Long-read metabarcoding approach for diagnosis and epidemiology in genetically heterogeneous *Prunus* sp. orchards

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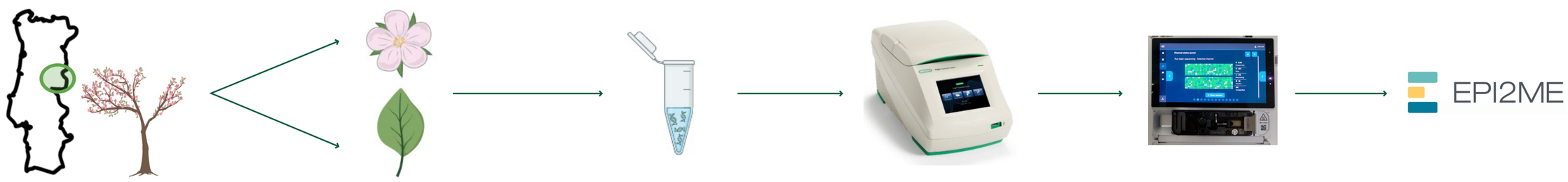
INTRODUCTION

Diagnostic challenges are increasing as globalisation and climate change favour the introduction of new plant pests. *Xylella fastidiosa, Xanthomonas arboricola, Pseudomonas syringae* and *Monilinia fructicola* are important pests affecting Prunus production with significant losses. Two different Prunus production systems were analysed: old orchards of *P. dulcis* and coexisting orchards of *P. avium* and *P. persica*, where significant areas of *P. dulcis* have recently been established. The high density of genetically heterogeneous *Prunus sp*. in which pests can coexist favours the development of highly adapted strains and/or the occurrence of coevolutionary phenomena associated with the spread and emergence of new diseases. This coexistence of phylogenetically close strains with drastically different phenotypes is a critical diagnostic challenge.

OBJECTIVE

In this context, the aim was to conduct molecular epidemiology studies by characterising the microbiota of *P. avium* and *P. persica* leaves and flowers using the Oxford Nanopore Technologies (ONT) long-read sequencing platform to identify bacteria and fungi. This strategy aims to detect the introduction and impact of pathogens on the structure and functions of the microbiota, and to identify taxonomic groups relevant to modulating the microbiota as part of potential bio-based solutions for disease control.

METHODS



1. Sampling in Cova da Beira orchards of *P. avium* and *P. persica*
2. Processing of flowers and leaves (collected in spring and autumn)
3. DNA extraction by the CTAB method
4. 16S rDNA and ITS18S-ITS1-5.8S-ITS2-28S amplification
5. Long chain sequencing ONT
6. Identification with the EPI2ME platform

