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BOOK OF ABSTRACTS

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The composition of apple and pear bark microbiota suggest microbial migrations from soil

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Highlights
- The migration of soil microbial communities possibly define the bark microbiota
- The bark microbiota is affected by the bark age and plant species

Introduction

The study of plant-associated microbial communities has mainly been focused on soil and rhizosphere habitats, rather than the aerial part of the plant (Vorholt, 2012). Soil represents a reservoir of microorganisms (Martins et al., 2013) that may migrate to the plant phyllosphere trough rain splash, wind or agricultural practices (Zarraonaindia et al., 2015), but scarce information is available on the relations between the bark and soil microbiota. Despite the importance of bark as a potential habitat of plant pathogens and biocontrol agents (Buck et al., 1998), knowledges on composition and dynamics of its microbial communities are lacking. The aim of this work was to optimise a method for the analysis of the bark-associated fungal and bacterial microbiota and to assess the influence of plant genotypes and bark age on its composition.

Material and methods

Bark samples were collected using a fire-sterilised scalpel from one year-old shoots (new) and 3-4 years-old branches (old) of Abate and Williams pear varieties and Golden and Gala apple varieties before budding. Each sample consisted of a pool of five plants and three replicates were collected for each variety. Bark samples were processed and the viability of culturable fungi and bacteria was assessed using the classical plating method to determine the number of colony forming units (CFU) per unit of bark fresh weight (CFU/g). DNA was extracted from the ground samples using the FastDNA spin kit for soil (MP Biomedicals). The internal transcribed spacer 2 (ITS2) and the V5-V7 region of 16S rDNA were amplified and libraries were sequenced using the Illumina MiSeq technology in order to identify fungi and bacteria, respectively. A PERMANOVA analysis was carried out in order to assess the influence of bark age, plant variety and plant species on the composition of fungal and bacterial communities. Pear and apple bark microbiota was screened for the presence of potential plant pathogenic and beneficial genera.

Results and discussion

The amount of culturable fungi and bacteria was higher in new as compared with old barks. In addition to the bark age, the number of fungal CFU was also affected by the plant species and apple

variety, while the number of bacterial CFUs was affected by the apple variety. After quality filtering, detection of chimeric, singleton and plant sequences, a total of 2,050,096 and 2,757,400 sequences and a total of 430 and 824 operational taxonomic units (OTU) were detected for fungi and bacteria, respectively. A PERMANOVA analysis revealed that the diversity of fungal and bacterial communities was influenced by the bark age, plant variety and plant species. The dominant fungal microbiota was composed by *Alternaria* and *Cryptococcus* with consistent abundance among bark samples. Conversely, the abundance of *Aureobasidium* and *Sporobolomyces* was higher in new as compared with old barks, while that of *Cystobasidium* and *Rhodotorula* was lower. Moreover, the dominant genera *Phaeosclera* was more abundant in apple barks as compared with pear barks. The bacterial microbiota was mainly composed by *Deinococcus* and *Frondinhabitans* that showed consistent abundance among bark samples. Moreover, the abundance of *Amnibacterium*, *Curtobacterium* and *Hymenobacter* was higher in new as compared with old barks, while that of *Massilia*, *Modestobacter* and *Sphingomonas* was lower.

Soil-derived fungal (*Alternaria*, *Cryptococcus*) and bacterial (*Massilia*, *Microbacterium*, *Solirubrobacter*, *Terrimonas*) genera (O' Brien et al., 2005; Nicola et al., 2017) were found on apple and pear barks, demonstrating that the bark microbiota possibly originated from soil microbiota. Particularly, genera that include potential pathogens for pear and apple were found, such as fungal agents of bark (*Diplodia*), root (*Rosellinia*), leaf (*Alternaria* and *Taphrina*) and fruit diseases (*Gibberella*, *Peltaster*, *Penicillium*, and *Stemphylium*). However, beneficial genera with potential biocontrol or plant growth promotion activities were found both for fungi (*Aureobasidium*, *Coniothyrium*, *Cryptococcus*, *Rhodotorula,* and *Sporobolomyces*) and bacteria (*Arthrobacter*, *Deinococcus*, *Lactobacillus*, *Pedobacter*, *Cohnella*, and *Promicromonospora*) on apple and pear barks.

This method allowed to study the viability and the structure of fungal and bacterial communities of bark and to assess factors that affect the microbiota composition. The presence of fungal and bacterial genera typically belonging to the soil microbiota suggests that bark communities are possibly influenced by migration of soil microorganisms. Moreover, bark could represent a reservoir of plant pathogens and beneficial microorganisms.

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