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Culture-Independent Characterization of Citrus Rhizospheric Bacterial and Fungal Microbiota from Piura, Peru

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Abstract

The "*limón sutil*" (*Citrus aurantifolia*) has been widely cultivated and well established for many years in Piura, northwestern Peru, because of its exceptional climate and soil conditions. However, decline and death of *C. aurantifolia* trees caused by different phytopathogens remain a common problem which has been observed in the last decades. It is known that the microbiota of soil plays an important role with their host and could be the starting point to understand the causes of citrus decline. In this study, we identified through culture-independent methods the bacterial and fungal microbiota associated to *C. aurantifolia*, *C. jambhiri* and *C. volkameriana* rhizospheres in the main areas of Piura. By using a 16S rRNA and ITS-metabarcoding analysis, we evaluated the taxonomic diversity between healthy trees and with decline symptoms and how this diversity could influence the health status of citrus trees. More than 600 and 200 bacterial and fungal ASVs were identified, respectively. Our metabarcoding analysis was able to identify *Proteobacteria, Cyanobacteria, Firmicutes, Bacteroidota* and *Acidobacteriota* prokaryotic phyla, while fungal phyla included *Ascomycota, Basidiomycota* and *Glomeromycota*. In addition, there were differences between microbial diversity indices in rhizospheres evaluated. Finally, bacterial and fungal genera were shared among the different citrus rhizospheres. These results have allowed us to obtain a preliminary identification of microbiota in the citrus rhizospheres of healthy trees and with decline symptoms.

Keywords: *Citrus aurantifolia*, *limón sutil*, Rhizosphere, Decline, Microbiota, Bacterial and Fungal Diversity, Non-culturable, Metabarcoding, Next Generation Sequencing

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INTRODUCTION

Citrus aurantifolia (Christm.) Swingle, known in Peru as "*limón sutil*" (the Key lime is called "*limón*" in much of South America and "*sutil*" from phonetic derivative "*ceuti*" gentilic term from Ceuta),[1] is an important citrus plant widely cultivated in coast and Amazonian regions of Peru. The "*limón sutil*" has been well established for many years in Piura, northwestern Peru, because of its exceptional agrometeorological and soil conditions, which allow for growing, flowering and fruiting throughout yearly. It is a highly valued plant for its culinary, industrial, nutritional, antioxidant and antimicrobial properties.[2,3] In 2022, Piura was main producer region with 204,000 t, allowing to achieve US$ 33 million in exports.[4]

The "*limón sutil*" grafted onto "*limón rugoso*" rootstocks (*C. jambhiri* or *C. volkameriana*), thrives in Piura in full sun in a warm and humid climate with moderate annual rainfall. Although it is very well adapted to unfavorable conditions such as drought, it succumbs to adverse climatic events such as "*El Niño*" phenomenon.[5] The diseases can be a limiting factor in these scenarios, mainly due to the proliferation of phytopathogens at the ground level and the opportunism of other microorganisms in the upper parts of the plant.[5-8]

This has led to identifying a typical problem of progressive decline and death of *C. aurantifolia* trees in Piura over the last two decades. It is believed to be caused by pathogens mainly from soil. *Phytophthora* spp., *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* have been associated with root rot,[6] and infestations of nematodes have also been described.[9] On the other hand, Citrus Tristeza Virus has been associated with citrus decay, whilst Huanglongbing (HLB), no presence in Peru, have made it possible to reinforcement the national surveillance of phytoplasmas-transmitting vectors.[10,11] Overall, different aspects have to be considered in the management and control of citrus decline.

It is known that certain soil microorganisms, interact with plants in an extraordinary manner generating physiological benefits for the host.[12] However, it is still poorly understood how microorganisms couple to plant tissues.[13] Microbial colonization of plant tissues generally begins with the establishment of microorganisms in the rhizosphere.[14-16] Some studies have shown that the microorganisms in the rhizosphere can influence in citrus plant physiology and health through different mechanisms as nutrient supply, phytohormone production and antimicrobial compounds synthesis.[14,16-18] All these features are mainly due to the formation of a complex community known as microbiome.

