ones. The bacterial community in mastitic milk contained high microbial diversity with sequences aligning predominantly to the phyla Firmicutes and Proteobacteria and belonging to the genera Staphylococcus, Lactococus, Enterococuus and Ralstonia. In the present work we have confirmed that NGS is a powerful technique to study the diverse and complex mastitic pathogens.

Workshop Sessions

BAM-WK124.07 - Streptococcus suis serotype 2 interacts with both human meningeal cells and astrocytes

Jean-Philippe Auger[1], Myron Christodoulides[2], Marcelo Gottschalk[1]
[1]GREMIP, University of Montreal, Saint-Hyacinthe, Canada, [2]Neisseria Research Group, University of Southampton, Southampton, UK

Streptococcus suis serotype 2 is an important bacterial pig pathogen and an emerging zoonotic agent. Meningitis, the hallmark of the central nervous system (CNS) infection, is characterized by a local exacerbated inflammatory response. Yet, the interactions of S. suis with human meningeal cells and astrocytes, which play an important role during infection of the CNS by different bacterial pathogens, have not been evaluated. In this study, strains isolated from both clinical human and pig cases of infection and mutants for the capsular polysaccharide (CPS) and suilysin were used. The strains were representative of different important sequence types (ST) found worldwide (ST1, 7, 25, and 28), as determined by multilocus sequence typing, including the strain responsible for the 2005 Chinese human epidemic. All STs adhered to meningeal cells and astrocytes. With the exception of the ST7, none of the other STs were capable of invading meningeal cells, while all capable of invading astrocytes. The CPS influenced both adhesion to and invasion of both cell types, while the suilysin influenced invasion of astrocytes only. However, only low levels of CCL5, and no levels of cytokines or chemokines, were induced by the different S. suis STs in infected meningeal cells and astrocytes, respectively. In summary, different S. suis serotype 2 STs showed similar patterns of adhesion, invasion and cytokine induction with human meningeal cells and astrocytes. Although the meninges and astrocytes have been shown to play an important role in inflammation during bacterial infection of the CNS, these cells do not appear to be the source of the inflammatory mediators observed during S. suis meningitis in humans, suggesting the contribution of other resident cells. Nevertheless, the interactions with both meningeal cells and astrocytes could possibly, to a certain extent, play a role in the pathogenesis of the infection in humans.

Workshop Sessions

BAM-WK124.08 - Superoxide dismutase of Streptococcus suis type 2 contributes to delayed autophagic response in infected macrophages

Lihua Fang[1], Weihuan Fang[1], Hongxia Shen[1], Xiaowu Jiang[1]
[1]Institute of Preventive Veterinary Medicine, Hangzhou, China

Streptococcus suis type 2 (SS2), an important swine and human pathogen, causes septic shock and meningitis. We have recently shown that superoxide dismutase (Sod) of SS2 is a virulence factor probably by scavenging reactive oxygen species (ROS) from phagocytes. We wanted to know if and how SS2 explores Sod to interfere with the autophagic cellular response. A sod deletion mutant (SS2Δsod) was constructed and compared with its parent strain in autophagic responses and ROS load in the murine macrophage cell line RAW264.7. With luminol enhanced chemiluminescence to analyze ROS produced in vitro by the hypoxanthine-xanthine system, we found that the wild-type and complemented strains cleared up far more ROS than the strain SS2Δsod. Purified Sod of SS2 expressed in E. coli could also eliminate ROS effectively, suggesting that SS2 Sod is an effective ROS scavenger. Western blotting showed that the strain SS2Δsod activated autophagy with considerably increased LC3 lipidation (LC3-II) at 1 h post-infection, which was about 1 h earlier than the wild-type strain. Further fluorescent analysis of the superoxide indicator dihydroethidium reveals that macrophage cells infected with the strain SS2Δsod contained more intracellular O2- and than those infected with its parent or complemented strains, shown as increased expression of p47-phox and p67-phox. The deletion mutant also increased nuclear translocation of NF-κB, as compared with its parent strain. These results indicate that more accumulation of ROS in the sod-deficient strain activated NF-kB and induced autophagy at early time. We also found that treatment of the SS2Δsod infected cells with 3-methyladenine improved survival of the mutant strain, while inhibiting the autophagic response. Taken together, S. suis type 2 makes use of superoxide dismutase for its survival not only by scavenging ROS but also by alleviating the host autophagic response.

Workshop Sessions

MEM-WK314.01 - Pathogenic cryptococci: iron metabolism and virulence

Jim Kronstad[1], Guanggan Hu[1], Sanjay Saikia[1], Melissa Caza[1], Debora Oliveira[1], Matthias Kretschmer[1], Won Hee Jung[2], Eunsoo Do[2], Jennifer Geddes[1]
[1]Michael Smith Laboratories, University of British Columbia, Vancouver, Canada, [2]Department of Systems Biotechnology, Chung-Ang University, Anseong, Republic of Korea

The yeast, *Cryptococcus neoformans*, causes an estimated one million cases of life-threatening meningitis in people with immunodeficiency due to HIV/AIDS. The global burden of this disease results in >600,000 deaths every year, with the majority occurring in Sub-Saharan Africa. The related species *Cryptococcus gattii* has emerged as a pathogen of people with normal immune systems, as demonstrated by an unprecedented outbreak in British Columbia and the Pacific Northwest over the past 15 years. Our work has contributed to transcriptome and genome resources and has provided an understanding of genome variability for the pathogenic cryptococci. One area of focus concerns the mechanisms of iron acquisition for *C. neoformans*. Iron is particularly important for this pathogen because it is essential for proliferation and iron levels also regulate elaboration of a key virulence factor, the polysaccharide capsule. We initially found that the high affinity, reductive iron acquisition system makes a partial contribution to disease and was critical for colonization of the central nervous system. We also recently deleted eight genes encoding candidate ferric reductases and identified a contribution of Fre2 to disease and brain colonization. These functions are important for iron acquisition from transferrin. We also characterized iron acquisition from heme, the most abundant source of iron in vertebrate hosts. This analysis revealed that the mannoprotein Cig1 and endocytosis mediated by ESCRT complexes are important for heme uptake and for disease. Interestingly, some ESCRT complexes also make a contribution to capsule elaboration. Overall, these studies reveal that *C. neoformans* has a number of mechanisms for iron acquisition and that each system makes a partial contribution to disease. Our analysis of regulatory pathways (e.g., protein kinase A) and factors (e.g., Cir1, HapX and Rim101) for iron sensing is revealing additional levels of integration of iron homeostasis with other nutrient uptake systems and metabolic functions.

Workshop Sessions

MEM-WK314.02 - Drug discovery using a Whole Animal Infection Model

Eleftherios Mylonakis[1]

[1]*Alpert Medical School of Brown University, Providence, USA*

Several virulence-related genes in a variety of bacterial and fungal pathogens previously shown to be involved in mammalian infection have also been shown to play a role in killing of invertebrate hosts. The model nematode Caenorhabditis elegans can be used to study microbial pathogenesis in a genome-wide scale and perform whole-animal, high throughput bioassays for novel antifungal compounds. Importantly, this infection model has been adapted to a high-throughput (HT) methodology to rapidly screen numerous compounds. Our findings indicate that this screening model permits data-driven decisions regarding the quality of a hit and eliminates preconceived biases about certain chemical classes or motifs. Notably, the whole-animal in vivo assay examines some compound characteristics (such as water solubility, stability and toxicity) in parallel and avoids inefficiencies associated with the sequential optimization hits with regard to desired properties, which must occur in a traditional optimization cycle. A pilot screen of 640 FDA approved drugs yielded compounds with antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA). Also, we found that the probiotic Gram-positive bacterium Lactobacillus acidophilus NCFM is not harmful to C. elegans. Conditioning with L. acidophilus NCFM significantly decreased the burden of a subsequent Enterococcus faecalis infection in the nematode intestine and prolonged the survival of nematodes exposed to pathogenic strains of E. faecalis and Staphylococcus aureus. Importantly, L. acidophilus NCFM activates key immune signaling pathways involved in C. elegans defenses against Gram-positive bacteria, including the p38 mitogen-activated protein kinase pathway (via TIR-1 and PMK-1) and the β-catenin signaling pathway (via BAR-1). In summary, HT screening of the whole animal infection model can identify compounds that cure bacterial infections associated with resistant pathogens.

Workshop Sessions

MEM-WK314.03 - Bat white-nose syndrome: an emerging fungal disease with major implications for bat conservation

David Blehert[1], Jeffrey Lorch[2], Michelle Verant[2]
[1]US Geological Survey - National Wildlife Health Center, Wisconsin, USA, [2]University of Wisconsin, Madison, USA

White-nose syndrome (WNS) is an emergent wildlife disease that has spread rapidly and caused unprecedented mortality among bats of eastern North America. Since first detected in New York during the winter of 2006-2007, WNS has spread to 23 US states and five Canadian provinces, and the disease is estimated to have killed over five million bats. Prior to emergence of WNS, such massive population declines in mammalian species due to an infectious disease were unprecedented. Also unusual is that WNS is caused by a fungus, a class of organisms that rarely cause large-scale disease outbreaks among mammals. Ongoing investigations have demonstrated that cutaneous infection of hibernating bats by the fungus *Pseudogymnoascus*. (formerly *Geomyces*.) *destructans*. disrupts vital physiological processes and causes behavioral disturbances. Furthermore, the pathogen is psychrophilic. While it grows well at temperatures consistent with bat hibernation (approximately 2 to 12°C), it cannot grow on metabolically active bats. The fungus does, however, persist throughout the year in soil of underground bat hibernation sites, including during summer months, indicating that these sites serve as persistent reservoirs for the pathogen. Finally, a growing body of evidence suggests that *P. destructans* was introduced to North America, possibly from Europe where bats seem to co-exist with the fungus, suggesting that WNS in North America is likely the result of a novel pathogen introduced into a naïve population of hosts.

Workshop Sessions

MEM-WK314.04 - Penicillium marneffei infection in mainland China

Liyan Xi[1], Sha Lu[1], Yongxuan Hu[1], Xiqing Li[1], Xiaowen Huang[1]
[1]*Dept of Dermatology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangdong,China*

Penicilliosis marneffei, an infection caused by P. marneffei, is an endemic disease in areas of South East Asia. It mostly occurred in AIDS patients, and increasing rapidly in mainland China recently years. We recently collected 668 cases in China from 1984 to 2009 and found that 99.4% cases in southern part of China, more than 80% from Guangxi and Guangdong province. Five hundred and eighty-six cases of penicilliosis marneffei reported with HIV/AIDS, 25 cases (3.8%) with other immunocompromised diseases. The 569 cases received antifungal therapy with mortality of 24.3%, 99 cases hadn't received antifungal therapy with 50.6% mortality. The routes of infection are poorly understood, bamboo rats have still been the only natural animal reservoir of P. marneffei in endemic area of southern China. We isolated the P. marneffei from the inner organs of bamboo rats and found the isolation rate of lung and liver was 100%, 83% respectively. We also isolated the pathogen around bamboo rat's holes. It indicates that P. mameffei actually exist in nature, but most patients haven't had the contacting history of bamboo rat. So the infectious route is still a mystery.

Workshop Sessions

MEM-WK315.01 - Photobiology in model and clinical fungi

Jennifer Loros[1], Kevin Fuller[1], Arko Dasgupta[1], Chen-Hui Chen[2], Jay Dunlap[1]
[1]Geisel School of Medicine, Hanover, USA, [2]Duke University, Durham, USA

Light is essential for life on earth and also serves as influential environmental information signaling daily and seasonal time to organisms across kingdoms. Within the fungi, the molecular mechanisms of light responsiveness are well characterized in the model system Neurospora crassa. 6% or more of the Neurospora genome is light regulated at the level of gene expression, controlled largely by the photoreceptive, LOV domain transcriptional activator White Collar-1 (WC-1) in complex with White Collar-2 (WC-2). This acute response to light results in cascades of transcriptional activators and repressors that eventually control a large number of biological outputs including stress responses, development and general metabolism. Vivid (VVD), an additional LOV photoreceptor, controls early repression of initial light responses, allowing eventual responsiveness to increases in light intensity, a process called photoadaptation. VVD facilitates precise entrainment of circadian rhythms. We have recently shown a complex response to light in the clinically important fungus Aspergillus fumigatus. This opportunistic pathogen responds to red and blue light, with melanin pigmentation, conidial germination and stress responses under control of multiple photoreceptors including the WC-1 homolog LreA and a phytochrome, FphA playing unique and overlapping roles. Both the light responsive physiology of this organism and its photobiology are complex and involves input from additional, as yet uncharacterized, photosensory pathways.

Workshop Sessions

MEM-WK315.02 - Regulators of chromatin structure governing global responses to changes in light and time

Bin Wang[1], Jennifer Hurley[1], Jennifer Loros[1], Jay Dunlap[1]
[1]*Geisel School of Medicine at Dartmouth, Hanover, USA*

Most fungi are highly responsive to their immediate environment, having developed sophisticated photoreceptors as well as circadian clocks to anticipate repeating environmental changes. In Neurospora, blue light is detected by FAD stably bound by the transcription factor WC-1, eliciting photochemistry that drives a conformational change in the complex of WC-1 and WC-2 (WCC) resulting in activation of gene expression from promoters bound by the WCC. The circadian system allows anticipation of recurring environmental changes, and comprises a negative feedback loop wherein this same WCC, in the dark, drives expression of frq. FRQ makes a complex with a putative helicase FRH and also with casein kinase 1, and after phosphorylation-mediated delays, this complex downregulates the WCC (Baker, Loros, & Dunlap, FEMS Microbiol. Rev.36: 95-106, 2012). Using the tools of next generation sequencing, recombineering, and luciferase reporters, the molecular details of the clock itself as well as network ramifying from the clock out to targets of light and clock control can now be described. Light and clock-regulated transcription factors (TFs) transduce regulation from light responses, or from the core circadian oscillator, to banks of output clock-controlled genes (ccgs), some of which are in turn other TFs. Structure/function analysis of WC-1 identified a region of 100 amino acids essential for frq circadian expression. A proteomics-based search for coactivators using this region uncovered the SWI/SNF complex: SWI/SNF interacts with WCC in vivo and in vitro, binds to the cis-regulatory Clock box in the frq promoter, and is required both for circadian remodeling of nucleosomes at frq and for rhythmic frq expression: WC-1 recruits SWI/SNF to remodel and loop chromatin at frq thereby activating frq expression to initiate the circadian cycle. Considered in the context of the genome, these data allow us to compare and contrast light-regulation of gene expression and clock-regulation of gene expression.

Workshop Sessions

MEM-WK315.03 - Inter-kingdom cross-talk and its bio-control applications

Elham Esmaeilishirazifard[1], Sterghios Moschos[1], Tajalli Keshavarz[1]
[1]*University of Westminster, London, UK*

Quorum sensing (QS) molecules are involved in the regulation of complicated processes helping bacterial population benefit from their cell-density. The co-evolution of prokaryotes and eukaryotes raises the prospect of the existence of inter-kingdom signalling pathways, promoting parasitic/symbiotic relationships. While the members of each kingdom possess hormone-like molecules for cell–cell communication, the members of any given kingdom also respond to the signals produced by another. So, QS plays a major role in this cross-talk. Bacterial antagonistic activity against fungi is considered as an inter-kingdom communication. Interestingly, several bacteria like Bacillus, Lactobacillus and Pseudomonas have shown in laboratory experiments the ability to inhibit fungal growth and production of aflatoxins by Aspergillus. During the screening of antagonistic bacteria against Aspergillus flavus (causes pre-/post-harvest diseases in seed-crops) in vitro, Bacillus subtilis was identified having high antifungal activity. Bacillus licheniformis has industrial application due to its production of antimicrobial compounds. B. licheniformis is related to B. subtilis genetically, whose control of competence-sporulation is regulated by a QS mechanism (comQXPA operon). QS process in B. subtilis are regulated by a specific molecule, ComX pheromone. Pheromone encoding genes have been identified in B. licheniformis NCIMB-8874. To further investigate cell-cell communication, we designed a primer pair to amplify the QSM encoding genes. The comQX locus was sub-cloned into a shuttle vector under the control of an inducible promoter. The shuttle vector was expressed in E. coli and pheromone was isolated by reverse phase chromatography. Pheromone as a QSM is potential signal for communicating between kingdoms and could be applied for biocontrol purposes. Identification of new antifungal peptides against A. flavus could lead to the development of biotechnological strategies which facilitate control of aflatoxin contamination and genetic engineering of plant resistance to fungi through the exploitation of genes related to the bacterial antifungal peptide molecules.

Workshop Sessions

MEM-WK315.04 - The pheromone response pathway of C. albicans; exactly the same except in every detail

Hanah Regan[1], Hui Lu[1], Yoalin Chen[1], Pierre Cote[1], Yuan Sun[1], Malcolm Whiteway[1]
[1]*Concordia University, Montreal, Canada*

Candida albicans has been recognized to have a sexual cycle since evidence for a mating type locus was uncovered from genome sequencing studies. Extensive investigation from a number of labs has established that the fundamental elements that are involved in the signaling pathway implicated in mating are similar to those of the well-studied mating pathway for the model yeast Saccharomyces cerevisiae. However, within this general framework, the details of the mating regulation between these two ascomycetes are surprisingly distinct. We have been studying the function of the C. albicans orthologs of the yeast G protein gamma subunit Ste18, the scaffold proteins Ste5 and Far1, and the transcription factor regulator Dig1. Our results emphasize that between the two ascomycetes the circuits are exactly the same, except in every detail.