RESULTS *PRELIMINARY*

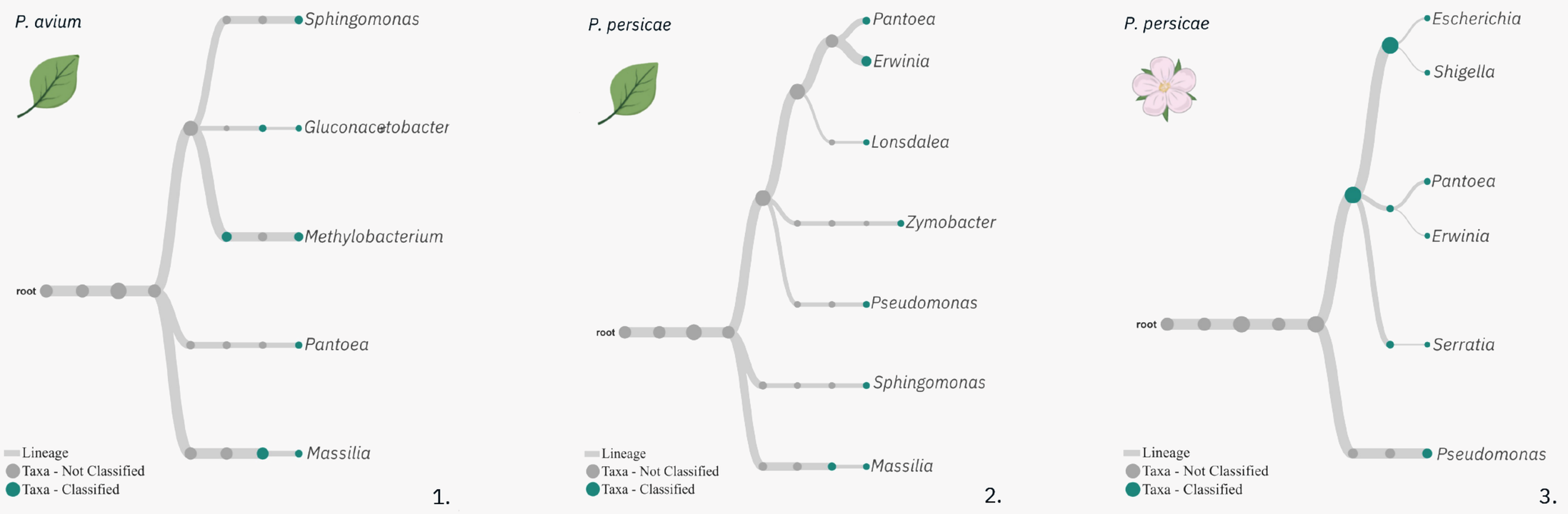


Figure 1. Identification by 16S rDNA sequencing of *Prunus avium* (1) and *P. persica* (2) leaf and *P. persica* flowers (3) microbiota using the EPI2ME platform.

DISCUSSION *PRELIMINARY*

Preliminary results showed that, in contrast to the leaf microbiome among Prunus species, significant differences were found between the flower and leaf microbiomes. Important pathogen-related groups were detected with significant impact on the microbiome structure, supporting the use of this approach for large screening phytosanitary surveys. It was possible to identify a microbiome common to the leaves and flowers of the *Prunus* sp. species analysed - *Massilia* sp., *Pantoea* sp. and *Sphingomonas* sp.

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4th European conference on Xylella fastidiosa 2023

Endophytic microbiomes of wild plants susceptible to *Xylella fastidiosa* infection

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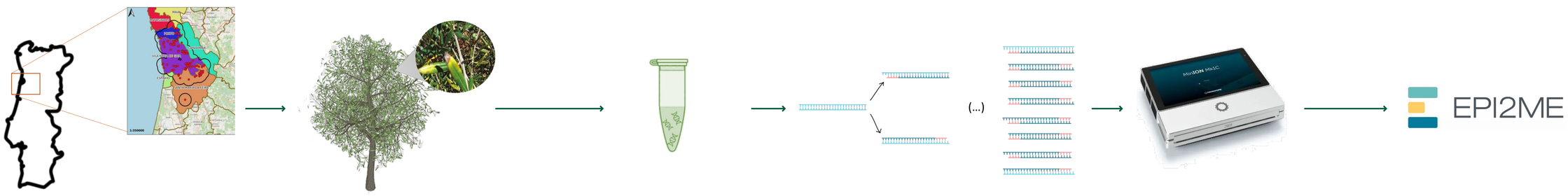
INTRODUCTION

Xylella fastidiosa (Xf) is a phytopathogenic bacterium responsible for a wide range of diseases with high economic, environmental and social impacts. The plant-associated microbiome plays a central role in maintaining fitness, but little is known about the diversity and structure of these endophytic microbial communities. It is therefore essential to study this diversity and the interactions between endophytes and plants in order to understand the biotechnological potential of these microorganisms.

OBJECTIVE

In this context, we aim to characterise the structural diversity of endophytic microorganisms in wild plant species susceptible to Xf infection and present in the demarcated area of Portugal.

METHODS



1. Sampling of wild plants in the focus area
2. Leaf and branch processing
3. DNA Extraction
4. Amplification of 16S rRNA gene with PNAs
5. Sequencing of 16S rRNA gene on Oxford Nanopore Technologies platform
6. Identification with the EPI2ME platform