The microbiome of rhizosphere has a high taxonomic diversity of bacteria and fungi that inhabit it. Culture-independent approaches have shown that microbiota diversity of rhizosphere is highly underestimated. Next Generation Sequencing (NGS) have demonstrated that only a minority of bacteria (< 5%) are culturable and a significant proportion of bacterial taxa detected by these technologies are unidentified.[19-22] A few studies have characterized the microbiome of citrus rhizosphere[23] and how plant health can be impaired when certain pathogens indirectly alter the host-microbiome interaction at the rhizosphere level.[24] In this study, by culture-independent methods, we characterized and compared the bacterial and fungal microbiota associated with rhizospheres of *C. aurantifolia*, *C. jambhiri* and *C. volkameriana* as a starting point to shed light on the possible causes about decline of citrus trees in Piura.

MATERIALS AND METHODS

Sampling and plant material

The estates evaluated were located producing-areas of citrus in Chira valley, Sullana, Piura (Figure S1; Table S1). In this valley, the "*limón sutil*" grafted onto a "*limón rugoso*" rootstock (*C. aurantifolia*/*C. jambhiri*) is the predominant plantation. Two kinds of plant status were evaluated:i) healthy trees with foliage, trunk and production remarkable and ii) decline or dieback trees (Figure 1). Also, *C. jambhiri* and *C. volkameriana* healthy trees, were evaluated.

For each tree, four sub-samples were collected around the trunk at 25 cm depth using a shovel previously disinfected with 1.5% NaClO. The sub-samples were homogenized together

until reaching one sample of 200 g (soil and roots) stored in zip lock bags. All samples were stored at 4°C and processed within 24 h.

Five samples for each citrus were collected: 1) *C. aurantifolia*/*C. jambhiri* healthy trees (code 01ML); 2) *C. aurantifolia* / *C. jambhiri* decline symptoms trees (code 05ML); 3) *C. jambhiri* healthy trees (code 02ML) and 4) *C. volkameriana* healthy trees (code 06ML). A total of twenty samples for four kinds of rhizospheres were analyzed in this study.

DNA extraction, metabarcoding and sequencing

The samples of roots were recovered and pooled (from the five samples of each citrus tree) and washed using 100 ml PBS (1%) buffer in 250 ml bottles with strong shaking. The soil adhered that was washed off from the roots (rhizosphere) was stored into 50 ml tubes. The metagenomics DNA was extracted from 250 mg rhizosphere using a DNeasy® PowerSoil Kit (QIAGEN, Germany) following the manufacturer's guidelines. The DNA concentrations were quantified using a BioPhotometer UV/Vis Spectrophotometer (Eppendorf, Germany) at a wavelength of 260 nm and adjusted to 10 ng/µl for subsequent analyzes. Aliquots of 25 µl of DNA were used for PCR amplification of V4 and ITS1 regions belonging to the 16S rRNA and ITS genes using primers from Walters et al.[25] The samples were purified using calibrated AMpure XP beads (Beckman Coulter, USA). Then, purified PCR products were used to prepare Illumina DNA library. The sequencing was performed at MR DNA (www.mrdnalab.com, USA) using Illumina MiSeq Systems, following the manufacturer's guidelines.

Bioinformatics and statistical analysis

Raw reads generated using 2 × 250 Illumina Miseq, were evaluated with FastQC v. 0.11.2 and demultiplexed on the Galaxy web platform (http://usegalaxy.org).[26] Removals of barcodes and primers were established according to the configuration of HEADCROP parameters (30 bp) of Trimmomatic v. 0.36.[27] The quality filtration was processed using the DADA2 workflow for bacteria and or fungi,[28] implemented in R v. 3.4.3 software. The filtered sequences were assigned to six taxonomy levels (from phylum to genus) through assignTaxonomy, using the SILVA database v. 138 for bacteria and UNITE ITS database v.8.2 for fungi. The final result was an amplicon sequence variant (ASV) table. The taxonomic analysis of the microbial diversity was developed through the Phyloseq v. 1.24.2 package.[29] The statistical significance of Chao1, Shannon and Simpson alpha diversity indices were evaluated in R v. 3.4.3. The rarefaction curves were obtained by Vegan v. 2.5.3. Venn diagrams were elaborated by Mothur software.[30]

RESULTS

Bacterial diversity analysis

The diversity indices were used to calculate the alpha diversity of bacterial communities of each rhizosphere (Table 1). The 02ML sample showed the highest indices (Chao1 = 540.0, Shannon = 3.75 and Simpson = 0.97).