Workshop Sessions

MEM-WK315.05 - WITHDRAWN - From minus to males: how sexes and multicellularity co-evolved in Volvocine Algae

James Umen[1], Takashi Hamaji[1], Ayano Miyagi[1], Sa Geng[1]
[1]*Donald Danforth Plant Science Center, St. Louis, USA*

Male and female sexes have evolved repeatedly in eukaryotes but the origins of dimorphic sexes and their relationship to mating types in unicellular species are not understood. Volvocine algae include isogamous species such as Chlamydomonas reinhardtii with two equal-sized mating types (minus and plus), and oogamous multicellular species such as Volvox carteri with sperm-producing males and egg-producing females. Theoretical work predicts the recruitment of gamete size regulatory genes into an ancestral mating-type locus as a possible step in the evolution of dimorphic gametes, but this idea has not been tested. Contrary to this prediction, we found that a single conserved mating locus (MT) gene in Volvocine algae--MID, which encodes a RWP-RK domain transcription factor--evolved from its ancestral role in C. reinhardtii as a mating type specifier, to become a determinant of sperm and egg development in V. carteri. Transgenic female V. carteri with ectopically expressed male MID produced functional sperm packets during sexual development. Transgenic male V. carteri with RNAi-mediated knockdowns of VcMID produced functional eggs, or self-fertile hermaphrodites with both eggs and sperm. Post-transcriptional controls were found to regulate cell-type-limited expression and nuclear localization of VcMid protein that restricted its activity to nuclei of developing male germ cells and sperm. Crosses with sex-reversed strains uncoupled sex determination from sex chromosome identity and uncovered sexually antagonistic interactions between genes in MT and the sexual development pathway. Our data for the first time reveal genetic continuity between mating types and sexes, and document the emergence of sexual antagonism between a pair of nascent haploid sex chromosomes. These findings will enable a deeper understanding of how a master regulator of mating-type determination in an ancestral unicellular species was reprogrammed to control sexually dimorphic gamete development in a multicellular descendant.

Workshop Sessions

MEM-WK315.05 - Signs of elusive sex and clonal reproduction in the genomes of relevant microbes

Nicolas Corradi[1], Adrian Pelin[1], Mohammed Selman[1]
[1]*University of Ottawa, Ottawa, Canada*

Sexual reproduction is a process that is vital for the production of new genetic diversity in most eukaryotes, and this process typically results from the fusion of distinct gametes (or nuclei in parasexual organisms) that are produced by either different donors or the same donor as in the case of selfing. In unicellular eukaryotes, the absence of sexual reproduction (i.e. clonality) has usually been assumed based on the lack of a recognizable sexual stage or apparatus, or of well-defined and clearly distinguishable meiotic processes. For this reason, many unicellular organisms, and particularly eukaryotic pathogens, have long been assumed to propagate exclusively through clonal reproduction simply based on lack of evidence of sexual reproduction. Today, however, these assumptions are being challenged, as many genes and genetic signatures that are generally linked to sexual processes are now commonly found in the genomes of many unicellular eukaryotes that had previously been thought to be clonal; suggesting they may be able to undergo "cryptic" sex. This presentation aims to highlight the importance of "Next Generation Sequencing" and comparative genomics tools to unravel the potential for cryptic sexual processes in unicellular eukaryotes. Specific examples will be given using data recently acquired on unicellular lineages that are often assumed to be clonal; namely the Arbuscular Mycorrhizal Fungi and the Microsporidia.

Workshop Sessions

MEM-WK316.01 - MALDI-TOF MS for the identification and characterization of yeasts and multicellular fungi

Markus Kostrzewa[1]
[1]*Bruker Daltonik GmbH, Bremen, Germany*

Matrix-assisted laser-desorption/ionization time –of-flight mass spectrometry (MALDI-TOF MS) has revolutionized culture based identification of yeasts and multicellular fungi during the recent years. After minimal or low-effort sample preparation, a specific mass fingerprint of the organism is recorded in a MALFI-TOF mass spectrometer and compared to the reference spectra stored in an extensive database. Based on the similarity with the best matching organism in the database, identification together with a quality assessment of this is being reported. Databases containing references for hundreds of yeasts and fungi have been established making the method broadly applicable. Besides the databases supplied by manufacturers, also libraries can be established by the users of the system which are dedicated to specific needs or application areas. Good to excellent performance has been reported for the method together with low consumable costs and a significantly reduced time to result. The talk will give an overview about the state-of –the-art, variations in sample preparation and databases, and performance in different areas. Further, it will give an outlook about the role of the technology in other areas like typing and resistance detection.

Workshop Sessions

MEM-WK316.02 - Detection and identification of opportunistic Exophiala species using Rolling Circle Amplification of ribosomal Internal Transcribed Spacers

Peiying Feng[1,3], M.J. Najafzadeh[2], G.S. de Hoog[3]

[1]3rd Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [2]Mashhad University of Medical Sciences, Mashhad, Iran, [3]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands

Deep infections by melanized fungi deserve special attention because of a potentially fatal, cerebral or disseminated course of disease in otherwise healthy patients. Timely diagnostics are a major problem with these infections. Rolling Circle Amplification (RCA) is a sensitive, specific and reproducible isothermal DNA amplification technique for rapid molecular identification of microorganisms. RCA-based diagnostics are characterized by good reproducibility, with few amplification errors compared to PCR. The method is applied here to species of Exophiala known to cause systemic infections in humans. The ITS rDNA region of four Exophiala strains (E. dermatitidis, E. oligosperma, E. spinifera, and E. jeanselmei) was sequenced and aligned in view of designing specific padlock probes to be used for the detection of single nucleotide polymorphisms (SNPs) of the Exophiala species concerned. The assay proved to successfully amplify DNA of the target fungi at the level of species; while no cross reactivity was observed. Amplification products were visualized on 1% agarose gels to verify the specificity of probe-template binding. Amounts of reagents were minimized to avoid the generation of false positive results. The sensitivity of RCA may help to improve early diagnostics of these difficult to diagnose infections.

Workshop Sessions

MEM-WK316.03 - Determination of yeast population diversity in Citrofortunella microcarpa and Citrus reticulata from the Philippines using Vitek™ MS-MALDITOF

Noel Tan Gana[1], Rosario Monsalud[1]
[1]Philippine National Collection of Microorganisms, University of the Philippines Los Banos, Philippines

Yeasts diversity in Philippine fruits, particularly in citrus, remains largely unexplored. The emergence of new technologies in the identification of microorganisms such as MS-MALDITOF (Mass Spectrometry Matrix-Assisted Laser Desorption Ionization Time of Flight) offers an opportunity to quickly identify a large number of microorganisms. This technology can be employed to rapidly explore overlooked microbial habitats such as Philippine fruits i.e. Citrofortunella microcarpa and Citrus reticulata which are common citrus fruits used in foods and drinks for both commercial and domestic preparations. Yeasts were isolated from Citrofortunella microcarpa and Citrus reticulata samples by spread plating fruit washings onto yeast malt extract agar supplemented with chloramphenicol and biphenyl. Fifty randomly picked yeast colonies from each fruit sample were identified by Vitek™ MS-MALDITOF. Yeast species common to both fruits were Candida duliniensis, C. famata, C. globosa, Cryptococcus neoformans, Pichia guiliermondii and Stenoascus ciferii. fruits. P. guiliermondii was the dominant yeast species in both fruits. Yeasts found only in Citrofortunella microcarpa were Candida albicans, Hypopichia burtonii and Kodamaea ohmerii while Candida aaseri, C. boidini , C. glabrata, C. orthosilopsis, C. parasilopsis, C. tropicalis, Cryptococcus uniguttulatus, Debaryomyces polymorphus, Kluyveromyces marxianus, Loderomyces elongisporus, Mechtsnikowia pullerchima, Saccharomyces kluyverii, Trichosporon mucoides and Zygosaccharomyces bailii were only isolated from the Citrus reticulata sample. C. microcarpa and C. reticulata had 3 and 21 yeasts respectively, which remained unidentified. These unidentified isolates represent possible novel yeasts which could be targeted for further detailed taxonomic analysis. This study represents the first attempt in the Philippines to investigate the ecological diversity of yeasts found in Philippine citrus fruits. The results from the techniques employed in this study also has a possibly great potential application in the control charting of indigenous organisms in raw products used to manufacture food and feeds.

Workshop Sessions

MEM-WK316.04 - Loop-mediated isothermal amplification (LAMP) of fungal DNA as a diagnostic tool in food safety and food quality

Ludwig Niessen[1], Carla Denschlag[1], Jie Luo[1], Rudi F. Vogel[1]
[1]Technische Universität München (TUM), Freising, Germany

Loop-mediated isothermal amplification (LAMP) is a technology for enzymatic amplification of target DNA at a constant temperature of 65 °C. The use of four primers hybridizing to six different binding sites within the target DNA as well as the application of a highly processive thermophilic DNA polymerase (Bst) make the process specific, rapid, and easy to operate both under lab and on-site conditions. Based on the LAMP-system we have developed several assays ranging from species specific diagnosis of *Fusarium graminearum* or specific diagnosis of typical aflatoxin producing species (*Aspergillus flavus, A. parasiticus, A. nomius*) over assays detecting and quantifying the presence of food quality related genes in a group of fungal species, e.g. gushing inducing *Fusarium* spp. and trichothecene producing *Fusarium* spp. to application of the technology in the detection of gene expression. Different approaches to LAMP signal detection and quantification were used and will be elucidated along with the different assays. Data obtained from the analysis of sample materials with the LAMP assays will be discussed during the presentation.

Workshop Sessions

MEM-WK316.05 - *Aspergillus* collagen-like genes, *acl*, as targets for PCR-based species-specific pathogen identification

Kiril Tutevski[1], Brandon C Durney[2], Anna K Snyder[3], P Rocco LaSala[4], Rita V M Rio[5], Lisa A Holland[6], Slawomir Lukomski[7]

[1]West Virginia University, Morgantown, USA

Aspergillus species are environmentally ubiquitous fungi that are associated with a variety of health care problems, including allergies, asthma, and invasive aspergillosis in immunosuppressed patients. To date, species-specific molecular diagnostic assays are commercially not available in in the United States and detection relies on time-consuming morphological characterization or non-standardized serological assays that are restricted to identifying a fungal etiology. In Europe, commercial qPCR assays were developed that are based on 18S-5.8S internal transcribed space region of rRNA genes. Our objective was to test the *Aspergillus* collagen-like genes, *cal*, as potential biomarkers for the specific detection of main pathogenic species, including *Aspergillus fumigates*, *A. flavus*, *A. nidulans*, *A. niger* and *A. terries*. Bioinformatic analysis and sequencing were used to assess genetic polymorphism and phylogeny among *cal* genes. Gene-specific PCR primers targeting various *cal* genes were designed and tested towards the identification of these five species in laboratory collection and in clinical cultures. Amplicon separations were compared by standard slab-gel and nanogel capillary electrophoresis. In addition, TaqMan probes targeting the *aclF1* gene were developed and successfully tested for the identification of *A. fumigates* organism. In conclusion, we demonstrated that *Aspergillus cal* genes could be used as PCR targets to discriminate between clinically relevant *Aspergillus* species. Detection of the specific *cal*-gene products can be achieved by microfluidic applications or by qPCR to determine the sensitivity and specificity of the identification of *Aspergillus* colonization and invasive aspergillosis in immunocompromised subjects.

Workshop Sessions

MEM-WK316.06 - Occurrence of nine Fusarium species and sixteen mycotoxins in Nigerian guinea corn (Sorghum bicolor) (L.) Moench

Timothy Adejumo[1], Raana Dastjerdi[2], Katharina Doell[2], Petr Karlovsky[2]
[1]Dept. of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Nigeria, [2]Phytopathology and Mycotoxin Research, Dept. of Crop Science, Georg-August University, Göttigen, Germany

Fifty seven (57) sorghum samples meant for human consumption from four states in Nigeria were tested for nine Fusarium species by species-specific polymerase chain reaction. Fusarium verticillioides was the most commonly detected fungi (61%), followed by F. proliferatum (58%), F. graminearum (9%), F. culmorum (4%) and F. avenaceum (2%), while F. equiseti, F. poae, F. subglutinans and F. tricinctum were absent in the samples. Quantification of sixteen mycotoxins was carried out with high performance liquid chromatography, coupled with mass spectroscopy (HPLC/MS); the limit of quantification varied between 8 and 68 ng g-1. The samples were contaminated with fumonisin B1 (mean: 191.0 ng g-1; range: 10.2-1100.3 ng g-1), diacetoxyscirpenol (mean: 132.1 ng g-1; range: 131.1–133.1 ng g-1), beavericin (mean: 48.7 ng g-1; range: 14.3–85.6 ng g-1), zearalenone (mean: 20.8 ng g-1; range: 10.1–31.6 ng g-1), detected in 39%, 3.5% and 17.5% and 3.5% of total samples respectively. There were no deoxynivalenol, 3, monoacetyldeoxynivalenol, 15, mono-acetyldeoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin, neosolaniol, fusarenone-X, enniatins A, A1 and B and apicidin detected in the samples. This is the first comprehensive tests for several mycotoxins and Fusarium species in sorghum for human consumption in Nigeria.

Workshop Sessions

VIR-WK233.01 - Emergence of a gut-adapted variant of coxsackievirus B3 in mice

Yao Wang[1], Julie Pfeiffer[1]
[1]*UT Southwestern Medical Center, Dallas, USA*

Coxsackievirus B3 (CVB3) is an enterovirus in the *Picornaviridae* family. During experiments to examine the effect of gut microbiota on CVB3 replication in orally inoculated mice, we noticed a major phenotypic change in shed viruses. While inoculum viruses generated small plaques in HeLa cells, fecal viruses generated both small and large plaques. The large plaques were over 500-fold larger than inoculum plaques and represented 11% of viruses shed by orally inoculated mice. Emergence of the large plaque variant appears to be a gut-specific event, since viruses isolated from muscle of intramuscularly injected mice generated very few large plaques. We determined that sulfated glycosaminoglycans (GAGs) present in agar inhibited plaque formation of the inoculum virus but not the large plaque fecal isolate. We identified a single amino acid change in the VP3 capsid protein, N63Y, which was present in large plaque viruses. Using reverse genetics, we determined that N63Y was sufficient for the large plaque phenotype. While WT CVB3 binds the GAG heparan sulfate (HS), N63Y CVB3 has reduced binding to HS. Experiments in cell culture revealed that N63Y CVB3 has reduced replication due to reduced cell attachment. Several different viruses undergo cell culture adaptation that selects for GAG binding, which can enhance viral growth in cultured cells but reduce replication and pathogenesis in mice. Indeed, we found that N63Y CVB3 has higher replication and virulence in orally inoculated mice than WT CVB3. Interestingly, N63Y and WT CVB3 have equivalent pathogenesis in intramuscularly inoculated mice, suggesting that N63Y CVB3 is a gut-adapted variant. We are now examining factors that may promote N63Y CVBV3 replication in the intestine and we are exploring N63Y as a more efficient model for oral CVB3 infection of mice

Workshop Sessions

VIR-WK233.02 - WITHDRAWN - Lymphocyte-monocyte heterokaryons induced by the HIV-1 envelope: quantitative analysis and functional studies

Leonor Huerta[1], David Martínez-Méndez[1], Evelyn Rivera-Toledo[2], Ileana Licona[1], Enrique Ortega-Soto[1]
[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico, [2]Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico

The presence of circulating HIV strains able to induce cell-cell fusion correlates with accelerated CD4+ T cell depletion and progression to AIDS. Likewise, multinucleated cells expressing HIV-antigens are frequently found in the lymphoid tissues and central nervous system of HIV infected individuals. T lymphocytes and cells from the monocyte/macrophage lineage interact closely during the immune response. We investigated whether fusion occurs in cocultures of T-lymphoid Jurkat cells expressing the HIV-1 Env proteins from the HXBc2 HIV-1 strain, and monocytic THP-1 CD4+/CXCR4+ cells. Analysis of fusion was performed by the differential labeling of fusion partners with DiO (green) and DiI (red) fluorescent dyes, followed by quantification of double fluorescent cells (fused cells) by flow cytometry. Up to 20% of double fluorescent cells were detected after 48 hours of coculture. Heterokaryon formation was inhibited by anti-CD4 antibodies and the specific HIV-1 fusion inhibitor T-20, demonstrating the participation of Env and CD4 molecules in the fusion process. A 3-day follow up showed that the expression of the lymphoid markers CD3 and CD28 was greatly and consistently reduced in heterokaryons, whereas CD32 and CD68, both markers of monocytic cells, remained unchanged. CD4 expression was barely detected in heterokaryons. Fifty percent of heterokaryons were phagocytic, as determined by a three color flow cytometry Fc receptor-dependent assay for phagocytosis of erythrocytes. Finally, fluorescence microscopy analysis showed that heterokaryons had a clear tendency to adhere to the bottom of plastic wells, a morphological change resembling monocyte differentiation to macrophages. These observations point to the possible generation of lymphocyte–monocyte heterokaryons with a myeloid phenotype during HIV infection. Outcomes of this event may include anomalous monocyte differentiation and function, and generation of a T-tropic-virus reservoir in non-cycling cells. (Research supported by grants PAPIIT-UNAM IA200414 and CONACYT-México 152946).