RESULTS *PRELIMINARY*

SAMPLE	HOST	MOST ABUNDANT SPECIES				
42B	*Adenocarpus sp.*	*Caballeronia sordidicola*	*Caballeronia terrestris*	*Massilia putida*	*Burkholderia catarinensis*	*Massilia pinisoli*
43L	*Lavandula dentata*	*Ralstonia pickettii*	*Bacillus mycoides*	*Delftia lacustris*	*Staphylococcus epidermis*	*Staphylococcus saccharolyticus*
45L	*Dimorphoteca*	*Xylella fastidiosa*	*Ralstonia picketii*	*Xhantomonas campestris*	*Duganella zoogloeoides*	*Delftia lacustris*
45B	*Dimorphoteca*	*Xylella fastidiosa*	*Ralstonia picketii*	*Staniera cyanosphaera*	*Aliterella antarctica*	*Loriellopsis cavernicola*
46B	*Gazania sp.*	*Xylella fastidiosa*	*Stanieria cyanosphaera*	*Aliterella antarctica*	*Loriellopsis cavernicola*	*Caballeronia sordidicola*
85L	*Cytisus scoparius*	*Xylella fastidiosa*	*Liberibacter crescens*	*Diplorickettsia massiliensis*	*Xanthomonas campestris*	*Pseudomonas boreopolis*
193L	*Dimorphoteca*	*Aliterella antarctica*	*Xylella fastidiosa*	*Staniera cyanosphaera*	*Caballeronia sordidicola*	*Ralstonia pickettii*
193B	*Dimorphoteca*	*Aliterella antarctica*	*Staniera cyanosphaera*	*Xylella fastidiosa*	*Loriellopsis cavernicola*	*Caballeronia sordidicola*
197L	*Gazania sp.*	*Aliterella antarctica*	*Staniera cyanosphaera*	*Ralstonia pickettii*	*Loriellopsis cavernicola*	*Xylella fastidiosa*
198L	*Dimorphoteca*	*Stanieria cyanosphaera*	*Aliterella antarctica*	*Xylella fastidiosa*	*Caballeronia sordidicola*	*Loriellopsis cavernicola*
198B	*Dimorphoteca*	*Xylella fastidiosa*	*Caballeronia sordidicola*	*Aliterella antarctica*	*Stanieria cyanosphaera*	*Stenotrophomonas rhizofhila*
199B	*Adenocarpus sp.*	*Caballeronia sordidicola*	*Caballeronia terrestris*	*Caballeronia udeis*	*Terriglobus aquaticus*	*Staphylococcus saccharolyticus*

Legend: B - Branches; L - Leaves

Table 1. Most common species in each of the wild host species.

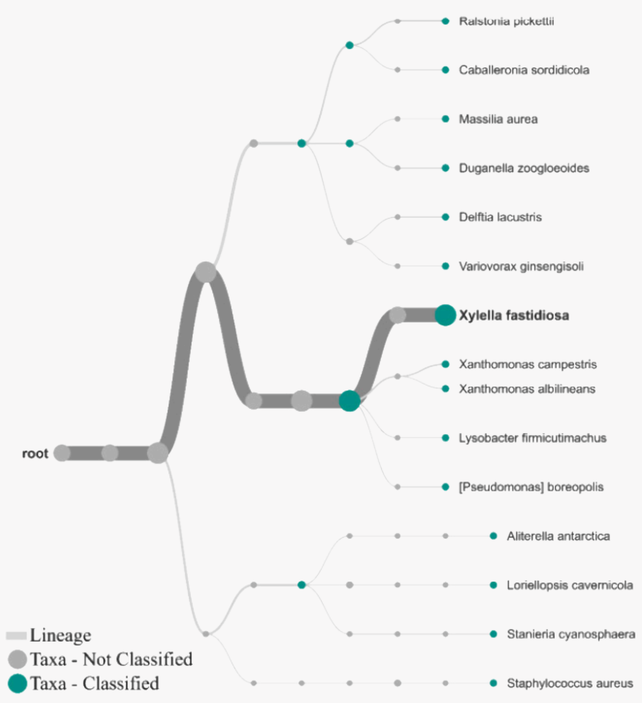


Figure 1. Identification by metabarcoding of the 16S rRNA gene of sample 45L using the EPI2ME platform.

DISCUSSION *PRELIMINARY*

The results suggest a functional importance of some core groups of the microbiome, related to the ability to degrade toxic substances, fix nitrogen and promote plant growth. They may also help to explain the lack of visible symptoms and signs of decline in these wild plants, suggesting that certain taxonomic endophyte groups may help to model infection. Notably, this is the first study in wild plants and the identification of relevant clusters contributes to the understanding of the impact of Xf on microbiome dysbiosis in nature.

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