The bacterial communities in the four groups of rhizosphere were mainly represented by following phyla: Proteobacteria (01ML = 23.09%, 05ML = 83.07%, 02ML = 33.86% and 06ML = 74.07%), Bacteroidota (01ML = 7.33%, 05ML = 0.66%, 02ML = 19.10% and 06ML = 0.25%), Firmicutes (01ML = 2.70%, 05ML = 16.27%, 02ML = 0.46% and 06ML = 25.69%), Cyanobacteria (01ML = 33.02%, 05ML = 0.0%, 02ML = 23.34% and 06ML = 0.0%) and Acidobacteriota (01ML = 7.38%, 05ML = 0.0%, 02ML = 7.13% and 06ML = 0.0%). Other phyla detected were Actinobacteriota, Chloroflexi, Myxococcota, Nitrospirota, Planctomycetota

Table 1. Alpha diversity indices of bacterial richness associated to citrus tree rhizospheres

Rhizospheres	Status	Code	Chao1	Shannon	Simpson
C. aurantifolia/C. jambhiri	healthy trees	01ML	340.0	3.23	0.95
C. aurantifolia/C. jambhiri	decline trees	05ML	100.0	1.81	0.79
C. jambhiri	healthy trees	02ML	540.0	3.75	0.97
C. volkameriana	healthy trees	06ML	190.0	2.50	0.88

and Verrucomicrobiota in lower abundances (Figure 2a).

At genera level, 01ML rhizosphere was mainly represented by *Bradyrhizobium* (2.50%), *Nitrospira* (2.40%), Pir4_lineage (2.28%), *Bryobacter* (2.07%), *Sulfurifustis* (1.91%), *Pseudolabrys* (1.76%), *Steroidobacter* (1.52%), *Chryseolinea* (1.50%) and *Pseudomonas* (1.40%). The 02ML rhizosphere had the highest bacterial diversity and was mainly represented by *Steroidobacter* (3.22%), *Sphingomonas* (2.71%), *Edaphobaculum* (2.50%), Ellin6055 (2.16%), *Acidibacter* (1.82%), *Lacibacter* (1.76%), *Terrimicrobium* (1.45%), *Stenotrophobacter* (1.43%) and *Pseudolabrys* (1.43%). In the 05ML rhizosphere, *Cronobacter* (13.78%) and *Pseudomonas* (13.58%) were the most representative genera, followed by *Klebsiella* (7.12%), *Acinetobacter* (4.07%), *Kosakonia* (1.94%), *Bacillus* (1.78%) and *Lactococcus* (1.63%) as markers genera. Finally, 06ML rhizosphere comprised *Pseudomonas* (14.24%), *Clostridium sensu stricto* (9.81%), *Cronobacter* (8.19%) as the most representative genera, while *Aeromonas* (9.73%), *Terrisporobacter* (2.15%), *Trabulsiella* (2.13%), *Paenibacillus* (1.69%), *Paraclostridium* (1.64%) and *Solibacillus* (1.29%) were markers genera. Others such as *Acinetobacter* (4.57%) and *Klebsiella* (3.45%) were also present (Figure 2b).

Over 600 bacterial ASVs were detected. The 02ML sample had the highest abundance (Figure 3a). The highest number of shared genera was observed between 01ML and 02ML, in addition, *Pseudomonas* was the only common genus the four rhizospheres evaluated in this study (Figure 3b).