Workshop Sessions

VIR-WK233.02 - Transient potential channels as potential therapeutic targets for virus induced asthma exacerbations: Studies in a human neuronal cell model

Haniah Abdullah[1], Hanagh Winter[1], John Corry[1], Liam Heaney[1], Lorcan McGarvey[1], Louise Cosby[1]
[1]Queen's University Belfast, Belfast, UK

Airway nerves have an important role in controlling crucial reflexes such as cough and bronchoconstriction. In asthma and other respiratory conditions these reflexes become hyperactive, typically triggered by exposure to environmental irritants (i.e. cigarette smoke, acidic air pollutants and cold air) and are exacerbated during viral infections. The precise mechanism of how respiratory virus infection induces hypersensitisation of the airway nerve causing bouts of coughing and wheezing has not yet been established. One possibility is the virus may sensitize or up-regulate the largest group of ion channel receptors which are capable of detecting noxious stimuli including thermal, chemical and mechanical stimuli. The transient receptor potential (TRP) channel family are present on both neuronal and non-neuronal airway cells. TRP channel subfamily A member 1 (TRPA1) has been implicated in the pathophysiology of acute and chronic cough and has been reported to be activated by temperatures below 15°C, noxious chemicals such as acrolein (presents in cigarette smoke), and compounds in common herbs such as allicin (garlic), cinnamaldehyde (cinnamon) and gingerol (ginger). Our previous study demonstrated that soluble factors induced by human rhinovirus (HRV) infection up-regulate TRPA1 in a neuronal cell model which consists of functional TRPA1 channels. Factors such as IL-8, IL-6, and NGF were implicated and may play a significant role (Abdullah et al., Thorax 69:46-54, 2013). Based on this finding we further explored the potential inflammatory factors which are responsible for up-regulating TRPA1 at the protein and mRNA levels using neutralising antibodies and inhibitors. Our preliminary results suggest that treatment of virus induced supernatants with neutralizing IL-8 or NGF antibodies inhibits the up-regulation of TRPA1 by HRV infection at the protein level. Understanding the mechanism by which virus induced hyper- expression/hyper-sensitization of the TRP channels occurs may indicate novel potential therapeutic targets for treatment of asthma and other respiratory conditions.

Workshop Sessions

VIR-WK233.03 - Neuron-specific NF-κB-dependent genes mediate reovirus neuropathogenesis

Andrea Pruijssers[1], Terence Dermody[1]
[1]*Vanderbilt University School of Medicine, Nashville, USA*

Pathological effects of apoptosis associated with viral infections of the central nervous system are an important cause of morbidity and mortality. Reovirus is a neurotropic virus that causes apoptosis in neurons, leading to lethal encephalitis in newborn mice. Reovirus-induced encephalitis is diminished in mice with germline ablation of NF-κB subunit p50. It is not known whether the pro-apoptotic function of NF-κB is mediated by neuron-intrinsic processes, NF-κB-regulated cytokine production by inflammatory cells, or a combination of both. To determine the contribution of cell type-specific NF-κB signaling in reovirus-induced neuronal injury, we generated mice that lack NF-κB p65 expression in neurons using the Cre/loxP recombination system. Following intracranial inoculation of reovirus, 50 percent of wild-type mice succumbed to infection, whereas more than 90 percent of neuron-specific NF-κB-deficient mice survived. Viral titers in brains of wild-type mice were significantly higher than those in brains of neuron-specific NF-κB-deficient mice. Histological analysis revealed that areas in the wild-type brain positive for reovirus antigen displayed enhanced cleaved caspase-3 immunoreactivity, while reovirus antigen-positive areas of the brain of neuron-specific NF-κB-deficient mice displayed little cleaved caspase-3 immunoreactivity. These data suggest that neuron-intrinsic NF-κB-dependent factors are essential mediators of reovirus neuronal replication and neurovirulence. To identify neuron-intrinsic factors under control of NF-κB p65 that mediate these effects, wild-type and neuron-specific NF-κB-deficient mice were inoculated intracranially, and at days 2 and 6 post-inoculation, RNA was extracted from cortices and gene expression quantified by RNA sequencing. Our results suggest that ablation of NF-κB p65 expression in neurons leads to significantly reduced upregulation of genes involved in cell death, innate immunity, and inflammation following reovirus infection. A better understanding of the contribution of cell type-specific NF-κB-dependent signaling to viral neuropathogenesis could inform the development of new therapeutics that target and protect highly vulnerable cell populations.

Workshop Sessions

VIR-WK233.04 - Sub-neutralizing concentrations of Chikungunya virus-specific antibodies exacerbates disease severity

Fok-Moon Lum[1,2], Thérèse Couderc[3], Marc Lecuit[3], Lisa FP Ng[1,2]
*[1]Singapore Immunology Network, A*STAR, Singapore, [2]Department of Biochemistry, National University of Singapore, Singapore, [3]Institut Pasteur, Paris, France*

Chikungunya virus (CHIKV) garnered the attention of many due to the unexpected epidemics in 2005-2008. The unprecedented outbreaks occurred in several Indian Ocean Islands, India, Southern Europe, and many parts of South East Asia, including Singapore. CHIKV is responsible for Chikungunya viral fever (CHIKF) that is often defined by the characteristic arthralgia. Persistent arthralgia has also been reported in infected individuals several months after disease onset. Moreover, severe CHIKV-associated pathologies including acute nephritis, cardiac myocarditis, pericarditis, ophthalmological retrobulbar neuritis and neurological complications have also been documented. Current understanding on the immuno-pathogenesis of CHIKV still remains insufficient. Thus, it is crucial to further study the immuno-pathology and pathophysiology of in vivo CHIKV infections. Using a mouse model of CHIKV infection, inoculation of CHIKV into the joint footpad of C57BL/6 adult mice recapitulated the viremic phase and joint inflammation observed in humans. In this study, we studied the disease progression of CHIKV infection in this adult mouse model performed in the presence of sub-neutralizing concentrations of CHIKV-specific antibodies. Enthrallingly, these mice not only suffered from higher viremia but also endured a greater degree of footpad swelling throughout the course of disease. To investigate if this phenomenon is due to the enhancement of CHIKV infection by the CHIKV-specific antibodies, in vitro CHIKV infections were performed in both primary cells and cell lines. Results showed that the presence of sub-neutralizing amounts of CHIKV-specific antibodies enhanced both infection and viral replication. In order to decipher the molecular mechanisms of this enhancement, specific immune subsets upon CHIKV infection were analysed and revealed the role of Fc receptors as crucial mediators. Efforts are currently ongoing to elucidate how sub-neutralizing CHIKV-specific antibodies augment the infection pathway and enhance disease severity. Understanding this anomaly will provide further insights into CHIKV pathogenesis and lead to better treatment and patient management.

Workshop Sessions

VIR-WK233.05 - Modulation of Necroptosis by the Human Neuroinvasive Coronavirus OC43

Mathieu Meessen-Pinard[1], Marc Desforges[1], Pierre Talbot[1]
[1]INRS-Institut Armand-Frappier, Laval, Canada

Human Coronaviruses (HCoV) are respiratory pathogens that infect upper and lower respiratory tracts where they cause diseases such as the common cold, pneumonia, exacerbation of asthma or Respiratory Syndromes (SARS, MERS). We have previously demonstrated that the OC43 strain of HCoV (HCoV-OC43) can invade the central nervous system (CNS), where the neuron is its main target cell. Moreover, using a murine model and cell culture, we showed that HCoV-OC43 induces neurodegeneration, suggesting its possible involvement in human neurodegenerative diseases. We recently reported that coronavirus-induced neurodegeneration was linked to activation of programmed cell death (PCD), an important hallmark of neurodegenerative diseases. However, the underlying mechanism is still poorly understood. Necroptosis, a type of neuronal PCD involves two Receptor-Interacting Protein kinases, RIP1 and RIP3, which play a role in regulation of cell survival. Using a human neuronal cell line (LA-N-5) and murine primary cultures from the CNS in the presence of chemical inhibitors of the RIP1 factor or by using RNA interference on RIP1 expression, we found that infected cells were protected from death, indicating that RIP1-dependent activation of necroptosis could play a role in neuronal cell death. Moreover, we observed that after HCoV-OC43 infection of LA-N-5, expression of the rip1 gene was upregulated. However, the corresponding protein appeared partially degraded. Furthermore, the HCoV-OC43 S protein is an important factor in the induction of neuronal PCD, as illustrated by a modulation of cell-death by different viral S mutants compared to wild-type virus. Taken together, these results suggest that necroptosis is activated following HCoV-OC43 infection and that the virus induces degradation of RIP1 protein to counteract this activation. (Supported by operating grant MT-9203 from CIHR (III) to Pierre J. Talbot. Mathieu Meessen-Pinard gratefully acknowledges a doctoral studentship from Fond de Recherche du Québec-Santé, FRQS).

Workshop Sessions

VIR-WK234.01 - TALEN knockout of the PSIP1 (LEDGF/p75) gene in human cells: analyses of HIV-1 replication and Allosteric Integrase Inhibitor Mechanism

Hind Fadel[1], Eric Poeschla[1]
[1]Mayo Clinic College of Medicine, Rochester, USA

HIV-1 utilizes the cellular protein LEDGF/p75 as an integration cofactor. Once inside the nucleus, the pre-integration complex is thought to dock to the chromosome when integrase binds to LEDGF/p75. This tethering process is largely responsible for the two-fold preference for integration into active genes, but the cofactor's full role in the lentiviral life cycle is not yet clear. The LEDGF/p75 gene, PSIP1, is a potential therapeutic target because, like CCR5, depletion of LEDGF/p75 is tolerated well by human CD4+ T cells, and knockout mice have normal immune systems. RNAi has been useful for studying LEDGF/p75, but the potent cofactor activity of small protein residua can be confounding. Here, in human cells with utility for HIV research (293T, Jurkat), we used transcription activator-like effector nucleases (TALENs) to completely eradicate all LEDGF/p75 expression. We performed two kinds of PSIP1 knockouts: whole gene deletion and deletion of the integrase binding domain (IBD)-encoding exons. HIV-1 integration was inhibited and spreading viral replication was severely impaired in PSIP1-/- Jurkat cells infected at high multiplicity. Furthermore, frameshifting the gene in the first coding exon with a single TALEN pair yielded trace LEDGF/p75 levels that were virologically active, affirming the cofactor's potency and the value of definitive gene or IBD exon segment deletion. Some recent studies have suggested LEDGF/p75 may participate in HIV-1 assembly. However, we determined that assembly of infectious viral particles is normal in PSIP1-/- cells. The potency of an allosteric integrase inhibitor, ALLINI-2, for rendering produced virions non-infectious was also unaffected by total eradication of cellular LEDGF/p75. We conclude that HIV-1 particle assembly and the main ALLINI mechanism are LEDGF/p75-independent. The block to HIV-1 propagation in PSIP1-/- human CD4+ T cells raises the possibility of gene targeting PSIP1 combinatorially with CCR5 for HIV-1 cure.

Workshop Sessions

VIR-WK234.02 - Host restriction factor SAMHD1 limits human T Cell Leukemia Virus Type 1 infection of monocytes via STING-Mediated Apoptosis

Alexandre Sze[1], Julien van Grevenynghe[1], Rongtuan Lin[1]
[1]*Lady Davis Institute-McGill University, Montreal, Canada*

Human T cell leukemia virus type 1 (HTLV-1) is the causative agent of adult T cell leukemia and HTLV-1-associated myelopathies. In addition to T cells, HTLV-1 infects cells of the myeloid lineage, which play critical roles in the host innate response to viral infection. Investigating the monocyte depletion observed during HTLV-1 infection, we discovered that primary human monocytes infected with HTLV-1 undergo abortive infection accompanied by apoptosis dependent on SAMHD1, a host restriction factor that hydrolyzes endogenous dNTPs to below the levels required for productive reverse transcription. Reverse transcription intermediates (RTI) produced in the presence of SAMHD1 induced IRF3-mediated antiviral and apoptotic responses. Viral RTIs complexed with the DNA sensor STING to trigger formation of an IRF3-Bax complex leading to apoptosis. This study provides a mechanistic explanation for abortive HTLV-1 infection of monocytes and reports a link between SAMHD1 restriction, HTLV-1 RTI sensing by STING, and initiation of IRF3-Bax driven apoptosis.

Workshop Sessions

VIR-WK234.03 - Novel transmembrane protein inhibitors of the HIV coreceptor CCR5

Daniel DiMaio[1], Sara Marlatt[1], Lisa Petti[1], Elizabeth Scheideman[1]
[1]*Yale School of Medicine, New Haven, USA*

The chemokine receptor CCR5 is a multi-pass transmembrane protein that serves as an essential co-receptor for most strains of HIV. We previously isolated artificial, 43- or 44-amino acid transmembrane protein aptamers (traptamers) that affect CCR5 expression and activity. Expression of these traptamers specifically blocks cell-surface expression of CCR5 and inhibits infection by CCR5-dependent pseudotyped HIV reporter viruses by 90% or more in human T-cells. Most of these traptamers are highly specific for CCR5 and do not affect cell-surface expression of CD4, CXCR4 (another HIV coreceptor), or the more closely related CCR2. Genetic and biochemical evidence suggests that the traptamers interact with the transmembrane domains of CCR5 and reduce cell-surface levels of CCR5 post-transcriptionally. Most of the traptamers require a specific lysine in CCR5 to cause down-regulation and appear to target CCR5 to the lysosome for degradation. However, one traptamer does not require this lysine nor induce lysosomal degradation, suggesting that different traptamers can use alternative mechanisms to mediate CCR5 down-regulation. Traptamers can be used as probes to investigate how the transmembrane domains of CCR5 specify its structure, function and metabolism, and study of these novel inhibitory proteins may suggest new anti-viral approaches.

Workshop Sessions

VIR-WK234.04 - Selection bias in the heterosexual transmission of HIV-1

Jonathan Carlson[1], Martin Deymier[2], Malinda Schaefer[2], Daniela Monaco[2], Daniel Claiborne[2], Jessica Prince[2], Zachary Ende[2], William Kilembe[3], Susan Allen[2,3], Eric Hunter[2]

[1]Microsoft Research, Los Angeles, USA, [2]Emory University, Atlanta, USA, [3]Zambia Emory HIV Research Project, Lusaka, Zambia

Heterosexual transmission of HIV-1 typically results in one genetic variant from the large virus quasispecies in the transmitting partner establishing systemic infection. Our previous studies showed that viruses initiating infection were not the most abundant in the genital tract and that they encoded more compact, less glycosylated envelope glycoproteins – indicative of selection for more transmissible variants. An analysis of more than 150 full-length HIV-1 genomic sequences for 6 transmission pairs has recently shown that the single transmitted variant in each case was closer, evolutionarily, to ancestral virus than the bulk of non-transmitted viruses, again consistent with selection. To determine the basis for the severe genetic bottleneck observed, we compared, for 137 linked transmission pairs, the amino acid sequences encoded by structural (gag), replicative (pol), and accessory (nef) genes of viruses in both partners. We demonstrate a highly significant selection bias for transmission of amino acids that are consensus in the cohort and that are predicted to confer increased in vivo fitness in the newly infected, immunologically naive recipient. A higher number of preferred amino acids predicts higher odds of transmission for individual viruses within a donor's quasispecies, while donor viral populations characterized by a predominance of preferred amino acids are more likely to transmit to their partners. Known transmission risk factors (e.g., donor viral load and recipient sex) reduce the selection advantage of fitter viruses, allowing for transmission of less fit variants and lower viral loads. Thus, individuals with a low susceptibility to transmission will typically experience a stronger genetic bottleneck, resulting in high fitness breakthrough viruses and poorer prognosis upon successful transmission. Conversely, preventative or therapeutic approaches that even marginally weaken the virus may reduce overall transmission rates via a mechanism that is independent from the quantity of circulating virus and may provide long-term benefits even upon successful transmission.

Workshop Sessions

VIR-WK234.05 - Promyelocytic leukemia (PML) protein as an intrinsic immunity factor against lentiviruses

Nasser Masroori[1], Lionel Berthoux[1]
[1]*UQTR, Trois-Rivières, Canada*

The tripartite motif (TRIM)-containing proteins are involved in many cellular activities, including antiviral responses. The potential antiviral activity of TRIM19, also called promyelocytic leukemia (PML), against several DNA and RNA viruses has been well documented. TRIM5α, another member of the TRIM family, is a retroviral restriction factor. Recently it was found that human and Rhesus TRIM5α shuttle into the nucleus and can transiently localize at PML nuclear bodies (NBs). This prompted us to analyze the effect of PML expression on lentiviral infections. Wild-type (WT) or PML-/- mouse embryo fibroblasts (MEF) cells and also MEFs over-expressing PML were infected with HIV-1, SIVmac, EIAV, and B-MLV vectors expressing GFP. The infectivity of the three lentiviral vectors was increased by up to 30-times in the absence of PML. Conversely, PML knockout decreased B-MLV infection by up to 10-fold. Analyzing the levels of GFP expression in cells infected by the SIVmac or HIV-1-based vectors demonstrated up to 8-times higher GFP expression in the absence of PML, suggesting that PML has the capacity to restrict lentiviruses at both early and late infection stages. Overexpression of PML in PML-depleted MEF conferred significant decrease in HIV-1 and SIVmac infectivity. Finally, IFN-mediated inhibition of HIV-1 and SIVmac was more efficient in WT cells compared to PML knockout cells, suggesting a role for PML in the regulation of interferon-mediated restriction of retroviruses. Our data suggest a role for PML in regulating the restriction of lentiviruses at several stages, pre- and post-integration. In addition, IFNs induce a stronger antiretroviral state in the presence of PML. Therefore, our results support a role for PML in the control of HIV-1 and other retroviruses. Future experiments will address the viral determinants for PML-mediated inhibition of HIV-1.

Workshop Sessions

VIR-WK234.06 - Structural analysis of APOBEC3F: insights to HIV-1 Vif and single-strand DNA binding.

