

A POLYPHASIC APPROACH FOR DEREPLICATION AND CHARACTERIZATION OF WOOD ENDOPHYTIC BACTERIA WITH BIO-PROTECTIVE POTENTIAL



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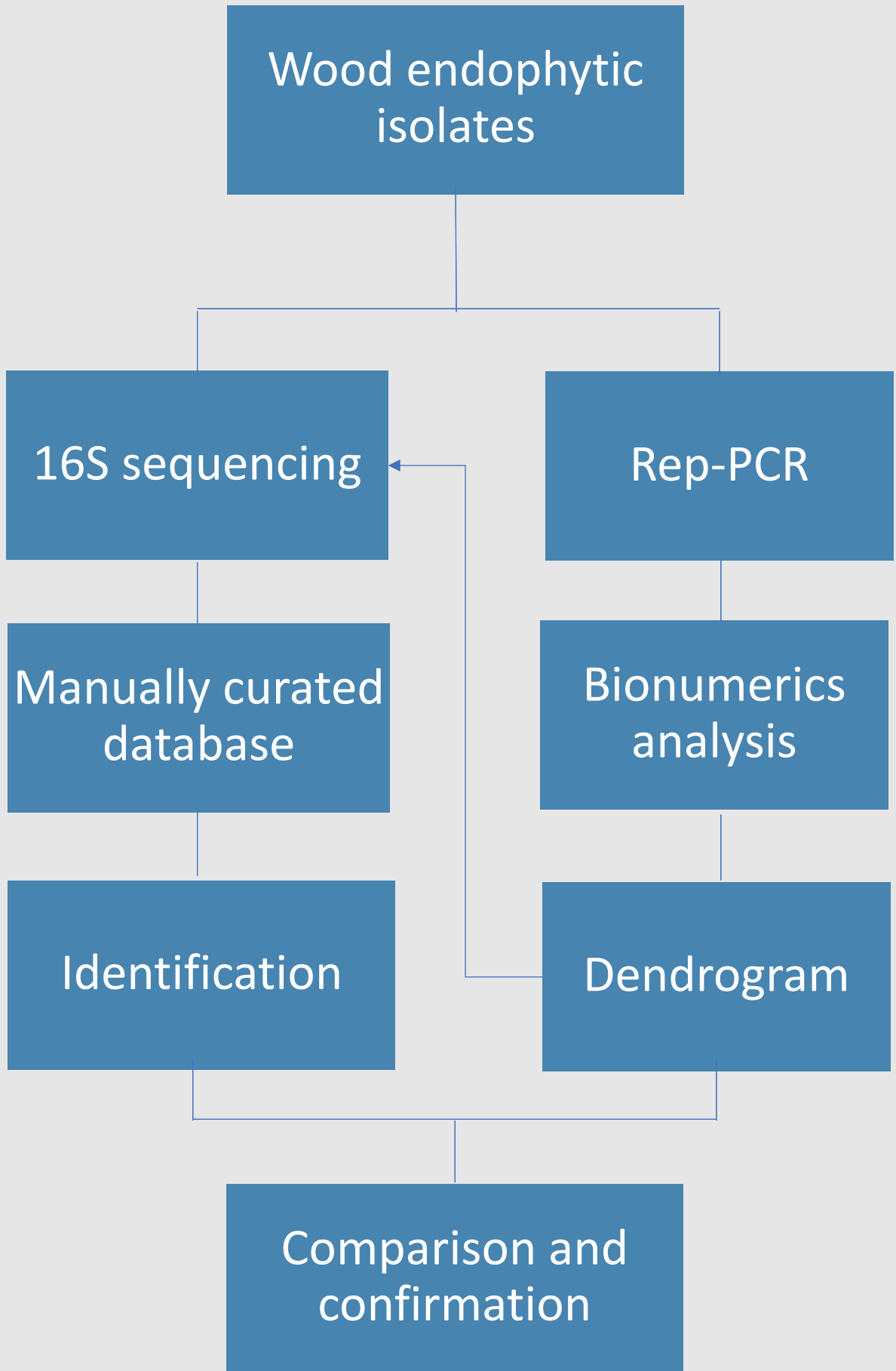


CONTEXT AND AIMS

The work hereby presented was carried out within a research project focusing on the exploration of the bio-protective potential of some bacterial isolates, mainly wood-endophytic Actinobacteria, against *Botrytis cinerea*, in the context of post-harvest biocontrol on fruits. The aim of this work was to evaluate the discriminatory power of a tailored method, combining 16S rDNA sequencing and Rep-PCR, to determine the isolates differentiation and their identification at genus level or below.