Fungal diversity analysis

The diversity indices were used to calculate the alpha diversity of fungal communities of each rhizosphere (Table 2). The 02ML sample showed the highest indices (Chao1 = 181.9 and Shannon = 1.97), while the 05ML sample had the highest Simpson index (Simpson = 0.69).

The fungal communities were mainly represented by following phyla: Ascomycota (01ML = 66.32%, 05ML = 90.32%, 02ML = 84.01% and 06ML = 89.76%), Basidiomycota (01ML = 15.64%, 05ML = 4.11%, 02ML = 5.0% and 06ML

Table 2. Alpha diversity indices of fungal richness associated to citrus tree rhizospheres

Rhizospheres	Status	Code	Chao1	Shannon	Simpson
C. aurantifolia/C. jambhiri	healthy trees	01ML	41.0	1.40	0.58
C. aurantifolia/C. jambhiri	decline trees	05ML	37.0	1.80	0.69
C. jambhiri	healthy trees	02ML	181.9	1.97	0.60
C. volkameriana	healthy trees	06ML	37.0	1.57	0.65

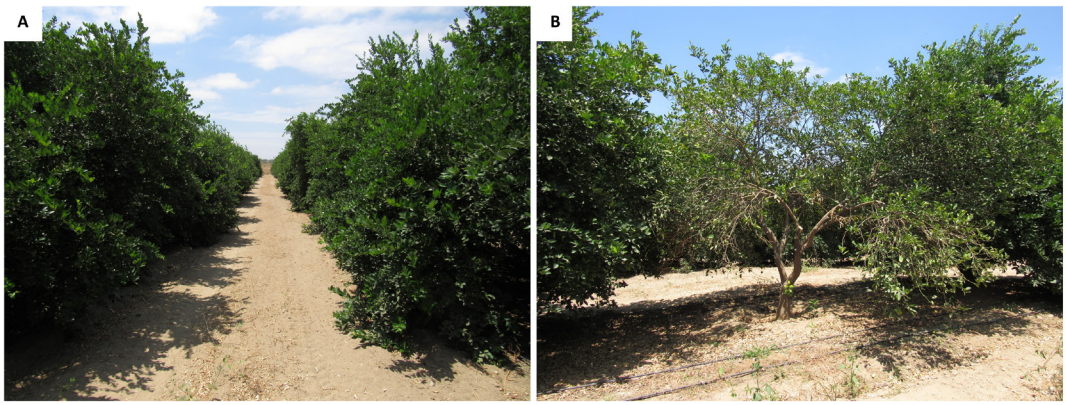

Figure 1. Agricultural property "Eloy", Sullana, Piura (4.5 ha). A) The picture shows a plantation of *C. aurantifolia*/*C. jambhiri* healthy trees of nine years old. B) A *C. aurantifolia*/*C. jambhiri* tree with decline symptoms, notice the difference in the foliage compared to the healthy trees on its sides. The samples were uptake from roots

= 3.64%) and Glomeromycota (01ML = 9.29%, 05ML = 0.0%, 02ML = 3.33% and 06ML = 0.47%) (Figure 4a).

Regarding the genera identified, the 01ML rhizosphere was mainly represented by *Fusarium* (14.48%), *Entoloma* (9.22%), *Trichoderma* (4.32%), *Rhizophagus* (2.95%) and *Glomus* (2.29%). The 02ML rhizosphere was represented by *Fusarium* (36.69%), *Kurtzmanomyces* (3.90%), *Trichoderma* (3.39%), *Rhizophagus* (3.20%), *Cladosporium* (2.42%) and *Acremonium* (1.82%). The 05ML rhizosphere, the genus *Fusarium* was the most representative (50.60%), followed by *Trichoderma* (6.32%), *Kurtzmanomyces* (2.69%), *Cladosporium* (2.14%), *Acrocalymma* (1.97%) and *Kazachstania* (1.83%) as genus marker and *Curvularia* (1.81%).