Karen Siu[1], Azmiri Sultana[1], Farshad Azimi[1], Jeffrey Lee[1]
[1]University of Toronto, Toronto, Canada

Human APOBEC3 proteins are host-encoded intrinsic restriction factors that inhibit the replication of diverse retroviral pathogens. Restriction is believed to occur as a result of its DNA cytosine deaminase activity; this activity converts cytosines into uracils in single-stranded DNA retroviral replication intermediates. APOBEC3F and APOBEC3G are the most potent factors against HIV-1. As a countermeasure, HIV-1 viral infectivity factor (Vif) targets APOBEC3s for proteasomal degradation. Here, we report the crystal structure of the Vif-binding domain in APOBEC3F. Moreover, we used biolayer interferometry and site-directed mutagenesis to identify residues involved in HIV-1 Vif and single-strand DNA binding. Our results point to an amphipathic surface that is conserved in APOBEC3s to be critical for Vif susceptibility in APOBEC3F. Moreover, structure-guided mutagenesis reveals a straight ssDNA-binding groove distinct from the Vif-binding site, and a novel ‘aromatic switch’ is proposed to explain DNA substrate specificities across the APOBEC3 family. This study opens new lines of inquiry that will further our understanding of APOBEC3-mediated retroviral restriction and provides an accurate template for structure-guided development of inhibitors targeting the APOBEC3-Vif axis. KK Siu, A Sultana, FC Azimi and JE Lee. (2013). Structural determinants of HIV-1 Vif susceptibility and DNA binding in APOBEC3F. Nature Communications. 4 2593.

Workshop Sessions

VIR-WK235.01 - Bornavirus research: exploring new species and old members

Keizo Tomonaga[1]
[1]*Institute for Virus Research Kyoto University, Kyoto, Japan*

Bornavirus research has entered a new era. Borna disease virus (BDV) was identified as the etiological agent of Borna disease, a progressive, nonpurulent encephalomyelitis in horses and sheep, in the early 20th century, and was long considered to be the only species in the genus Bornavirus and the family Bornaviridae. Studies of BDV characterized many properties unique to this RNA virus, but the lack of species diversity in the genus had long remained an enigma. However, after the discovery of viruses that are clearly related to, but distinct from, mammalian BDV in psittacine birds in 2008, our knowledge regarding bornaviruses has been rapidly growing. Since the initial discovery of avian bornaviruses (ABVs), eleven additional ABV genotypes have been detected, and one has been found in a reptile sample. Furthermore, in 2010, endogenous elements highly homologous to current bornaviruses, called endogenous bornavirus-like elements, were discovered in the genomes of many mammalian species, including humans. Each of these epoch-making discoveries in bornavirus research has not only had great impact on the analysis of mammalian BDV, but is relevant to various research areas outside virology, such as genetics and evolution. In addition, bornavirus research has been recently applied to the development of new biological tools and technologies, such as bornavirus vectors. In this presentation, I will provide an overview of recent progress and implications for future directions of bornavirus research.

Workshop Sessions

VIR-WK235.02 - Identification of novel Interferon-stimulated genes that attenuate Ebola virus infection

Lindsay Hill-Batorski[1], Peter Halfmann[1], John Schoggins[2,3], Charles Rice[2], Yoshihiro Kawaoka[1,4,5]
[1]Department of Pathobiological Sciences, University of Wisconsin–Madison, Madison, USA, [2]Virology & Infectious Disease, The Rockefeller University, New York, USA, [3]Department of Microbiology, UT Southwestern Medical Center, Dallas, USA, [4]Department of Microbiology and Immunology, University of Tokyo, Tokyo, Japan, [5]ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama, Japan

Ebola virus (EBOV), a member of the Filoviridae family, is a negative-sense RNA virus, and the causative agent of Ebola hemorrhagic fever. With no approved therapeutics to combat viral infection, and case fatality rates associated with EBOV outbreaks as high as 90%, EBOV is categorized as a biosafety level-4 (BSL-4) select agent. To identify novel host proteins with antiviral properties against EBOV, we developed a cell-based assay utilizing our previously established biologically-contained EBOV (EbolaΔVP30 virus). In the assay, an EbolaΔVP30 virus expressing the luciferase reporter gene was used to screen a library of interferon-stimulated genes (ISGs) to identify ISGs that attenuate EBOV growth. Several novel anti-EBOV ISGs were identified in the screen, along with a few ISGs previously identified to inhibit EBOV growth. The top ten ISGs that attenuated EBOV growth most effectively (as determine by virus-driven luciferase expression) without significantly inhibiting cellular viability were selected for further validation. These top ISGs were examined for their effects on EBOV growth kinetics. All 10 ISGs significantly attenuated EBOV growth, with the most potent ISG inhibiting virus growth by nearly 1000-fold. Additionally, in order to determine the steps within the viral life cycle that may be targeted by these ISGs, we examined the effect of each ISG on EBOV entry, transcription/replication, and budding. Collectively, the screen identified several novel ISGs that restricted EBOV growth, providing new venues of study to potentially combat EBOV infection.

Workshop Sessions

VIR-WK235.03 - Identification of the sensing mechanism of Borna disease virus ribonucleoprotein in the nucleus

Tomoyuki Honda[1], Daisuke Okuzaki[2], Akiko Makino[1], Shoko Nakamura[1], Keizo Tomonaga[1]
[1]Institute for Virus Research, Kyoto University, Kyoto, Japan, [2]Research Institute for Microbial Diseases, Osaka Unversity, Osaka, Japan

Borna disease virus (BDV), a nonsegmented, negative-strand RNA virus, is characterized highly neurotropic and noncytopathic infection. Unique among RNA viruses, BDV replicates in the nucleus and readily establishes a long-lasting persistent infection. This characteristic makes BDV the only animal-derived RNA virus capable of intranuclear parasitism. Therefore, the study of BDV allows us to detect previously unknown interactions between RNA virus and host cells. Recently, we demonstrated that BDV ribonucleoprotein (RNP) interacts directly with high mobility group box protein 1 (HMGB1), a host chromatin-binding protein, and that this interaction may play crucial roles for BDV replication and persistent infection. On the other hand, HMGB1 has also been shown to be a promiscuous sensor of nucleic acids. Thus, we further investigated the role of HMGB1 in BDV infection and identified HMGB1-binding proteins (HBPs) in the nucleus of BDV-infected cells. Here we demonstrate that an HBP, HBP-1, associates with both HMGB1 and BDV RNP and may sense BDV replication in the nucleus. We found that HBP-1 co-immunoprecipitates with BDV RNP components, including genome RNA, and co-localizes with BDV nucleoprotein in the nucleus. Knockdown of HBP-1 using siRNA increased the levels of viral RNAs in the persistently infected cells. Interestingly, we revealed by using microarray analysis that antimicrobial and inflammatory responses are enhanced in a HBP-1-dependent manner in BDV-infected cells. Moreover, HBP-1 seemed to translocate BDV RNP components from the nucleus and generate stress granule-like structures in the cytoplasm in BDV-infected cells. All these results suggested that HBP-1 interacts with BDV-HMGB1 complex in the nucleus and induces antiviral responses by the generation of cytoplasmic granule structures. Our data may provide a novel mechanism for the sensing of RNA virus replication in the nucleus.

Workshop Sessions

VIR-WK235.04 - Putative mammalian RNA-dependent RNA polymerase genes derived from an ancient bornavirus

Masayuki Horie[1,2], Yuki Kobayashi[3], Tomoyuki Honda[4], Takumi Akasaka[5], Nadine Gillich[2], Marcel A. Mueller[6], Victor M. Corman[6], Yoshiyuki Suzuki[7], Martin Schwemmle[2], Keizo Tomonaga[4]

[1]Transboundary Animal Diseases Center, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan, [2]Institute for Virology, University Clinic Freiburg, Freiburg, Germany, [3]College of Bioresource Sciences, Nihon University, Fujisawa, Japan, [4]Department of Viral Oncology, Institute for Virus Research, Kyoto University, Kyoto, Japan, [5]Laboratory Forest Ecosystem Management, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan, [6]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, [7]Graduate School of Natural Sciences, Nagoya City University, Nagoya, Japan

Viral RNA-dependent RNA polymerases (RdRps) are essential for RNA viruses to replicate the viral genomes. We and others have reported that elements derived from bornavirus L genes (endogenous bornavirus-like L: EBLL), which encode viral RdRps, are present in the genomes of many eukaryotes. However, an impact of EBLLs in the evolution of their hosts remains to be shown. To gain insights into biological significance of EBLLs, we analyzed sequences of EBLLs from various species, including bats in more detail. Surprisingly, we found that one of the EBLLs in Eptesicus fuscus (big brown bat) retains an intact and complete open reading frame (ORF) consisting of 1718 codons and designated efEBLL-1. We further identified EBLLs orthologous to the efEBLL-1 in the genomes of E. serotinus and E. nilssonii by PCR and sequencing analyses. The existence of these orthologous genes implies that these Eptesicus EBLL-1s (eEBLL-1s) originated from the integration of an ancient bornavirus L gene before the divergence of the different Eptesicus species 11.8 million years ago. Intriguingly, using independent algorithms, we showed that the eEBLL-1s have been under natural selection. Moreover, all eEBLL-1s possess a specific set of amino acid residues that are known to be conserved among RNA-dependent polymerases. Finally, eEBLL-1s retain known functional motifs essential for mononegaviral RdRp activities. Together, these observations strongly suggest that eEBLL-1s have exapted during the evolution and might still act as RdRps in their host cells. Our findings could provide novel insights into cell biology and co-evolution of RNA viruses and their hosts.

Workshop Sessions

VIR-WK235.05 - A polymorphism in TIM-1 is associated with cell susceptibility to filovirus infection

Makoto Kuroda[1], Daisuke Fujikura[2], Osamu Noyori[1], Eri Nakayama[1], Masahiro Kajihara[1], Junki Maruyama[1], Hiroko Miyamoto[1], Reiko Yoshida[1], Ayato Takada[1]
[1]Div. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido Univ., Sapporo, Japan, [2]Div. of Infection and Immunity, Research Center for Zoonosis Control, Hokkaido Univ., Sapporo, Japan

While several cellular molecules have been proposed to serve as receptors or co-receptors for entry of filovirus into cells, the molecular mechanism of the virus-receptor interaction has not been fully understood. T-cell immunoglobulin and mucin domain 1 (TIM-1) is one of the host proteins that has been shown to be involved in filovirus attachment to cells. In this study, we generated a monoclonal antibody M224/1 recognizing TIM-1 expressed on African green monkey kidney Vero E6 cells, which are highly susceptible to filovirus infection. We first confirmed that M224/1 efficiently blocked infection with vesicular stomatitis virus (VSV) pseudotyped with viral glycoproteins (GPs) derived from all known filovirus species and that the ectopic expression of Vero E6-derived TIM-1 in 293T cells, which do not express TIM-1, significantly enhanced the infectivity of VSV pseudotyped with filovirus GPs. Next, we compared the infectivity of the viruses in 293T cells expressing TIM-1 clones derived from several different origins. Interestingly, Vero E6-derived TIM-1 had greater ability to enhance the viral infectivity than TIM-1s derived from other African green monkey kidney cell lines (e.g., Cos1 cells) tested. Using chimeric TIM-1 mutants between Vero E6- and Cos1-derived TIM-1s, we found that the difference in the infectivity-enhancing ability of TIM-1 was most likely due to a single amino acid difference between these TIM-1s. Interestingly, it was also found that the interaction between TIM-1 and M224/1 was also regulated by the same amino acid. These results suggest that M224/1 binds directly to the focal site for the TIM-1 function to promote filovirus entry and that polymorphism of the TIM-1 molecules is one of the factors that influence cell susceptibility to filovirus infection.

Workshop Sessions

VIR-WK235.06 - Structural transformation begets multiple functions in the viral life cycle

Zachary Bornholdt[1], Takeshi Noda[2], Dafna Abelson[1], Peter Halfmann[3], Malcolm Wood[1], Yoshihiro Kawaoka[2,3], Erica Ollmann Saphire[1]

[1]The Scripps Research Institute, La Jolla, USA, [2]University of Tokyo, Tokyo, Japan, [3]University of Wisconsin, Madison, USA

Proteins, particularly viral proteins, can be multifunctional, but the mechanism(s) behind this trait are not fully understood. Here, we illustrate through multiple crystal structures, biochemistry and cellular microscopy that the VP40 matrix protein of ebolavirus rearranges into different structures, each with a distinct function required for the virus life cycle. A butterfly-shaped VP40 dimer trafficks to the cellular membrane. There, electrostatic interactions trigger rearrangement of the polypeptide into a linear hexamer. These hexamers construct a multi-layered, filamentous matrix structure that is critical for budding and resembles tomograms of authentic virions. A third structure of VP40, formed by a different rearrangement, is not involved in virus assembly, but instead uniquely binds RNA to regulate viral transcription inside infected cells. These results provide a functional model for ebolavirus matrix assembly and the other roles of VP40 in the virus life cycle, and demonstrate how a single, wild-type, unmodified polypeptide can assemble into different structures for different functions.

Workshop Sessions

VIR-WK235.07 - Protective mechanism of glycoprotein-specific monoclonal antibodies against lethal Ebola virus challenge

Xiangguo Qiu[1], Gary Wong[2], Jonathan Audet[2], Hugues Fausther-Bovendo[1], Larry Zeitlin[3], Gary Kobinger[1,2,4,5]
[1]Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada, [2]Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada, [3]Mapp Biopharmaceutical, Inc., San Diego, USA, [4]Department of Immunology, University of Manitoba, Winnipeg, Canada, [5]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, USA

Ebola virus (EBOV) causes severe hemorrhagic fever in humans and nonhuman primates (NHPs) with mortality rates of up to 90%. Currently, there are no effective treatments available. A panel of 8 monoclonal antibodies (mAbs) against the viral glycoprotein (GP) was generated and has been characterized in vitro and in vivo. Three mAbs (ZMAb, composed of 1H3, 2G4 and 4G7) showed neutralization activity and demonstrated protective efficacy in mice, guinea pigs and NHPs. The aim of this study is to identify the protective mechanism of ZMAb. FcRgamma, FcRn and NK knockout mice were employed to elucidate the role of antibody-dependent cell-mediated cytotoxicity (ADCC) and FcRn-mediated cross-presentation (FCP). The role of complement-dependent cytotoxicity was evaluated by mutating the Fc portion of the mAbs to eliminate C1q binding. In vitro assays were used to assess mAb activity for ADCC and neutralization at ingress and egress. Our results suggest that each mAb activates a number of different pathways. It is very likely that the recruitment of such a wide array of immune mechanisms is required to reduce the viral load to sub-lethal levels and to stimulate a timely and effective immune response.

Workshop Sessions

VIR-WK235.08 - The VP35 and VP24 proteins of Ebola Virus suppress maturation of dendritic cells and skew the T Cell response

Ndongala M. Lubaki[1,3], Philipp A. I Ilinykh[1,3], Colette Pietzsch[1,3], Bersabeh Tigabu[1,3], Alexander N. Freiberg[1,3], Richard A. Koup[4], Alexander Bukreyev[1,2,3]

[1]Departments of Pathology, University of Texas Medical Branch, Galveston, USA, [2]Departments of Microbiology & Immunology, University of Texas Medical Branch, Galveston, USA, [3]Galveston National Laboratory, University of Texas Medical Branch, Galveston, USA, [4]Immunology Laboratory, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, USA

Ebola virus (EBOV) infections are characterized by deficient T lymphocyte responses, T lymphocyte apoptosis and lymphopenia. Dendritic cells (DC) do not undergo maturation despite EBOV infection. We investigated the contribution of EBOV proteins on DC maturation and T cell responses by generating recombinant viruses carrying point mutations which disabled innate response antagonist domains (IRADs) in either VP35, R312A (EBOV/VP35m), VP24, K142A (EBOV/VP24m) or both (EBOV/VP35m/VP24m) and their testing in BSL-4 biocontainment. Disabling IRADs in VP35, and to some degree, VP24, effectively unblocked DC maturation and increased the secretion of cytokines and chemokines by up to several-hundred fold compared to wt-EBOV. The blocking effect of IRADs did not significantly depend on interferon signaling or the released interferon. The mutations also induced formation of homotypic DC clusters, which presumably facilitate transfer of antigen from migratory DC to lymph node DC (Lubaki et al., 2013, J.Virol. 87:7471-85). Furthermore, we investigated the role of IRADs on the activation of T lymphocytes. PBMC from cytomegalovirus (HCMV)-seropositive donors were stained with CFSE, infected with the viruses and stimulated with HCMV pp65 peptides. In CFSE- (proliferated) lymphocytes, EBOV/VP35m infection resulted in a strong increase in percentages of CD4+ T cells secreting IFNγ and TNFα, as compared to wt-EBOV, while no cytokine-secreting cells were detected in non-proliferated lymphocytes. We also observed a moderate increase in percentages of IFNγ+ and TNFα+ lymphocytes following infection with EBOV/VP24m or EBOV/VP35m/VP24m. To determine if the T cell effects are related to the maturation state of DC after infection with wt-EBOV or the mutants, DC derived from monocytes of CMV-seropositive donors were infected with the panel of viruses and co-cultured with autologous CD4+ T lymphocytes; again, the mutations increased the percentages of IFNγ+ and TNFα+ lymphocytes. Thus VP35 and VP24 suppress DC maturation, which results in a skewing of the T cell response.

Workshop Sessions

VIR-WK235.09 - Characterization of viral proteins from a newly identified filovirus from Spain, Lloviu virus

Peter Halfmann[1], Takeshi Noda[2,3], Amy Ellis[1], Yoshihiro Kawaoka[1,3,4]
[1]Department of Pathobiological Sciences, School of Veterinary Medicine, Influenza Research Institute, University of Wisconsin, Madison, USA, [2]PRESTO, Japan Science & Technology Agency, Saitama, Japan, [3]Department of Microbiology & Immunology, Division of Virology, Research Center for Infectious Diseases, University of Tokyo, Japan, [4]ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama, Japan

Filoviruses such as Ebola and Marburg viruses are the causative agents of a severe hemorrhagic fever in humans and nonhuman primates. Epidemic to central Africa, filoviruses cause sporadic disease outbreaks with case fatality rates ranging from 20%–90% depending on the species of virus. In 2002, a novel filovirus was discovered in bats during a massive bat die-off in Spain. Named for the cave in Spain where the virus was isolated, Lloviu virus (LLOV) has a negative-sense, single-stranded RNA genome containing seven open reading frames that is organized similarly to other filoviruses. Besides genomic sequence information, very little is known about this virus. Given the discovery of an uncharacterized and potentially pathogenic filovirus in Spain, there is a need to determine the potential of LLOV to cause infection and disease in humans. Although viral RNA was isolated from bats, to date, no live LLOV has been isolated or propagated to help answer basic scientific questions regarding viral tropism, replication, and pathogenicity. Therefore, to begin to characterize LLOV, we performed various cell-based assays to compare the function of the viral proteins of LLOV with those of Zaire Ebola virus, a highly pathogenic Ebola virus. Here, we present our findings regarding the similarities and differences in the functions of the viral proteins of these two filoviruses.

