Overexpression of a *Bacillus subilis* amylase in *E.coli* and application in bread making

Karima Salem[1], Massimiliano Perduca[2], Adel Sayari[3] & Aïda Hmida-Sayari[1]

(1) Laboratory of Microbial Biotechnology and Engineering Enzymes
Center of Biotechnology of Sfax (CBS)-TUNISIA

(2) Biocrystallography and Nanostructure Laboratory, University of Verona-ITALY

(3)Laboratory of Biochemical and Enzyme Engineering of lipases (ENIS), University of Sfax-TUNISIA
E-mail: karima.selem@gmail.com

Background and aim:
Bacterial alpha-amylases (EC3.2.1.1) have potential use in wide number of industrial applications such as textile, paper, detergent, food, fermentation and pharmaceutical industries. Recombinant DNA technology for amylase production involves the selection of an amylase gene, its insertion into an appropriate vector system, transformation in an efficient bacterial system to produce high amount of recombinant protein. In this context, the aim of this work is the overexpression of an α-amylase gene from *Bacillus subtilis* US572 in *E.coli* strain, the characterization of the recombinant enzyme and test the effect of different quantities added of amylase on wheat flours and bread characterization.

Methods:
PCR "Polymerase Chain Reaction", extraction of Plasmid DNA, amylolytic activity measured by the DNS method. The purification of the enzyme carried out by Ni-NTA column affinity ™ column affinity. Texture analyzer (TPA), Alveograph, Farinograph.

Results:
The DNA of the α-1,4-endoamylase of *Bacillus subtilis* US572 was cloned and expressed in *E.coli* BL21. The maximum activity obtained was 900U mL^{-1}, which was about 4-fold higher than that obtained with the native species. The purified enzyme showed a specific activity of 664.28 U $mg^{-1}$and a molecular mass of 136.6 kDa. It had an optimal activity at pH 7 and 70 °C, stable in a wide range of pH and in the presence of some detergents and organic solvents. r-AmyKS (recombinant amylase) was used as an additive in bread making. A rheological dough properties and bread quality was investigated by the measurement of rheological and texture parameters.

Conclusion:
In this study, the amylase activity expressed in *E.coli* reached 900 U mL^{-1}, which was about 4-fold higher than that obtained with the native species *B. subtilis* US572. The r-AmyKS-His6 was incorporated in wheat breads leading to a significant improvement of bread characteristics.

Keywords: Alpha-amylase, overexpression, *E.coli* BL21, purification, biochemical characterization, bread making.