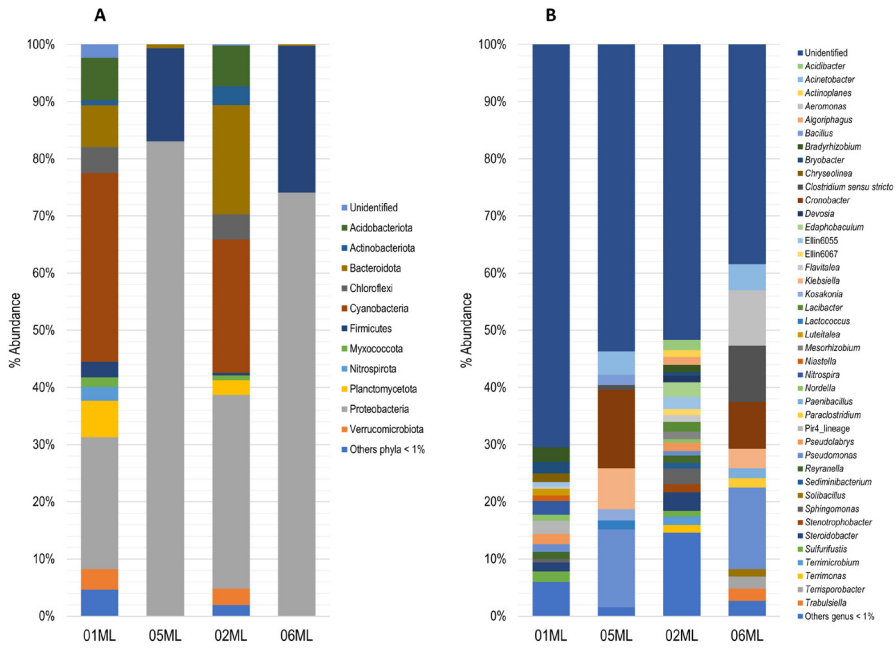

Figure 2. Relative abundance of bacterial microbiota. A) Barplots showing relative abundance of the bacterial phylum. B) Barplots showing relative abundance of the bacterial genera (metabarcoding analysis based 16S rRNA) present in the different kinds of citrus rhizospheres analyzed in this study

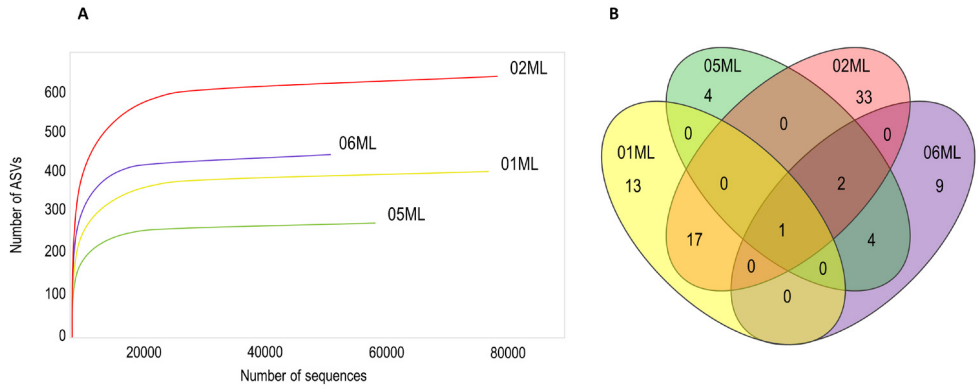

Figure 3. Rarefaction curves and Venn diagram of shared bacterial genera of citrus rhizospheres. A) Shown the number of bacterial ASVs observed for different citrus rhizosphere. The rhizosphere of *C. jambhiri* trees (02ML) showed the highest number of ASVs. B) *Pseudomonas* was the only genus shared for all rhizospheres

Finally, 06ML rhizosphere was represented by *Fusarium* (33.94%), *Aspergillus* (3.04%), *Clonostachys* (2.11%), *Acrocalymma* (2.08%) and *Arthrocladium* (1.50%). Figure 4b summarizes the abundance of the fungal genera identified.