Workshop Sessions

VIR-WK235.10 - A single residue in the Ebola virus receptor NPC1 controls cellular host range

Esther Ndungo[1], Rohan Biswas[1], Andrew Herbert[2], Matthijs Raaben[3], Gregor Obernosterer[4], Thijn Brummelkamp[4], Sean Whelan[3], John Dye[2], Kartik Chandran[1]

[1]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, USA, [2]Virology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, USA, [3]Netherlands Cancer Institute, Amsterdam, The Netherlands, [4]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, USA

Despite the increased frequency of filovirus outbreaks in recent decades, molecular determinants of viral host range remain poorly defined. We identified Niemann-Pick C1 (NPC1), a multispanning membrane protein involved in endosomal cholesterol transport, as a critical filovirus receptor. To assess the potential role of NPC1 in determining filovirus cellular host range, we tested a broad range of vertebrate cell lines for susceptibility to infection. These studies revealed that Ebola virus (EBOV) cannot infect a cell line from the Russell's viper, Daboia russellii, because it cannot utilize the viper NPC1 ortholog for entry. To understand why viper NPC1 lacks viral receptor activity, we cloned and expressed a soluble form of its second luminal domain (domain C). While human NPC1 domain C bound with high affinity to the viral glycoprotein, GP, the viper domain C recognized GP poorly. To map this difference in GP-NPC1 binding, we analyzed the behavior of panels of viper-human domain C chimeras and point mutants. Remarkably, a single, conservative amino acid change in domain C could bidirectionally alter its GP binding activity–introducing the human residue into viper domain C conferred high-affinity GP binding, whereas introducing the viper residue into human domain C abrogated GP binding. The results of these in vitro studies were congruent with the capacities of full-length human NPC1 proteins containing human or viper domain Cs to support EBOV entry. Intriguingly, in contrast to their differential activities as EBOV receptors, these full-length NPC1 chimeras could redistribute endosomal cholesterol. Our findings identify a region of NPC1 that is crucial for its viral receptor function and that may influence filovirus host range in nature. They also suggest that hosts under pressure to evade filoviruses could adapt via modest genetic changes to NPC1 that do not perturb its essential cholesterol transport function.

Workshop Sessions

VIR-WK236.01 - Evidence of increased virulence in Rabbit Haemorrhagic Disease Virus associated with genetic resistance in rabbits

Tanja Strive[1,6], Peter Elsworth[2,6], Brian D Cooke[3,6], John Kovaliski[4,6], Ronald Sinclair[4,6], Peter J Kerr[1], Edward C Holmes[5]

[1]CSIRO Ecosystem Sciences, Canberra, Australia, [2]Biosecurity Queensland, Department of Agriculture, Fisheries and Forestry, Toowoomba, Australia, [3]University of Canberra, Institute for Applied Ecology, Canberra, Australia, [4]Biosecurity South Australia, Adelaide, Australia, [5]Marie Bashir Institute for Infectious Diseases & Biosecurity, The University of Sydney, Sydney, Australia, [6]Invasive Animals Cooperative Research Centre, University of Canberra, Canberra, Australia

European rabbits are one of the worst introduced pest species in Australia, causing serious damage to the environment and agricultural industries. The two rabbit pathogens myxoma virus (MYXV) and Rabbit Haemorrhagic Disease Virus (RHDV) are used in Australia as biological control tools. While this has greatly reduced the damage caused by rabbits, it has also provided a unique opportunity to study the initial spread and establishment of emerging pathogens and the co-evolution with its host. In contrast to myxoma virus which attenuated shortly after its introduction and has become the text book example for the trade-off hypothesis of virulence evolution, rapid attenuation of RHDV has not been observed in the 16 years since its release. However, in recent years rabbit numbers have started to recover, and genetic resistance to RHDV has been described in some Australian rabbit populations. A study comparing key virulence parameters of three recent field isolates of RHDV in rabbits from a genetically resistant rabbit population indicated that the recent field isolates from 2006, 2007 and 2009 caused increasingly higher case fatality rates, killed rabbits faster and produced higher titres of viral particles in the livers of dead animals than the original virus. This demonstrated for the first time that there may selective pressure for RHDV to maintain high levels of virulence in the face of developing genetic resistance in Australian wild rabbits. These findings suggest that, similar to myxoma virus, RHDV virulence phenotypes may also be optimised towards maximum virus transmission, with the key difference that rabbit carcasses, and not the diseased animals are the likely source of mechanical transmission by insects. This is an important contribution to understanding host-pathogen co-evolution of rabbits and RHDV, and will help to understand the mechanisms promoting high levels of virulence in emerging diseases in general.

Workshop Sessions

VIR-WK236.02 - High impact of environmental surveillance employing advanced molecular methods on management of wild Poliovirus circulation in a highly vaccinated population in Israel in 2013

Yossi Manor[1], Lester Shulman[1], Musa Hindiyeh[1], Danit Sofer[1], Michael Gdalevitz[2], Kobi Moran-Gilad[3], Udi Kaliner[3], Itamar Grotto[3], Roni Gamzu[3], Ella Mendelson[1]
[1]Central Virology Laboratory, Sheba Medical Center, Tel – Hashomer, Israel, [2]South District Health Office, Ministry of Health, Be'er Sheva, Israel, [3]Public Health Services, Ministry of Health, Jerusalem, Israel

Israel was certified as polio-free in 2002 documenting this status by the standard acute flaccid paralysis (AFP) surveillance, and the supplementary environmental surveillance consisting systematic country-wide sewage monitoring for early detection of wild poliovirus penetration. Since April 2013 wild poliovirus type 1 (WPV1) has been detected in sewage samples in Southern Israel in the absence of clinical cases. The WPV1 isolates were genetically identified as the South Asian lineage (SOAS R3A) that circulates in Pakistan, was isolated from sewage in Egypt in Dec 2012, and most recently caused paralytic cases in Syria. To investigate the event standard protocols were revised and modified to increase the number and geographic distribution of sampling sites and sampling frequency. In addition, a highly sensitive real time RT-PCR assay specific for the WPV1 circulating in Israel was rapidly developed and used in parallel with classical tissue culture-based methods for WPV1 detection. Between June 2013 and December 2014 456 samples from 91 sewage treatment facilities and sewer systems were sampled covering about 80% of the entire Israeli population with 90% coverage in the Southern Health district. This intensified surveillance provided critical epidemiological information in real-time: it pinpointed communities in Southern and Central Israel with sustained or intermittent circulation of WPV1, provided a semi-quantitative indication of the extent of transmission before and after the immunization campaigns and aloud to follow the dynamics of the virus circulation and its gradual elimination from the population. Over all the entire program supported decision making and risk communication by the Ministry of Health to health professionals and the general public. The experience gained and the lessons learned enabled us to design a model for an improved post-event routine surveillance program and for contingency plans for response and intervention during similar events. These lessons are essential for the Global Poliovirus Eradication Initiative.

Workshop Sessions

VIR-WK236.03 - Arboviral infections of the central nervous system in patients with underlying diseases

Luiz Figueiredo[1], Michele Bastos[2], Felipe Maia[1], Maria Mourao[2]
[1]Virus Research Center, School of Medicine of USP, Rib Preto, Brazil, [2]Tropical Medicine Foundation of Manaus, Brazil

It is known that some microbial infections of the Central Nervous System (CNS), such as those caused by toxoplasma, cryptococcus and cytomegalovirus, can be related to predisposing underlying diseases. In the Brazilian Amazon region, the tropical climate favors proliferation of large quantities of insect vectors and their vertebrate reservoirs, thus supporting the natural cycles of many arboviruses that can infect man. The present report highlights examples of human CNS arboviral infections that could be related to underlying diseases. In a study done in the state of Amazonas, cerebrospinal fluid (CSF) from 110 meningoencephalitis patients were tested by reverse transcription-polymerase chain reaction (RT-PCR) for Orthobunyavirus and Flavivirus. Lymphomonocytosis predominated in all CSF cell counts. Sequencing of RT-PCR products obtained from 3 patients identified the Orthobunyavirus Oropouche (OROV). Two of the 3 OROV patients, a 54-year-old man with headache, dizziness, cloudy vision and Romberg sign, and a 37-year-old woman with headache, nausea, vomiting, and paraplegia, had underlying diseases affecting CNS and immune system (neurocysticercosis and AIDS). In a similar study using the same methods, 2 out of 23 patients from the state of Amazonas had the Flavivirus Rocio (ROCV) identified in the CSF. They were a 53-year-old man and a 30-year-old woman, both with AIDS and one of them with tuberculosis, both with headache, behavioral changes and seizures. All 4 patients in this report survived after lengthy hospital stays. It is possible that CNS invasion by OROV and ROCV was facilitated by immune deficiency or previous blood-brain barrier damage. It is important to be aware of arboviruses in patients from tropical regions with underlying diseases and CNS manifestations.

Workshop Sessions

VIR-WK236.04 - Avian Bornaviruses: A common and widespread emerging infection

Jianhua Guo[1], Susan Payne[1], Baroch John[2], H.L. Shivaprasad[3], Ian Tizard[1]
[1]Texas A&M University, College Station, USA, [2]USDA/APHIS Wildlife Services, Fort Collins, USA, [3]California Animal Health and Food Safety Lab, Tulare, USA

Bornaviruses (BV) are negative sense RNA viruses (Order Mononegavirales, genus Bornaviridae). Borna disease virus, the type strain, causes a neurological disease in horses and ruminants. The pathology of Borna disease is a nonsuppurative encephalomyelitis characterized by lymphocytic infiltrates. In the 1970s a fatal disease emerged among captive parrots. Called proventricular dilatation disease (PDD) clinical signs included encephalitis and gut paralysis leading to proventricular impaction, starvation and death. In 2008, 2 groups presented strong evidence of links between PDD and BV. Six distinct avian BV (ABV) genotypes were described. The virus was found worldwide, with no correlation among bird species, locale or ABV genotype. Two additional genotypes were found in captive passerines in Europe. While PDD is invariably fatal, most birds are healthy ABV carriers, and triggers for disease development are not known. Many infected captive parrots live for years (probably decades) without developing PDD. PDD was described in wild Canada geese in 1991, prior to the identification of ABV. Follow-up studies on stored specimens showed ABV antigen associated with lymphocytic infiltrates in the brain. Additional cases of PDD in urban/captive waterfowl have since been reported. Our group is performing an extensive survey of waterfowl across North America and we have detected the virus in brain samples from urban and migratory birds. Urban flocks of Canada geese have infection rates up to 52% while infection rates are up to 10% among migratory waterfowl. In 2011 we described a unique Canada goose genotype (ABV-CG) and have recently identified a second genotype in mallard ducks (ABV-MA). Additionally we have found ABV-CG in encephalitic raptors, suggesting an association with waterfowl predation. Thus a complex picture of ABV phylogeny, epidemiology and disease is emerging and the impact of ABV on wildlife and environmental health deserves additional study.

Workshop Sessions

VIR-WK236.05 - Phylogenetic analysis of Hepatitis E virus and contamination sources in swine production network

Nicolas Nantel-Fortier[1], Virginie Lachapelle[1], Yvan L'Homme[2], Ann Letellier[1], Philippe Fravalo[1], Julie Brassard[3]
[1]Université de Montréal, Saint-Hyacinthe, Canada, [2]Agence Canadienne d'Inspection des Aliments, Saint-Hyacinthe, Canada, [3]Agriculture et Agroalimentaire Canada, Saint-Hyacinthe, Canada

Viral contamination along the production chain is an important aspect of food safety and livestock have been reported to act as a reservoir for zoonotic, sometimes emerging viruses. Epidemiological studies have shown direct links between the consumption of uncooked pork meat and cases of hepatitis caused by the hepatitis E virus (HEV) genogroup 3 in humans. HEV can resist to low pH, to heat and to multiple freezing and thawing, making it persistent in the environment. Little is known on HEV distribution in pork meat primary production in Quebec. In order to type HEV genogroup 3 strains and evaluate the contamination sources in a swine production network, 486 environment and faecal samples were collected from 10 farms and a slaughterhouse representing a single production network, including samples of trucks transporting animals, over a period of 11 months. Viral RNA was extracted and a nested RT-PCR was performed. Positive samples were sequenced and a phylogenetic analysis was performed. HEV RNA was found in 38 samples both inside and outside the farm buildings, on the trucks, but most of them were found around the slaughterhouse, especially on the skid-loader. Phylogenetic analysis showed a wide diversity of HEV strains amongst samples. Interestingly, one strain detected in the slaughterhouse's field was homologous at 99% to a strain previously found in liver-transplanted immunosuppressed child who developed chronic hepatitis E infection. According to the results, trucks and skid-loader circulations might play an important role in HEV dissemination on the slaughterhouse's site, and most likely beyond. HEV was mainly detected outside the farms and slaughterhouse, but few samples from inside the farms were positive. The cleaning work made inside seems effective and should be extended outside the farms, slaughterhouse and trucks, in order to maintain similar conditions throughout the swine production process.

Workshop Sessions

VIR-WK236.06 - Development and validation of a novel rapid method for the simultaneous detection of viral pathogens causing acute encephalitis syndrome

Ravi Vasanthapuram[1], Sunil Govekar[1], Anita Desai[1], Latha Lakshman[2], Ravikumar Banda[2]
[1]Department of Neurovirology, NIMHANS, Bangalore, India, [2]XCyton Diagnostics Ltd., Bangalore, India

Providing rapid etiological identification of pathogens causing acute encephalitis syndrome (AES) has a tremendous impact on clinical management and outcome of patients. However, at present there is no single method available for the simultaneous detection of the multitude of pathogens that cause AES. In this study, we report for the first time, the development and validation of an AES Syndrome Evaluation System (SES) for the simultaneous detection of 16 different viral pathogens (JE, DEN, WNV, Enteroviruses, Measles, Mumps, Nipah, Rubella, Rabies, Chandipura, Chikungunya, HSV 1 and 2, Cytomegalovirus, Varicella Zoster, HHV-6, JC virus) in a single CSF sample. The SES technology comprised of nucleic acid extraction, multiplex conversion of RNA to cDNA and PCR amplification followed by sequence specific hybridization on a SES platform. The final results were read visually as a colored signal on a grid. The whole procedure takes 5-7 hours. The limit of detection (LOD) of the assay ranged between 0.1 to 50 viral particles per ml of CSF for the various viruses. The AES-SES was validated using a total of 406 well characterized CSF samples obtained from AES cases and Controls. Amongst them, the AES SES identified the correct pathogens in 41/43 CSF samples obtained from laboratory proven AES cases, 73/145 CSF samples (50.34%) obtained from suspected AES cases all of which were negative for the 16 viruses by any of the conventional diagnostic methods. The positive results were further confirmed by sequencing. None of the 50 control CSF samples obtained from patients undergoing spinal anesthesia for minor surgical problems were positive in the AES SES thereby indication high specificity. 20/ 168 CSF samples obtained form from non-infectious neurological cases were positive by the AES SES. All these 20 AES SES positive cases were either immunocompromised or had brain tumors and were on chemotherapy.

Workshop Sessions

VIR-WK237.01 - Targeting HBV cccDNA for inactivation with an endonuclease

Christoph Seeger[1], Ji Sohn[1]
[1]*Fox Chase Cancer Center, Philadelphia, USA*

Hepatitis B virus persists in infected hepatocytes with the help of covalently closed circular DNA (cccDNA), the template for the transcription of viral RNAs. cccDNA is stable in resting cells and is diluted and lost when hepatocytes divide. Moreover, it is possible that certain cytokines can induce noncytolytic destruction of cccDNA. Antiviral therapies with nucleoside analogues inhibit replication of HBV DNA in capsids present in the cytoplasm of infected cells, but do not reduce or destroy nuclear cccDNA. To investigate whether cccDNA could be directly targeted for destruction, we produced HepG2 cells expressing the HBV receptor NTCP (sodium taurocholate cotransporting polypeptide) and the endonuclease Cas9. We infected these cells with lentiviruses expressing guide RNAs of the CRISPR (clustered regularly interspersed short palindromic repeat) system to target HBV DNA for endonucleolytic cleavage by Cas9. We designed different guide RNAs targeting the core promoter region and the core gene of HBV. Subsequently, we infected the cells with HBV and determined the fraction of infected cells with antibodies specific for the HBV core antigen (HBcAg). The results showed that expression of certain guide RNAs inhibited production of core protein, indicating that Cas9 cleaved cccDNA. Experiments to determine whether cccDNA is degraded or repaired by non-homologous end joining are in progress. So far our results suggest that the CRISPR system can prevent productive infection of HBV in tissue culture cells.

