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Exploring the diversity of the root-associated microbiome of *Ilex paraguariensis* St. Hil. (Yerba Mate)

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ABSTRACT

Ilex paraguariensis St. Hil. (Yerba Mate) is an important crop for which a decrease in yields associated to unsustainable agricultural practices is well documented. The aim of this study is to investigate the diversity of bacteria and fungi inhabiting roots of Yerba Mate. This is an important pre-requisite for the use of microorganisms inhabiting roots to modulate plant nutrition and health as an ecologically friendly agricultural alternative for this crop. The diversity of the root-associated microbiome from eleven plantations with different agricultural practices was analyzed by high throughput sequencing of the 16S rRNA gene as a bacterial marker, whereas the fungal communities were targeted by amplifying the ITS region of the ribosomal RNA gene cluster. A comparison of the bacterial and fungal communities between plantation sites and cultivation practices was made to address the major factors contributing to the structure of the root microbiome of this crop. Operational taxonomic units (OTUs) related to well-known plant growth promoting bacteria such as *Burkholderia, Bradyrhizobium, Weissella, Enterobacter* and *Rhizobium* were detected. Those might constitute targets for future enrichment efforts of plant growth promoting clades. The analysis of the fungal community composition demonstrated that arbuscular mycorrhizae colonize Yerba Mate roots, and that the frequency of this group is favored in degraded soils. The detection of other groups harboring potential phytopathogens might help to broaden the understanding of the ailments affecting this crop. This study provides the first description of the root-associated microbiome of Yerba Mate and constitutes a stepping-stone towards harnessing the role of microbes in the sustainable cultivation of this crop.

1. Introduction

Degradation of agricultural soils is known to significantly impact crop productivity around the globe (Bindraban et al., 2012). Land degradation affects seriously the productivity of *Ilex paraguariensis* St. Hil. (Yerba Mate, Aquifoliaceae). Yerba Mate is a native tree from Northeastern Argentina, Paraguay, South Brazil and part of Uruguay (Grondona, 1954). The leaves of Yerba Mate are used to produce an energizing beverage widely consumed as an alternative to coffee. Aside South America, beverages derived from Yerba Mate are becoming increasingly popular in others regions, including the Middle East, Europe, and the United States, where it is appreciated as a natural energizing drink due to its high content of antioxidants and its nutritional benefits (Heck and De Mejia, 2007). Currently, soil degradation due to inadequate agricultural practices maintained over time is affecting a considerable fraction of the area of Yerba Mate production (INYM-INTA, 2008). This is the case in Misiones (INYM-INTA, 2008), the leading productive region of this crop in Argentina. In order to maintain and improve soil quality, more sustainable agricultural practices have been implemented for Yerba Mate including zero-tillage, use of green covers and of associative trees (Burtnik 2003; Day et al., 2011; Ilany et al., 2010).

In agricultural soils, the microbiome associated to plant roots play a key role in regulating plant growth through the production of phytohormones (Spaepen and Vanderleyden, 2011) or facilitating access to mineral nutrients (Rodrigues et al., 2008). In other cases, the soil microbiome provides plant protection by exerting antagonistic effects against phytopathogens (Compant et al., 2005). Currently, high-throughput DNA sequencing approaches

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are used to analyze the root-associated microbiome of different plant species in order to reveal the taxonomic and functional diversity of microbes involved in plant growth promotion (Hacquard et al., 2015). Such culture-independent methods can help to discover potentially beneficial microbial taxa that could be subsequently isolated. For instance, the isolation and selection of diazotrophic bacteria associated to sugarcane was based on the enriched taxa present in the roots of this crop (Paungfoo-Lonhienne et al., 2014).

The root-associated microbiome of Yerba Mate remains unexplored and its analysis might constitute a guide for the isolation and selection of particular microbial taxa as potential bio-inoculants contributing to plant nutrition and protection in low-input soils. In a previous study, it has been shown that bio-inoculation with plant growth promoting rhizobacteria (PGPR) isolated from Yerba Mate roots increased the biomass yields of seedlings in nursery (Bergottini et al., 2015). In addition, endomycorrhizal associations in this crop have been reported in native trees of the Atlantic forest (Andrade et al., 2000) and in seedlings cultivated in nurseries (Gaiad and Lopes, 1986). We hypothesized that the root-associated microbiome of Yerba Mate may present microbial taxa involved in functions of plant growth promotion and protection. According to this, the aim of this study was to analyze the root-associated microbiome of Yerba Mate in plantations with different agricultural management practices and located at different sites within the province of Misiones, the main productive area of this crop in South America. The bacterial and fungal communities of the root microbiome were compared between plantation sites and cultivation practices to address the major factors contributing to structure the root microbiome in this crop.