EXPERIMENTAL DESIGN AND METHODS

Fifty isolates were recovered from wood of different grapevine cultivars and cultured using appropriate growth media. The genomic characterization of isolates was firstly undertaken on 16S rDNA gene sequencing. The obtained sequences were compared against a proofread, robust and previously manually curated database through BLASTn algorithm. Then, the Rep-PCR was chosen as molecular technique to dereplicate the isolates using the amplification of conserved sequences distributed in the bacterial genome, with primers such as GTG5 and BOX-A1R. Then, fingerprinting outputs were analyzed using the software Bionumerics.



RESULTS

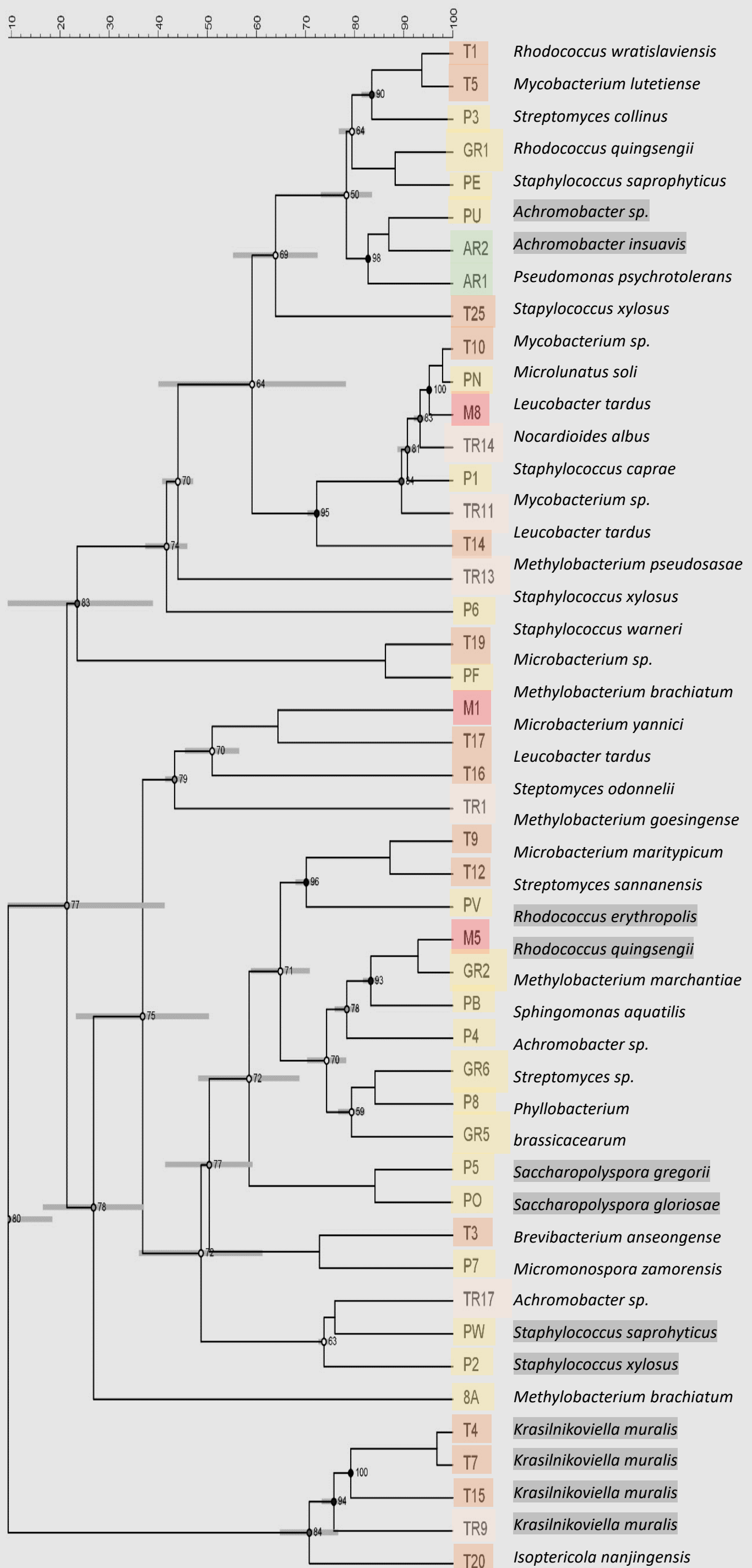
Phylum	Genus	Species	Isolate	Source of isolation	Year	
Betaproteobacteria	<i>Achromobacter</i>	<i>insuavis</i>	AR2	Arneis	2020	
		<i>sp.</i>	GR6	Garganega		
			TR17	Tocai roots		
			PU	Garganega		
Actinobacteria	<i>Brevibacterium</i>	<i>anseongense</i>	T3	Tocai	2020	
Actinobacteria	<i>Isoptericola</i>	<i>nanjingensis</i>	T20	Tocai	2020	
Actinobacteria	<i>Krasilnikoviella</i>	<i>muralis</i>	T4	Tocai	2020	
			T7			
			T15			
			TR9	Tocai roots		
Actinobacteria	<i>Leucobacter</i>	<i>tardus</i>	M8	Merlot	2020	
			T14	Tocai		
			T16			
Aphaproteobacteria	<i>Methylobacterium</i>	<i>marchantiae</i>	PB	Garganega	2021	
		<i>brachiatum</i>	8A	Merlot	2020	
		<i>pseudosasa</i>	TR13			Tocai roots
		<i>goesingense</i>	T9			Tocai
Actinobacteria	<i>Microbacterium</i>	<i>sp.</i>	PF	Garganega	2021	
		<i>yannici</i>	T17	Tocai	2020	
		<i>maritypicum</i>	T12			
		Actinobacteria	<i>Microlunatus</i>	<i>solii</i>	PN	Garganega
Actinobacteria	<i>Micromonospora</i>	<i>zamorensis</i>	P7	Garganega	2021	
Actinobacteria	<i>Mycobacterium</i>	<i>sp.</i>	TR11	Tocai roots	2020	
			T10	Tocai		
		<i>lutetiense</i>	T5			
Actinobacteria	<i>Nocardioiides</i>	<i>albus</i>	TR14	Tocai roots	2020	
Aphaproteobacteria	<i>Phyllobacterium</i>	<i>brassicacearum</i>	GR5	Garganega	2020	
Gammaproteobacteria	<i>Pseudomonas</i>	<i>psychrotolerans</i>	AR1	Arneis	2020	
Actinobacteria	<i>Rhodococcus</i>	<i>quingsengii</i>	GR1	Garganega	2020	
		<i>wratislaviensis</i>	GR2	Tocai		
			T1			
			<i>erythropolis</i>			M5