Workshop Sessions

VIR-WK237.02 - Major blood transmissible viral pathogens (HBV, HCV and HIV) among residents of emerging cities in South Eastern Nigeria

Ogbonnaya Ogbu[1], Moses Alo[3], Okoro Nworie[3], Chidiebube Nwuzo[1], Eucaria Okonkwo[1], Juliet Onwe[2]
[1]Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University Abakaliki, Nigeria, [2]Department of Medical Laboratory Science, Faculty of Health Science and Technology, Ebonyi State University Abakaliki, Nigeria, [3]Department of Biological Science, Faculty of Sciences, Federal University, Ndufu Alike, Ebonyi State, Nigeria

Serum samples from healthy looking individuals (180 males and 140 females) screened with ELISA showed 11.9%, 1.9%, and 3.8% persons had HBV, HCV and HIV respectively. Gender outcome revealed that 12.8%, 2.2%, and 3.8% males were HBV, HCV and HIV positive as against 10.7%, 1.4% and 3.5% females. Age, 18 – 30 years recorded 12.8% HBV, 1.5% HCV and 4.6% HIV, compare to 10.9%, 0.9% with 3.6% for 31 – 40 years, whereas above 40 years were 10.0%, 3.8% and 3.6% positive respectively. Habits showed, promiscuous persons 17%, 3.3% and 4.5% HBV, HCV, and HIV, heavy drinkers 16% HBV and 3.3% HIV, marijuana smokers 16% HBV and 3.3% HIV, cigarette smokers 12.5%, 1.3% and 2.5% HBV, HCV and HIV positive, while 10%, 2% and 4% unsterilized sharp objects users were positive for HBV, HCV and HIV respectively. Occupational distribution recorded; business people 13.6% HBV, 2.1% HCV and 4.2% HIV, politicians 12%, 1.3%, and 4.0%, commercial sex workers 15%, 02 5.0%, 5.0% and other professionals 9.1%, 0.95%, and 2.7% carriers in that order. Socio economic status demonstrated 1.1%, and 4.4% of high income group for HBV with HIV, middle income persons 10%, 1.3%, and 4.0%, low income individuals 12.6%, 2.1% and 3.2% and those on daily income below one US Dollar ($1), had 12.3% HBV, 2.9% HCV and 3.8% HIV positive persons.

Workshop Sessions

VIR-WK237.03 - Development of Tupaia belangeri for small animal infection model of Hepatitis B virus, according to the genomic research

Takahiro Sanada[1], Kyoko Tsukiyama-Kohara[2], Sayeh Ezzikouri[2], Naoki Yamamoto[1], Michinori Kohara[1]
[1]*Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan,*
[2]*Transboundary Animal Diseases Centre, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan*

Background & Aims: Natural hosts of hepatitis B virus (HBV) are human beings, and most of laboratory animals, are not susceptible hosts for HBV. Recently, we revealed that the tree shrew (Tupaia belangeri) a small animal is susceptible to HBV infection. The purpose of this study is improvement of the efficacy of viral infection to tupaia by genetic approach and establish a small animal model. Materials &Methods: We have isolated genomic DNA from liver tissues of tupaia and determined genome sequences by HiSeq2000 sequencer (Illumina Co.). Over 100 kinds of peptide antibodies for immune system analysis were established, according to the genome sequence. We characterized HBV pathogenesis during the course of infection in tupaias. Results & Discussion: The total data of genome sequence is 239.88 (R5) and (384) (R6) Gb. Mean sequence depath is 77.98 (R5) and 84.27 (R6) and coverage of mapping sites are over 97.8%. Genome alignment of tupaia and H.sapience showed 87.94% of target genome coverage rate. The results of genome sequence revealed that most of the molecules showed higher homology between human and tupaia than human and mouse, especially, tupaia sodium taurocholate cotransporting polypeptide showed 85% homology with human and 77% homology with mouse. Tupaia CD81, SRB1, CLD1, OCLDN1 showed 96, 88, 93, 98 % homoloby with human. Thus, these molecules can be a “missing link” during the evolution of tupaia and human. Tupaia is evolutionally more close to human than mice and applicable for not only the research of viral pathogenesis but also the evaluation of drugs before clinical trial in humans.

Workshop Sessions

VIR-WK238.01 - Helping themselves: Optimal virus-specific CD4 T cell responses require help via CD4 T cell licensing of dendritic cells

Matthew Oslon[1], Shirley Seah[2], Kathryn Edenborough[3], Peter Doherty[3], Andrew Lew[2], Stephen Turner[3]
[1]University of Purdue,Lafayette, USA, [2]Walter and Eliza Hall Institute, Melbourne, Australia, [3]The University of Melbourne, Melbourne, Australia

While CD4+ T cell help (TH) is critical for inducing optimal B cell and CD8+ T cell responses, it remains unclear whether induction of CD4+ TH responses after infection, are also dependent on CD4+ T cell help. Here we show that activation of adoptively transferred TH cells during primary influenza A virus (IAV) infection enhances both the magnitude and functional breadth of endogenous primary IAV-specific CD4+ T cell responses. This enhancement was dependent on CD154-CD40 dependent DC licensing and resulted in a greater recall capacity of IAV-specific CD4+ and CD8+ T memory responses after heterologous IAV infection. These data suggest that engaging pre-existing CD4 responses at the time of priming maybe a strategy for improving cellular immunity after vaccination.

Workshop Sessions

VIR-WK238.02 - IFN-λ3 plasma levels and polymorphism affect NKG2A expression and IFNγ production by NK cells during acute HCV

Marion Depla[1], Sandy Pelletier[1,2], Camille Brunaud[1,2], Julie Bruneau[1,3], Naglaa Shoukry[1,4]
[1]Centre de Recherche du CHUM (CRCHUM), Montreal, Canada, [2]Département de microbiologie et immunologie, Faculté de médecine, Université de Montréal, Montreal, Canada, [3]Département de médecine familiale, Faculté de médecine, Université de Montréal, Montreal, Canada, [4]Département de médecine, Faculté de médecine, Université de Montréal, Montreal, Canada

Single nucleotide polymorphisms (rs12979860) located in the region of the IL28B gene encoding the type III interferon, IFN-λ3, can predict spontaneous resolution of acute hepatitis C virus (HCV) infection. The predictive value is enhanced when combined with polymorphisms in the killer cell immunoglobulin-like receptor (KIR) genes controlling the activity of natural killer (NK) cells. We hypothesized that polymorphism in the IFNλ-3 region may modulate the expression of type III IFNs during acute HCV, that may in turn modulate the induction and cross-talk between innate and adaptive immunity. We performed longitudinal quantification of type III IFNs by ELISA in plasma samples collected from a cohort of 29 injection drug users during acute HCV that progressed to spontaneous resolution or chronic infection. We used multiparametric flow cytometry to monitor the phenotype and the activity of NK cells. IFNλ-3 plasma levels are increased during acute HCV. IFNλ-3 plasma levels correlated positively with the expression of the inhibitory receptor NKG2A on CD56DimCD16+ NK cells. In addition, decreased expression of NKG2A on this subset was associated with both HCV spontaneous resolution and CC IFNλ-3 genotype. In individuals with CC IFNλ-3 genotype, decreased expression of NKG2A on CD56BrightCD16- NK cells also correlated with decreased IFNλ-3 plasma levels and spontaneous resolution of acute HCV. IFNλ-3 plasma levels did not correlate with NK cells activity. Nevertheless in individuals with *T IFNλ-3 genotype decreased production of IFNγ during acute HCV correlated with chronic evolution. This suggests that in individuals with CC IFNλ-3 genotype, decreased expression of NKG2A might be predictive of spontaneous resolution whereas in individuals with *T IFNλ-3 genotype decreased production of IFNγ during acute HCV might be predictive of chronic evolution. These results demonstrate that IFNλ-3 polymorphism affect NK cell function during early acute HCV but other factors may also act to determine the outcome of infection.

Workshop Sessions

VIR-WK238.03 - Inhibition of CD8+ T cell memory by suppression of NF-kB activation during the acute phase of vaccinia virus infection

Hongwei Ren[1], Brian Ferguson[1], Carlos Maluquer de Motes[1], Rebecca Sumner[1], Laura Harman[1], Geoffrey Smith[1]
[1]Department of Pathology, University of Cambridge, Cambridge, UK

The host response to acute virus infection includes the activation of cytotoxic T cells that lyse virus-infected cells and promote the development of long-lived memory cells to fight future infection by the same virus. Understanding the factors that influence the quality, magnitude and longevity of T cell responses is incomplete and is important for vaccine development. Here vaccinia virus (VACV) protein N1 is shown to impair the development of both effector and memory CD8+ T cells via its inhibition of nuclear factor kappa B (NF-kB) activation. Infection with VACVs that either have the N1L gene deleted or engineered to express N1 with a single amino acid mutation that abrogates its ability to block NF-kB activation (vN1.I6E) resulted in increased populations of central and memory CD8+ T cells, increased CD8+ T cell cytotoxicity and lower virus titers upon challenge. The function of CD8+ memory T cells following infection with vN1.I6E was also enhanced. These cells produced more IFNg upon challenge with VACV and conferred greater protection to VACV infection following passive transfer to naïve mice compared to CD8+ T cells from mice infected with wild type virus (vN1.WT). This study demonstrates the importance of NF-kB activation within virus-infected cells for the development of long-term CD8+ T cell memory and vaccine efficacy. Further, it provides a rationale for deleting N1 from VACV vectors to enhance CD8+ T cell immunogenicity while simultaneously reducing virulence to improve vaccine safety.

Workshop Sessions

VIR-WK238.04 - Influence of the infection by the lymphocytic choriomeningitis virus on the humoral response to the nitrophenyl

Matthieu Daugan[1], Alain Lamarre[1]
[1]*Institut National de la Recherche Scientifique, Montreal, Canada*

Persistent viral infections are a major public health problem. One common characteristic of these infections is their tendency to provoke the late appearance of neutralizing antibodies as opposed to acute viral infections. In this project, we studied the humoral response mounted against 4-hydroxy-3-nitrophenyl (NP) in the context of an infection by lymphocytic choriomeningitis virus (LCMV), a well-characterized model of chronic infection. We showed that, in opposition to VSV-infected or uninfected mice, LCMV-infected animals produced increased amounts of total antibodies whereas the NP-specific response was dramatically impaired. This delay was T-dependent, appeared in the first days after immunization and lasted for approximately 20 days. It was accompanied by a polyclonal activation of B cells, triggering an unspecific hypergammaglobulinemia. During LCMV infection, follicular helper T cells (TFH) numbers increased whereas total CD4 numbers declined. This was accompanied by an increased secretion of IL-21 and IL-4 by TFH, cytokines implicated in the affinity maturation process. Serum levels of B cell activation factor (BAFF), favouring B cell survival, concomitantly rose. It is noteworthy that increased TFH numbers also correlated with an over expression of activation markers like ICOS and PD-1 on their surface and PD-L1/L2 on B cells. These results suggest that modulations in TFH numbers and/or function might be responsible for impaired B cell responses characterizing persistent viral infections.

Workshop Sessions

VIR-WK238.05 - The role of eosinophils in the pathogenesis of respiratory Syncytial Virus infection

Gia Green[1], Natasha Petrov[1], Devra Huey[1], Krista La Perle[1], Stefan Niewiesk[1]
[1]Department of Veterinary Biosciences, The Ohio State University, Columbus, USA

The role of eosinophils in the pathogenesis of respiratory syncytial virus (RSV) infection remains poorly understood. Eosinophils are of interest during RSV infection for three reasons. First, an anti-viral role has been suggested for esosinophils during RSV infection. Secondly, pulmonary eosinophilia is considered to be a hallmark of “vaccine-enhanced disease” in experimental animal models, and vaccine-enhanced disease has been a major obstacle in creating an RSV vaccine. Lastly, there is evidence to suggest that RSV infection may be linked to the development of asthma/allergy, a hallmark of which is also eosinophilia. Infection of cotton rats with RSV, measles virus, and influenza virus did not increase eosinophil levels in the lung. These results are consistent with the “textbook” expectation for virus infections. In contrast and again consistent with expectations, Staphyloccocus aureus infection leads to a marked increase in neutrophils. To determine the effect of RSV infection on pulmonary eosinophil levels in an allergy model, cotton rats were treated with house dust mite antigen and challenged with different combinations of allergen and RSV. Following treatment with house dust mite antigen, pulmonary eosinophil levels were significantly increased. Infection with RSV, however, did not alter the percentage of pulmonary eosinophils. After immunization with formalin-inactivated vaccine and subsequent challenge, a high percentage of eosinophils was induced. However, a part of that increase was due to the presence of cellular proteins in both the vaccine and challenge virus preparation. In summary, RSV infection did not lead to an increase in eosinophils after primary infection and in conjunction with an allergen, but did in a model of vaccine-enhanced disease.

Workshop Sessions

VIR-WK238.06 - Fas-binding pro-apoptotic protein 1 (FBPAP-1) is critical for induction of type I interferon signaling and the antiviral response

Jae-Hoon Kim[1], Min-Eun Park[1], Tae-Hwan Kim[1], Jong-Soo Lee[1]
[1]College of Veterinary Medicine (BK21 Plus program), Chungnam National University, Daejeon, Republic of Korea

The Fas-binding pro-apoptotic protein 1 (FBPAP-1) is a component of the death-inducing signaling complex in Fas-mediated apoptosis and regulates NF-kb activity as well as ubiquitination and proteaomal degradation. Here, we show that FBPAP-1 is involved in NF-kb and type I interferon signaling pathway as a positive regulator. Overexpression of FBPAP-1 in various cell types enhances the production of inflammatory cytokines and type I interferon upon Vesicular Stomatitis, Influenza, Newcastle disease virus infection. Controversially, knockdown of FBPAP-1 in raw 264.7 cell line and MEF cells or BMDM cells derived from FBPAP-1(gt/gt)mice markedly enhances susceptibility to virus infection and shows lower level of inflammatory cytokines and type I interferonproduction. Consistent with in vitro data, upon infection of Vesicular stomatitis virus (Indiana strain), FBPAP-1(gt/gt)mice exhibited marked susceptibility to virus infection. FBPAP-1(gt/gt)mice showed increased mortality and decreased level of IFN-β and IL-6 secretion from serum. These findings suggest that FBPAP-1 is a positive regulator that enhance the NF-κB and type I interferon signaling pathway against virus infection. [This research was supported by National Agenda Project Grant from KRCFST (KGM 0821113)].

Workshop Sessions

VIR-WK238.07 - MAP kinase phosphatase 5 expression induced by influenza virus infection negatively regulates IRF3 activation and type I interferon response

Sharmy James[1], Hong-Ying Teh[1], Hirotaka Takahashi[1], Huipeng Jiao[1], Yongliang Zhang[1]
[1]Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Influenza virus infection causes substantial morbidity and mortality globally. Type I interferon-mediated responses are essential for immunity against influenza. MAP kinase phosphatases (MKPs) are the major negative regulators of MAP kinases, which play important role in immune responses against microbial infection. However, the roles of MKPs in type I interferon-mediated immune responses to viral infection is not clear. Here, we showed that H1N1 and H3N2 influenza virus infection induces the expression of MKP5, one of the MKP members, in host cells. Mice deficient in MKP5 were resistant to H1N1 influenza virus infection, which was associated with increased type I interferon expression in the lung compared with WT mice. The increased type I interferon expression caused by the deficiency of MKP5 was associated with enhanced IRF3 phosphorylation and activation. Overexpression of MKP5 in macrophages inhibited IRF3 phosphorylation and nuclear translocation in response to influenza virus infection. The non-structure protein 1 (NS1) from H1N1 and H3N2 viruses induce the expression of MKP5 in macrophages, but not required for the inhibition of MKP5 on type I interferon expression. We further found that MKP5 interacts with IRF3 to regulate its activation. Both the N-terminal and C-terminal regions of MKP5 and the C-terminal region of IRF3 are required for their interaction. Our study, therefore, revealed a novel mechanism by which influenza viruses inhibit type I interferon expression in host through MKP5.

Workshop Sessions

VIR-WK239.01 - Identification of emerging adaptive intermediates of RNA viruses during experimental evolution

Marco Vignuzzi[1]
[1] *Institut Pasteur, Paris, France*

The high mutation rates and vast sequence heterogeneity of RNA virus populations permits rapid adaptation to novel host environments, although the rate of emergence and rise to dominance of new strains is dependent on ecological factors. Since deep sequencing technology permits an unprecedented sensitivity in characterizing virus populations, we explored how coupling deep sequencing with experimental evolution studies may improve the identification of emerging variants. Using Coxsackie virus as a model, we examined how a virus evolves during adaptation to a novel host environment in tissue culture. Our analysis identified a two-step adaptive process involving refinement of receptor usage that correlates with the availability of the primary and co-receptors. Using chikungunya virus to study virus evolution in vivo, we identified another two-step adaptive process in the infected mosquito. These new variants increase virus fitness in both insect and mammalian hosts, and the emerging strain is able to supplant the parental strain during the mosquito-to-mammal transmission cycle. Our data suggest that deep sequencing and experimental evolution is a powerful strategy to identify the variants most likely to emerge during the short-term evolution of RNA viruses.

Workshop Sessions

VIR-WK239.02 - Evidence of RNA mediated long-lasting antiviral immunity in insects

Juan A. Mondotte[1], Vanesa Mongelli[1], Hervé Blanc[1], Lionel Frangeul[1], Maria-Carla Saleh[1]
[1]Institut Pasteur, Viruses and RNA interference, Paris, France

Insects lack adaptive immunity and rely on the innate immune system to mount a defense response against pathogens. For decades, it was assumed that the innate immune system is hardwired and unable to establish immunological memory. But memory-like responses have been recently described in some invertebrates, a phenomenon that was termed immune priming. In the current work, using the fruit fly Drosophila melanogaster and the positive strand RNA virus Sindbis, we demonstrate the existence of long-lasting immunity and we put in evidence that this protection is RNA-mediated. Our results suggest not only that the defense system of the fly can react more efficiently against a virus after a previous encounter with the parasite, but also, and more importantly, that this initial host pathogen interaction can be vertically transmitted and confer resistance to the progeny. To our knowledge this is the first evidence of the existence of a mechanism of long-lasting protection against RNA viruses in insects. Unraveling the mechanism of vertical transmission of immunity in Drosophila could open the door to new concepts of immunology and in the field of host-pathogen relationships.