2. Material and methods

2.1. Roots collection and soil sample analysis

Eleven plantations located across the principal region of Yerba Mate production in Northeast Argentina were selected to analyze the root-associated microbiome of Yerba Mate. The selected plantations presented different historical agricultural management practices and contrasting productivity yields. A detailed description of each plantation is given in Table 1. In each plantation, seven plants were randomly selected to collect three subsamples of roots (up to a depth of 10 cm) per plant and pooled to constitute one sample per plantation. Bulk soil samples were collected to determine extractable phosphorus (Bray and Kurtz, 1945), nitrogen (by the semi-micro-Kjeldahl method) (Kjeldahl, 1883), potassium (K), sodium (Na), magnesium (Mg), and calcium (Ca) (measured in an ammonium acetate extract) concentrations. The soil chemical properties are described in Supplementary Table 1. The sampling was held in the winter season of July 2013. Mean annual temperature (MAT) values were 19.7 °C in Santo Pipó, 14.3 °C in Jardín América, and 19.7 °C in Andresito. Mean annual precipitation (MAP) values were 1720.5 mm in Santo Pipó, 1857.4 mm in Jardín América, and 2341.0 mm in Andresito.

2.2. DNA extraction, PCR and pyrosequencing

To access the root-associated microbiome, roots were washed with sterile distillated water (under a laminar flow cabinet) in order to remove the rhizospheric soil fraction not closely attached to Yerba Mate roots. One gram of washed roots per sample was processed for DNA extraction with the FastDNA Spin Kit for Soil (MP Biomedicals, California) according to the manufacturer's instructions. Amplicon generation and further 454-pyrosequencing analyses were performed by Eurofins Genomics GmbH (Switzerland). To target the bacterial communities the 16S rRNA gene was amplified with the primer pair 27F (Lane, 1991) and 1492R (Stackebrandt and Liesack, 1993) whereas the fungal communities were targeted by amplifying the ITS region with the primer pair ITS1-F/ITS4 (White et al., 1990; Gardes and Bruns 1993). The sequencing of the 16S rRNA gene and the ITS region was performed unidirectionally using the forward primers.

2.3. Sequence processing

Bacterial and fungal amplicon sequences were analyzed independently, using the mothur software version 1.34.4 (Schloss et al., 2009). Bacterial reads were processed largely following the Schloss standard operating procedure (Schloss et al., 2011). First, sequencing errors were reduced by implementation of the AmpliconNoise algorithm (minflows = 360 and maxflows = 720) and low-quality sequences were removed (minimum length of 360 bp, allowing 1 mismatch to the barcode, 2 mismatches to the primer, and homopolymers no longer than 8 bp). Barcode and primer sequences were removed. Subsequently sequences were

Table 1
Agricultural management of the plantations selected to investigate the diversity of the root-associated bacterial and fungal communities of Yerba Mate (*Ilex paraguariensis* St. Hil.).

Plantation location[b]	Latitude	Longitude	Productivity[a]	Agricultural practices			Age of plants
				P fertilization (kg/ha)	N fertilization (kg/ha)	Type of plantation	
AI1	25°49′58.80″S	53°55′56.32″W	Medium	15	72	Monoculture, tillage-zero, green covers	25
AI2	25°50′1.28″S	53°.55′56.00″W	High	25	125–150	Monoculture, tillage-zero, green covers	25
AI3	25°50′4.25″S	53°55′54.76″W	High	25	125–150	Monoculture, tillage-zero, green covers	25
AL	25°47′40.15″S	53°58′26.69″W	Low	0	0	Monoculture, conventional tillage, no fertilization, no green covers	25
AM	25°50′1.21″S	53°56′8.87″W	Medium	15	72	Rainforest converted into an agroforestry system with native tree, tillage-zero, green covers	25
AA	25°50′0.76″S	53°55′51.11″W	Medium	15	72	Co-cultivated with few tree species, native green covers	25
JA1	26°59′32.67″S	55°14′01.68″W	Medium	15	72	Monoculture, tillage-zero, green covers	25
JA2	26°59′35.43″S	55°14′2.15″W	High	25	125–150	Monoculture, tillage-zero, green covers	25
JA3	26°59′34.75″S	55°14′0.96″W	High	25	125–150	Monoculture, tillage-zero, green covers	25
SPB	27°8′18.07″S	55°25′27.11 ″W	Low	10	50	Monoculture, conventional tillage, fertilization, no green covers	30
SPA	27°6′10.96″S	55°18′55.26″W	Medium	10	75	Co-cultivated with few tree species, native green covers	30

[a] High: 16.000-18.000 kg/ha, Medium: 13.000 kg/ha, Low: 7.000 kg/ha.
[b] Plantation location: Andresito (A), Jardín América (JA), Santo Pipó (SP).