Actinobacteria	<i>Saccharopolyspora</i>	<i>gregorii</i>	P5	Garganega	2021	
		<i>gloriosae</i>	PO			
Aphaproteobacteria	<i>Sphingomonas</i>	<i>aquatilis</i>	P4	Garganega	2021	
Actinobacteria	<i>Staphylococcus</i>	<i>saprophyticus</i>	PE	Garganega	2021	
		<i>caprae</i>	PW			
			P1			
			P2			
		<i>xylosus</i>	P6	Tocai	2020	
			T25			
<i>epidermidis</i>	T24					
Actinobacteria	<i>Streptomyces</i>	<i>warneri</i>	T19			
		<i>odonnellii</i>	TR1	Tocai roots	2020	
		<i>sp.</i>	P8	Garganega	2021	
		<i>sannanensis</i>	PV			
Actinobacteria	<i>Streptomyces</i>	<i>collinus</i>	P3			

As a first result, thanks to 16S sequencing, 100% of the isolates were identified at the genus level and 80% of them reached the species level. The outcome showed that 90% of taxa belonged to the Actinobacteria phylum, Gram-positive and GC rich. Bacteria were isolated from different cultivars and sources, furthermore the analysis of the 16S identified 19 genera and 31 species.

For this reason, the Rep-PCR was chosen as molecular technique to dereplicate the isolates taking advantage from the well-known power to discriminate bacteria using the amplification of conserved sequences distributed throughout the genome.

The results of Rep-PCR were compared obtaining a dendrogram with the threshold of 95% similarity as cut-off for dereplication.

The comparison of the results between the two methods could show that Rep-PCR can go further the species attribution for strain dereplication in this database. In fact, in some cases in which the isolates were identified as the same species with 16S sequencing, it displayed the actual presence of different strains within the same species (e.g., 3 on 4 isolates for *K. muralis*).



CONCLUSIONS

In this work, we have shown how a polyphasic approach was useful for the correct characterization of our collection. In fact, the combination of the two tested methods can go further than the simple identification, displaying the correlation between two or more isolates. In this way, we were able to obtain a good dereplication for endophytic bacteria, together with a reliable genetic identification, both essential preconditions to make a correct assessment of bio-protection.