Workshop Sessions

VIR-WK239.03 - 25-hydroxycholesterol stimulated antiviral microRNAs regulate lipid metabolism

Ragunath Singaravelu[1,2], Daniel Jones[3], Shifawn O'Hara[1], Prashanth Srinivasan[1,2], Rodney Lyn[1], Rod Russell[3], John Pezacki[1,2]

[1]National Research Council of Canada, Ottawa, Canada, [2]University of Ottawa, Ottawa, Canada, [3]Memorial University of Newfoundland, St. John's, Canada

An emerging focus in immunology is the relevance of lipid metabolism in innate immunity. Recent work demonstrated a novel role for the macrophage secreted oxysterol, 25-hydroxycholesterol (25HC), in the host antiviral response and interferon signalling. While these studies demonstrate 25HC inhibits viral-cell fusion through modification of cellular membranes, it is unclear whether 25HC possesses additional membrane-independent antiviral functions. We demonstrate that 25HC activates the expression of microRNAs, miR-130b and miR-185, in hepatitis C virus (HCV) infected hepatoma cells. We show that miR-185 and miR-130b overexpression potently inhibits HCV replication. Conversely, miR-130b inhibition increases viral replication. miR-185 directly regulates SREBP2, a master transcriptional regulator of cholesterol biosynthesis, as well as SCD, a key enzyme in the synthesis of unsaturated fatty acids. Similarly, miR-130b regulates the expression of LDLR, a crucial receptor for cholesterol uptake. The miRNAs' antiviral activity is consistent with the previously reported crucial roles of SREBP2, SCD, and LDLR in the HCV life cycle. HCV hijacks hepatic lipid metabolism to facilitate its pathogenesis; clinically, these HCV-induced metabolic alterations results in steatosis for over 50% of patients. Interestingly, we demonstrate that HCV infection down-regulates miR-185 and miR-130b expression. Coherent anti-Stokes Raman scattering microscopy demonstrates that inhibition of miR-185 or miR-130b activity results in lipid accumulation – highlighting a novel mechanism of HCV-induced steatosis. As there is increasing evidence that cholesterol and unsaturated fatty acids play a critical role in viral entry and replication, 25HC's activation of miRNAs repressing LDLR, SCD, and SREBP2 expression may play a role in the broad antiviral response. Furthermore, HCV's repression of miR-130b and miR-185 represents a novel mechanism of innate immune evasion. Our data highlight miRNAs as a novel link between lipid metabolism and innate immunity.

Workshop Sessions

VIR-WK239.04 - The role of RNA silencing in viroid infection

Charith Raj Adkar Purushothama[1], Chantal Brosseau[2], Peter Moffett[2], Jean-Pierre Perreault[1]
[1]Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Canada, [2]Département de Biologie, Université de Sherbrooke, Sherbrooke, Canada

Viroids are single-stranded phytopathgenic circular RNAs with a size range of 246-400 nucleotides. These non-coding RNAs replicate autonomously via double-stranded RNA intermediates using the host machinery. As might be expected from their highly base-paired structure and their dsRNA mode of replication, viroids have been shown to induce RNA silencing. Previous investigations have hypothesized the involvement of vd-sRNA in the induction of host symptoms, but conclusive proof is lacking. In the present study, based on bioinformatic tools we predicted that PSTVd (Potato spindle tuber viroid)-sRNA can down-regulate cell division cycle proteins, hop-interacting protein and defense related genes in tomato plants by RNA interference. To analyze the influence of viroids on these genes, we infected tomato plants with different strains of PSTVd and viroid titre was evaluated over a four-week time-course. Relative Expression (RE) of the predicted vd-sRNA target genes was analyzed by qRT-PCR techniques. This confirmed the down-regulation of five genes, including Cell division cycle associated 7-like (CDCA7L) protein, Cell division cycle 48-homolog (CDC48), Hop interacting protein (Hip), and an LRR-serine threonine kinase and a serine/threonine kinase. Interestingly, all tomato plants infected with a PSTVd intermediate strain inhibited gene expression two-fold, while PSTVd mild and PSTVd severe strains resulted in one- and three-fold inhibition, respectively. The ability of vd-sRNAs to inhibit gene expression via predicted target gene binding sites was confirmed by artificial microRNA (amiRNA) experiments. In addition, target genes were silenced by VIGS (Virus Induced Gene Silencing) and plants were infected with PSTVd to investigate the role of these genes in viroid infections. Recent findings will be discussed in relation viroid infection mechanisms and disease symptom exhibition.

Workshop Sessions

VIR-WK239.05 - Inhibition of exoribonuclease XRN1 by beet necrotic yellow vein virus RNA3 coremin sequence

Alyssa Flobinus[1], Tanja Seissler[1], Elodie Klein[1], Salah Bouzoubaa[1], David Gilmer[1]
[1]CNRS, Strasbourg, France

Beet necrotic yellow vein virus (BNYVV) is a multipartite positive stranded RNA virus inducing the rhizomania disease in sugar beet. BNYVV RNA3 containing the coremin sequence of 20 nucleotides is essential for long distance movement. In the viral context, RNA3 undergoes a cleavage that produces a non-coding RNA3 (ncRNA3) stabilized by the coremin sequence at its 5'end. This ncRNA3 is not retrieved using an RNA3 mutant containing a modified coremin sequence. In vitro, incubation of wild-type (wt) or mutated RNA3 with wheat germ gives similar results. Expression of wt or mutated RNA3 in Saccharomyces cerevisiae FY4 allowed accumulation of ncRNA3 only with wt RNA species. Screen of S. cerevisiae ribonuclease mutants allowed us to demonstrate that XRN1 exonuclease was responsible for the RNA3 processing. Yeast FY4ΔXRN1 strain was successfully complemented with vectors expressing wt XRN1 and more interestingly with Arabidopsis thaliana XRN4 orthologue. Using a biochemical approach, we demonstrated an inhibitory effect of the ncRNA3 on XRN1 in vitro. These preliminary results, suggest an essential role of ncRNA3 in the inhibition of the exonuclease that could modulate the systemic spread of the virus.

Workshop Sessions

VIR-WK240.01 - Class II fusion proteins in viral entry and in embryogenesis

Rey Felix[1]
[1]*Institut Pasteur, Paris, France*

Class II proteins are viral membrane fusogenic molecules folded essentially as β-sheet and having an internal fusion peptide. In particular, they lack the characteristic central alpha-helical coiled coil present in the post-fusion conformation of all other viral fusion proteins. The regular, icosahedrally symmetric enveloped viruses that have been studied so far, such as flaviviruses, alphaviruses and phleboviruses have been shown to have class II fusion proteins, which in their pre-fusion conformation make an icosahedral shell surrounding the viral membrane. Yet despite having very similar envelope proteins, these viruses belong to three different viral families with totally different genome replication machineries. We have recently identified the rubella virus fusion protein as belonging to class II, although the virus particles appear pleomorphic and lack icosahedral symmetry. In spite of the lack of any detectable sequence conservation, the available structures indicate that class II proteins have undergone divergent evolution from a distal, ancestral gene. We have now discovered that the cellular fusion protein EFF-1, involved in syncytium formation during the genesis of the skin in nematodes (C. elegans) and in other multicellular organisms, is also folded as a class II viral fusion protein. This finding strongly indicates a common ancestry for the corresponding gene, and highlights an unprecedented amount of exchange of genetic information between viruses and cells. My talk will discuss the implications of this finding, which highlights the intricate exchange of genetic information that has taken place between viruses and cells during evolution. Nevertheless, the fusogenic mechanism of the cellular fusion protein EFF-1 appears to require trans-oligomerization of proteins anchored in the two membranes to be fused, similar to the SNAREs and in contrast to the viral proteins. My talk will describe the characterization of the fusogenic mechanism of this novel cellular class II fusion protein.

Workshop Sessions

VIR-WK240.02 - Host cell Autophagy promotes BK Virus infection

Stephanie Bouley[1], Aisling Dugan[1]
[1]*Assummption College, Worcester, USA*

Autophagy is important for a variety for virus life cycles. We sought to determine the role of autophagy in human BK polyomavirus (BKPyV) infection. The addition excess amino acids during viral infection reduced BKPyV infection. Perturbing autophagy levels using inhibitors, 3-MA, bafilomycin A1, and spautin-1, also reduced infection, while rapamycin treatment of host cells increased infection. siRNA knockdown of autophagy genes, ATG7 and Beclin-1, corresponded to a decrease in BKPyV infection. BKPyV infection not only correlated with autophagosome formation, but also virus particles localized to autophagy-specific compartments early in infection. These data support a novel role for autophagy in the promotion of BKPyV infection.

Workshop Sessions

VIR-WK240.03 - Grouper heat shock cognate protein 70 is involved in the cellular entry of nervous necrosis virus

Jui-Shin Chang[1], Shau-Chi Chi[1]
[1]*National Taiwan University, Department of Life Science, Taipei, Taiwan*

Nervous necrosis virus (NNV) is a devastating pathogen of cultured marine fish, and has affected more than 40 species of fish. NNV belongs to betanodavirus of Nodaviridae, and is a non-enveloped icosahedral particle with two single-stranded positive-sense RNAs. The knowledge of NNV entry into host cell is limited, and no NNV-specific receptor protein has been identified. Using grouper fin cell line GF-1 and purified NNV capsid protein in virus overlay protein binding assay (VOPBA), grouper heat shock cognate protein 70 (GHSC70) and grouper voltage dependent anion selective channel protein 2 (GVDAC2) were assumed to be NNV receptor protein candidates of GF-1 cells. The genes of GHSC70 and GVDAC2 were cloned and sequenced, and were expressed in Escherichia coli for the anti-serum preparation. Knockdown the expression of GHSC70 and GVDAC2 by specific short interfering RNA (siRNA) significantly down-regulated the NNV RNA expression in NNV-infected GF-1 cells. By immuno-precipitation assay, GHSC70 were reconfirmed to interact with NNV capsid protein, while VDAC2 could not. Through immuno-fluorescence staining and flow cytometry analyzing, GHSC70 protein was detectable at the cell surface. Furthermore, NNV RNA level detected at 1 hour post infection (hpi) significantly lower in the cells pretreated with anti-GHSC70 antiserum than that in the non-treated cells. According to the above data, we suggest that GHSC70 plays an important role in NNV entry of GF-1 cells.

Workshop Sessions

VIR-WK240.04 - When a push comes to a shove: dispersal and release of vaccinia virus

J. Horsington[1], H. Lynn[1], G. Karupiah[4], C.B. Whitchurch[2], L. Turnbull[2], D. Cheng[3], F. Braet[3], T.P. Newsome[1]
[1]School of Molecular Bioscience, University of Sydney, Sydney, Australia, [2]The ithree institute, University of Technology Sydney, Sydney, Australia, [3]Australian Centre for Microscopy & Microanalysis, University of Sydney, Sydney, Australia, [4]John Curtin School of Medical Research, Australian National University, Canberra, Australia

Vaccinia virus (VACV) is a large DNA virus that following replication in the cytoplasm generates two infectious forms: wrapped virus (WV) and intracellular mature virus (MV). Wrapped virus is released from infected cells by fusion of the outermost of two trans-Golgi derived membranes with the host plasma membrane. Extracellular, cell-associated WVs possess the capacity to activate actin polymerisation in the underlying cytoplasm, thereby promoting cell-to-cell spread. Here we report an analysis of VACV during release using fluorescent recombinant viruses, achieving a resolution of ~20 nm with three-dimensional structured illumination microscopy (3D-SIM). We have imaged recombinant viruses that differentially label the virus core and viral membranes, we were able to resolve the trans-Golgi-derived membranes as spheres that encompass the viral core of intracellular WV. As WV fused with the plasma membrane, the outer WV membrane redistributed to form a platform adjacent to extracellular WV. The viral protein responsible for initiating actin polymerisation, the transmembrane protein A36, was found to be tightly associated with de novo formed F-actin filaments at the onset of actin polymerisation. We describe a role for actin nucleation in the untethering of virus into the surrounding extracellular space.

Workshop Sessions

VIR-WK240.05 - Membrane binding of Retroviral Gag proteins – roles played by acidic phospholipids and RNA

Jingga Inlora[1], Balaji Olety[1], Gabrielle Todd[1], Akira Ono[1]
[1]University of Michigan Medical School, Ann Arbor, USA

HIV-1 particle assembly, a process driven by a viral structural protein Gag, takes place predominantly at the plasma membrane (PM). A PM-specific acidic phospholipid, PI(4,5)P2, plays an important role in proper localization of Gag to the PM. We and others demonstrated that PI(4,5)P2 depletion inhibits PM localization of Gag and efficient virus release and that Gag interacts with PI(4,5)P2 via a highly basic region (HBR) in the matrix (MA) domain of Gag. Previous in vitro studies showed that HBR also interacts with RNA. Interestingly, we observed that this MA-RNA interaction inhibits Gag binding to liposomes containing another acidic lipid phosphatidylserine but not binding to liposomes containing PI(4,5)P2. We further found that both MA-phosphatidylserine and MA-RNA interactions are determined by the total positive charge of HBR, whereas PI(4,5)P2 binding requires not only the positive charge but also a specific sequence of MA HBR. These results collectively support a working model in which the electrostatic interaction of RNA with HBR prevents HIV-1 Gag from binding to membranes containing prevalent acidic lipids (e.g., phosphatidylserine), but PI(4,5)P2 interaction allows Gag to overcome this block, thereby ensuring specific binding of Gag to the PM. To determine the generality of this mechanism for membrane binding regulation, we examined HIV-1 Gag derivatives, where MA is replaced with MA from other retroviruses. We observed that liposome binding via MA from HTLV-1, MLV or HERV-K does not require PI(4,5)P2 and is insensitive to RNA, whereas liposome binding via HIV-1 or RSV MA is both RNA-sensitive and PI(4,5)P2-dependent. This suggests a correlation between RNA suppression and PI(4,5)P2 dependence of retroviral MA membrane binding. Importantly, these characteristics also correlate with the sensitivity of Gag localization and VLP release to PI(4,5)P2 depletion. These results suggest that the competition between RNA and acidic lipids over MA defines two types of retroviral-MA-mediated membrane binding.

Workshop Sessions

VIR-WK240.06 - A topological assay to detect virus membrane fusion in intact cells

Anna Wec[1], Eva-Maria Mittler[1], Kartik Chandran[1]
[1]*Department of Microbiology & Immunology, Albert Einstein College of Medicine, New York, USA*

The Ebola and Marburg filoviruses are enveloped nonsegmented negative-strand RNA viruses that cause outbreaks of hemorrhagic fever. Filovirus entry into host cells culminates in fusion of the viral lipid envelope with the limiting membrane of one or more cellular compartments. This process is mediated by the viral envelope glycoprotein (GP), and is driven by large-scale GP conformational changes in response to an unknown cellular trigger. While filovirus particles must undergo trafficking to late endosomes, where they gain access to essential entry host factors, it is unclear whether late endosomes are the sites of membrane fusion or merely waypoints to other compartments where fusion and cytoplasmic escape ultimately take place. Here we describe an approach to visualize the intracellular sites of viral membrane fusion by selective detection of the post-fusion conformation of GP in intact cells. This approach takes advantage of the distinct compartmentalization of the GP C-terminal tail (C-tail) in its pre- and post-fusion states. Prior to fusion, the C-tail is located within the viral lipid membrane; after fusion, it becomes exposed on the cytoplasmic face of the targeted cellular compartment. To detect cytoplasmic access of the GP C-terminus, we fused the biotin acceptor peptide (BAP) sequence to the C-tail of Ebola virus GP, and generated infectious virus particles containing this fusion protein. We then exposed this virus to cells over-expressing the bacterial biotin ligase enzyme (BirA) in the cytoplasm. BirA catalyzes the covalent attachment of biotin to BAP, affording the detection of cytoplasmically exposed GP C-termini with streptavidin-fluorophore conjugates. Our current work is focused on combining biotin labeling with compartment-specific cellular probes to define the fusion compartment(s) and to place them within the context of GP's interaction with its intracellular receptor, Niemann-Pick C1.

Plenary Sessions

BAM-PL10.01 - The *Legionella pneumophila* strategy: Molecular mimicry to hijack host-signaling pathways

Carmen Buchrieser[1]
[1]*Institut Pasteur, Paris, France*

Legionella pneumophila is the etiological agents of Legionnaire's disease, a Gram-negative bacterium present in fresh and artificial water environments that replicates in protozoan hosts. When aerosolized bacteria are inhaled, they are able to colonize the respiratory tract, invade alveolar macrophages and replicate therein causing the disease. Replication within protozoa is essential for the survival of the bacterium. Genome sequencing and analyses led to a giant step forward in our understanding how *L. pneumophila* replicates intracellularly by suggesting new ways how this bacterium might subvert host functions. Our analyses revealed that the *L. pneumophila* genome encodes a high number and great diversity of eukaryotic-like proteins. We suggested that these are mimicking host proteins to subvert host-signaling pathways. Indeed, we and others showed that *L. pneumophila* employs its F-box encoding proteins to exploit the ubiquitin signaling pathways of the host. Recently we characterized a SET-domain encoding protein and showed that it is secreted in the host cell where it induces epigenetic regulations to down regulate the host transcriptional response to infection. Comparative and evolutionary genomics analyses of the eukaryotic like proteins demonstrated that lateral gene transfer from eukaryotic hosts contributed to the evolution of these proteins within *Legionella*. Collectively these data shed new light on the virulence strategies of *L. pneumophila*, a major aspect of which is molecular mimicry.

Plenary Sessions

BAM-PL10.02 - Burkholderia cenocepacia: an intracellular opportunistic pathogen full of surprises

Miguel Valvano[1]
[1]*Queen's University Belfast, Centre for Infection and Immunity, Belfast, UK*

Burkholderia cenocepacia is a member of the B. cepacia complex (Bcc), a group of Gram-negative opportunistic pathogens that chronically infect the airways of cystic fibrosis patients, but they can also infect patients with various types of immunosuppressive disorders. B. cenocepacia are multidrug resistant bacteria that have the ability to persist in the infected host and also elicit robust inflammatory responses. Studies using macrophages, combined with dramatic advances in the ability to genetically manipulate these microorganisms, have contributed to increase our understanding of the molecular mechanisms of virulence in these pathogens and the molecular details of the cell host responses triggering inflammation. One of the highlights of the intracellular infection by this bacterium is its ability to compromise the phagosomal membrane and then initiate a process of abortive autophagy, which leads to a pronounced pro-inflammatory response mediated by the NLRP3/pyrin inflammasome. Even more remarkable is the synergy between the bacterial infection and ability to halt autophagy and the endogenous autophagy defect present in cells with a defective CFTR protein. Finally, this bacterium displays a tremendous capacity to resist antibiotics and can escape killing by antimicrobial peptides. All of this properties provide B. cenocepacia with the ability to thrive in immunocompromised hosts, becoming a persistent pathogen that is very difficult to eradicate.

Plenary Sessions

BAM-PL11.01 - The three "A"s of microcin E492: Antimicrobial, Antitumor, and Amyloidogenic

Rosalba Lagos[1], Andrés Marcoleta[1], Eduardo Bignon[1], Pablo Lobos[1], Paulina Aguilera[1], Octavio Monasterio[1]
[1]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

Microcin E492 (MccE492) is a low-molecular-mass pore-forming bacteriocin produced by Klebsiella pneumoniae RYC492 that exerts its antibacterial action on related strains. MccE492 has also a cytotoxic effect on malignant human cell lines through apoptosis, a desired mechanism for cancer therapy. In addition, this bacteriocin generates amyloid structures that modulate the antibacterial activity by sequestering the toxic species. For the production of active MccE492, this bacteriocin has to undergo post-translational modification with a catechol-type siderophore at the C-terminal, which is essential for the recognition of the outer membrane receptors. After recognition, MccE492 is translocated to the periplasm and inserted into the inner membrane of the target cell. The modification however is not essential for the pore-formation activity, because both unmodified and modified MccE492 can form pores in membranes. The fiber morphology for both forms is the same, but the aggregation kinetics of the modified form presents a considerable delay compared to unmodified MccE492. This can be explained by a different conformation of the precursors, which present a different content of secondary structure, that affects the nucleation process. An amyloidogenic region was identified through mutagenesis, and the precursor for the toxic species identified. The amyloidogenic region turned out to be different to that required for pore formation. MccE492 appears to be a helpful model for studying the toxic structure of an amyloid, because a conserved folding pathway with oligomeric intermediates characterizes most amyloid fibers. Supported by FONDECYT grant 1140430.

Plenary Sessions

BAM-PL11.02 - Electromicrobiology: Biocommodities, Bioenery, Biolectronics, and Biogeochemistry

Derek Lovely[1]
[1]*Department of Microbiology, University of Massachusetts, Amherst, USA*

Electromicrobiology is a rapidly emerging field of study that investigates microbial electron exchange with electrodes, minerals, and other organisms, as well as novel electronic properties of microorganisms with applications in bioelectronics. For example, biofilms of the microbe Geobacter sulfurreducens have recently been shown to have conductivities rivaling those of synthetic conducting polymers, as well as supercapacitor and transistor properties. The ability of microorganisms to directly exchange electrons with electrodes has led to novel applications. For example, microbial electrosynthesis is an artificial form of photosynthesis in which electrical energy derived from solar technologies is used to feed electrons to microbial biofilms on electrodes. The microbes use the electrons to reduce carbon dioxide to multi-carbon organic compounds that are excreted from the cells. Producing commodity chemicals and transportation fuels with microbial electrosynthesis is much more efficient and potentially more environmentally sustainable than biocommodity strategies that rely on biological photosynthesis. Direct interspecies electron transfer (DIET), is a recently discovered form of extracellular electron transfer in which microorganisms exchange electrons to cooperatively degrade organic compounds under anaerobic conditions. DIET appears to be an important mechanism for electron exchange in wastewater digesters converting organic wastes to methane, a proven bioenergy strategy. Artificially enhancing DIET can accelerate methane production. One of the most surprising discoveries in electromicrobiology is microbial nanowires, which contribute to microbe-electrode and microbe-microbe electron exchange. In Geobacter sulfurreducens the microbial nanowires are pili that are capable of conducting electrons along their length via metallic-like conductivity. The possibility of metallic-like conductivity in a biological protein is a paradigm shift, differing dramatically from typical biological electron transfer, which is accomplished via electron hopping/tunneling between discrete redox-active proteins. Recently elucidated structural insights into biological metallic-like conductivity are leading to new synthetic biology strategies for enhancing microbial electrical connections in a growing diversity of applications, including biocomputers.

Plenary Sessions

MEM-PL10.01 - A temporal metagenomic survey of a soil microbial community following willow planting in petroleum hydrocarbon-contaminated soil

Y. Terrat[1], S. Halary[1], E. Yergeau[1], M Hijri[1], M. St-Arnaud[1]
[1]*Institut de recherche en biologie végétale (IRBV), Montreal, Canada*

Contamination attributed to toxic pollution of industrial sites is a serious problem worldwide, with a public health impact comparable to Tuberculosis, HIV/AIDS or Malaria. Of particular importance is the remediation of contaminated soils with the view of feeding a growing human population. Among the different existing depollution approaches, phytoremediation has proven itself to be extremely cost-effective and environment-friendly, but its underlying mechanisms are poorly understood. This study aimed at understanding how the indigenous soil microbial community surrounding plants is affected by petroleum contamination, and how it reacts to pollutants over time. Willows were planted in crude oil-contaminated (900-1500 mg/kg C10-C50 and 60 mg/kg HAP), and non-contaminated soils in a greenhouse, and the taxonomic and functional composition of microbial communities was monitored in the rhizosphere and the bulk soil at three different time points using deep Illumina sequencing of genomic DNA. Our comparative metagenomic analysis shows that the overall soil bacterial content is moderately affected in contaminated soils with a moderate but significant promotion of some known hydrocarbon-degrading taxa mainly belonging to β and γ-proteobacteria. However, the microbiome of contaminated soil tends to resemble the non-contaminated one after less than one year. Interestingly, we observed that contaminated soils exhibit a highly significant increase in plasmid and virus-related sequences, and that these mobile entities carry genes involved in metal-trace element resistance and petroleum hydrocarbon degradation. Taken together, our results show that the soil microbiome possesses the intrinsic capacity to overcome petroleum contamination over a relatively short period of time. Our data confirm that known petroleum degrading taxa are implicated in this remediation process and suggest that a community of generalist bacteria can participate in this process through the acquisition of new genes carried by plasmids and viruses. These findings open new perspectives to improve bioremediation processes using metagenomic toolkits.

Plenary Sessions

MEM-PL10.02 - The skin mycobiome in human health and disease

Julia Oh[1], Keisha Findley[1], Joy Yang[1], Allyson Byrd[1], Sean Conlan[1], Clayton Deming[1], NIH Intramural Sequencing Center[2], Heidi Kong[3], Julia Segre[1]

[1]Genetics and Molecular Biology Branch, National Human Genome Research Institute, NIH, Bethesda, USA, [2]NIH Intramural Sequencing Center, National Human Genome Research Institute, Bethesda, USA, [3]Dermatology Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, USA

Foundational studies characterizing the endogenous microbial flora of our bodies have focused on bacteria, yet fungi and higher eukaryotes have major roles in community dynamics, human health, and disease. Given the lag behind bacterial methodologies targeting the 16S rRNA gene for taxonomic classification, we developed a pipeline and custom databases to analyze the fungal ITS1 region. To define broad ecological principles of the skin mycobiome, we characterized the fungal communities at multiple skin sites of healthy individuals and patients with skin diseases, also sequencing patients with atopic dermatitis(AD) and AD-like eczema associated with primary immunodeficiency syndromes. In healthy adults, 11 core body & arm sites were dominated by Malassezia with species-level compositional differences between sites. In contrast, 3 foot sites showed marked fungal diversity not mirrored by increased bacterial diversity at these sites. The more diverse and less lipophilic bacterial communities of pre-adolescent skin were accompanied by increased fungal diversity and less predominance of lipophilic Malassezia species than healthy adult skin. While AD is characterized by dramatic shifts in the bacterial microbiome, fungal diversity and community composition remained markedly stable. However, in primary immunodeficiency patients, we observed an increase in fungal diversity and an increase in rare pathogenic species Aspergillus & Candida that are associated with recurrent lung and mucosal infections in these patients. Our work, analyzing co-occurring bacterial and fungal communities, demonstrates that physiologic attributes differentially shape these communities. Our current studies address the challenges of examining bacterial and fungal communities directly with shotgun metagenomic sequencing, revealing novel functional capacities of the skin metagenome. These results provide a framework for future investigation into the fungal-bacterial interactome and its role in disease pathogenesis and maintenance of human health.

Plenary Sessions

MEM-PL11.01 - Damp buildings, fungi and the rise of non-atopic asthma

J David Miller[1]
[1]*Carleton University, Ottawa, Canada*

Twenty five years ago the first epidemiological data appeared that suggested that living in a damp house with ca. 1% of the floor area with signs of dampness and mold increased both allergic and other respiratory symptoms, regardless of atopic status. The early data indicated that increased allergy to all allergens was co-incident with mold exposure, which was certainly unanticipated. Within the last decade, these remarkable observations have confirmed in many epidemiology studies in many populations. Discerning a mechanism for these unexpected results has been a decade long journey. Very low exposures to triple helical beta 1, 3 D glucan and the metabolites that are produced by common damp building fungi up-regulate inflammatory pathways resulting in non-atopic asthma and other symptoms. This research required an investment to understand the fungi that were present, resolving their identities and characterizing many new metabolites from fungi that had largely been ignored because they are not important in food or agriculture. Very pure metabolites were used to study patterns of inflammatory response in relevant animal models leading to the identification of unambiguous markers of asthma after exposure. Providing a partial mechanism for the epidemiological data has contributed to recent stronger advice on mold from the public health, industrial hygiene, engineering and allergy community from 2012.

Plenary Sessions

MEM-PL11.02 - Fungi and the Indoor Challenge

Jan Dijksterhuis[1], Frank Segers[1], Karel Van Laarhoven[2], John E. Hallsworth[3], Mirjam Bekker[2], Henk Huinink[2], Jos Houbraken[1], Olaf Adan[2], Robert A. Samson[1]
[1]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, [2]Eindhoven University of technology, Eindhoven, The Netherlands, [3]Queens University Belfast, Belfast, UK

Fungi are present in every cubic meter of air. This can be as survival structures as spores, but there is also evidence that non- viable fungal cells and fragments of cells are present. The total number of fungal species in air is variable and indoor fungal growth is expected to change the composition of indoor fungal mixture. Recent studies demonstrate that the diversity of the mycobiota indoors is much higher than known from traditional detection methods. In addition, there are strong indications that the role of xerotolerant fungi is underestimated. If fungal spores are able to deposit and conditions become conducive for growth they may perform outgrowth. This is not an easy task, nutrients are generally low and water availability is transient as it may drop below the levels that support fungal growth. Due to these restrictions indoor fungal growth mostly occurs in another time scale as observed in the laboratory. But, fungi do grow in, most probably, the majority of houses on surprising surfaces. This contribution reflects on fungal growth, surfaces and water.

Plenary Sessions

VIR-PL06.01 - Host pathogen interactions at the maternal-fetal interface

Carolyn Coyne[1]
[1]*University of Pittsburgh, Pittsburgh, USA*

Placental trophoblasts form the interface between the fetal and maternal environments, and serve to limit the maternal-fetal spread of viruses. We have shown that cultured primary human placental trophoblasts are highly resistant to infection by a number of viruses, and importantly, confer this resistance to non-placental recipient cells by exosome-mediated delivery of specific microRNAs (miRNAs). We show that miRNA members of the chromosome 19 miRNA cluster (C19MC), which are almost exclusively expressed in the human placenta, are packaged within trophoblast-derived exosomes, and attenuate viral replication in recipient cells by the induction of autophagy. Together, our findings identify an unprecedented paracrine and/or systemic function of placental trophoblasts that utilizes exosome-mediated transfer of a unique set of placental-specific effector miRNAs to directly communicate with placental or maternal target cells and regulate their immunity to viral infections.

Plenary Sessions

VIR-PL06.02 – Innate Immune Sensing of DNA Viruses and Retroviruses

Zhijian 'James' Chen[1]
[1]HHMI, University of Texas Southwestern Medical Center, Dallas, USA

The inappropriate presence of DNA in the cytosol is a danger signal that alerts the host of potential microbial invasion and triggers innate immune responses including the production of type-I interferons. DNA can be delivered to the cytosol by functional as well as defective microbial particles such as DNA viruses and retroviruses. Under certain pathological conditions, self DNA, which normally reside in the nucleus or mitochondria, could also trigger autoimmune responses from the cytosol, resulting in human diseases such as lupus. Cytosolic DNA induces interferons through a signaling pathway that involves the adaptor protein STING, the kinases IKK and TBK1, and the transcription factors NF-kappaB and IRF3. Through a biochemical approach, we have identified the cytosolic DNA sensor that activates STING and triggers type-I interferon production. This sensor turns out to be a novel enzyme which we named cyclic GMP-AMP synthase (cGAS). cGAS is activated by its binding to DNA and upon activation it catalyzes the synthesis of a unique cyclic dinucleotide, cyclic GMP-AMP (cGAMP), which contains both 2'-5' and 3'-5' phosphodiester linkages. This cGAMP isomer, termed 2'3'-cGAMP, functions as a second messenger that binds to and activates STING, leading to the induction of interferons and other cytokines. Genetic experiments show that cGAS is essential for innate immune responses triggered by DNA viruses and retroviruses, including HIV. This work uncovers a cyclic dinucleotide signaling pathway that was previously not known to exist in metazoa, reveals a new signaling mechanism in innate immunity, provides cGAS as a new therapeutic target for the treatment of autoimmune diseases, and offers cGAMP as a candidate molecule for the development of new vaccines and immune therapeutics.

Plenary Sessions

VIR-PL06.03 - T Cell Responses during Chronic Infections

John Wherry[1]
[1]*University of Pennsylvania, Philadelphia, USA*

“No abstract available at time of publication”

Plenary Sessions

VIR-PL06.04 - Dissecting the antibody response to pathogens

Antonio Lanzavecchia[1]
[1]Institute for Research in Biomedicine, Bellinzona and Institute of Microbiology, ETH Zurich

Memory B lymphocytes and long-lived plasma cells represent a repository of the antigenic experience of an individual. By analyzing the specificity and function of these cells we can gain insights into the human immune response to pathogens and vaccines, identify correlates of protection, and isolate neutralizing antibodies and protective T cells. To interrogate human memory B cell and plasma cell repertoires we developed two culture-based high-throughput methods that are used to isolate, with high efficiency, human monoclonal antibodies of distinctive specificities. Unusually potent neutralizing antibodies against human cytomegalovirus were isolated from infected donors and used to identify the viral ligands and to design an experimental vaccine. We also isolated antibodies of exceptional breadth, such as a pan-influenza A neutralizing antibody and an antibody that neutralizes both respiratory syncytial virus and metapneumovirus. By targeting conserved structures, these broadly neutralizing antibodies are less prone to select escape mutants and are therefore promising candidates for prophylaxis and therapy of infections as well as tools for the design of improved subunit vaccines. We also isolated and characterized autoantibodies from patients with pemphigus, pulmonary alveolar proteinosis and other autoimmune diseases. By reverting the antibodies to the germline we investigate the role of somatic mutations in the generation of broadly neutralizing antibodies and autoantibodies.

Closing Ceremony and Lecture

PL12.01 - Gut microbiota: after metagenomics, experimentomics

Phillippe Sansonetti[1]
[1]*Institut Pasteur, Paris, France*

Recent metagenomic analyses have revealed the extraordinary diversity of the genes catalogues that constitute the gut human microbiome (150 x the number of human genes). This « second genome » corresponds to the stable and resilient assemblage of hundreds of species that collectively contribute the metabolic and immunological (and other) functions representing the bacterial part of the mutualistic deal established by co-evolution. From descriptive, metagenomic studies have become correlative, thus providing preliminary evidence that microbiota with abnormal gene/species composition – also called dysbiosis – were associated with disease states such as inflammatory bowel diseases, obesity and diabetes. These dysbioses are essentially marked by a decrease in gene diversity. In addition, establishment of eubiosis in the newborn and infant seems essential for child's health and early-acquired dysbioses may lead to late health consequences, such as allergy, atopy and obesity. Stemming from this descriptive and correlative period, metagenomics needs to become experimental, thus the aim to develop « experimentomics », a « cellular microbiology » of symbiosis. This new period will largely capitalize on the lessons of cellular microbiology applied to pathogens : choice of model organisms (symbiont/pathobiont and host), development of dedicated methods of reverse genetics, genome editing of host cells and animal models, establishment of in vitro and in vivo screens in combination with various omics, particularly metabolomics. Decrypting of the cross-talks that structure the symbiotic interaction will open new avenues for better understanding of homeostasis and its rupture in essential areas of physiology such as nutrition and metabolism, maturation and tuning of the immune system, tissue regeneration, brain maturation, metabolism of drugs and xenobiotics. Experimentomics is expected to produce novel biomarkers and bioreagents that may have a great impact in future translational medicine that is already marked by significant successes of fecal transplantation.