Over 200 fungal ASVs were detected. The 01ML sample had a greater richness (Figure 5a). Finally, *Acremonium*, *Acrocalymma*, *Aspergillus*, *Coprinellus*, *Fusarium*, *Gibberella* and *Trichoderma* were the seven common genera in the four kinds of rhizospheres (Figure 5b).

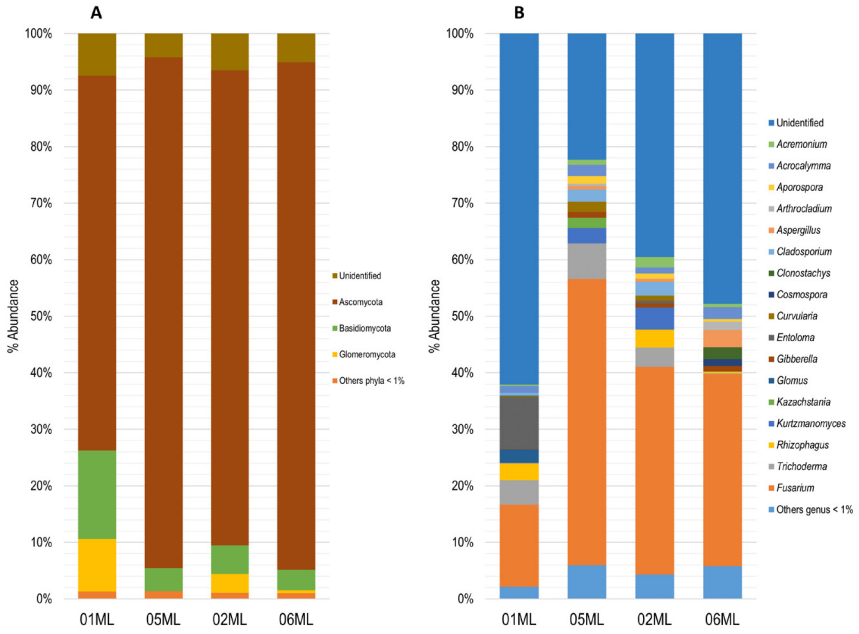

Figure 4. Relative abundance of fungal microbiota. a) Barplots showing relative abundance of the fungal phylum and b) Barplots showing relative abundance of the fungal genera (metabarcoding analysis based ITS) present in the different kinds of citrus rhizospheres analyzed in this study

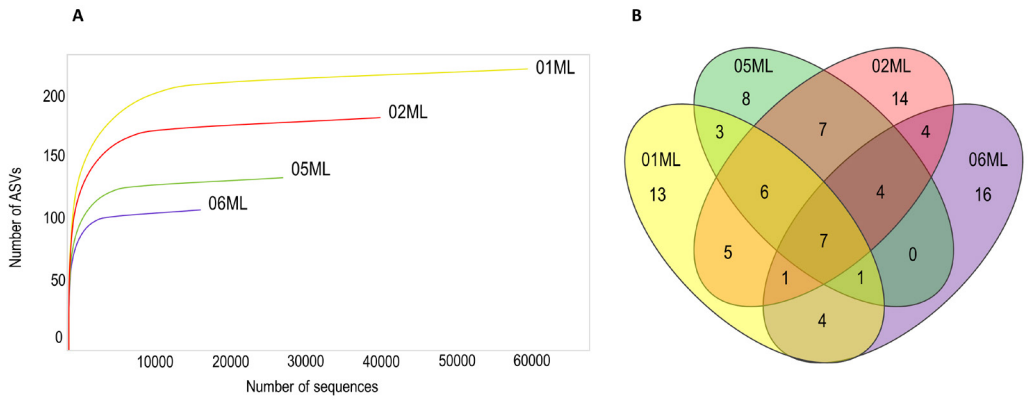

Figure 5. Rarefaction curves and Venn diagram of shared fungal genera of citrus rhizospheres. A) Rarefaction curve showing the number of fungal ASVs observed for different citrus rhizosphere. The rhizosphere *C. aurantifolia*/*C. jambhiri* healthy trees (01ML) showed the highest number of ASVs. B) Seven fungal genera were shared in the different kinds of citrus rhizospheres

DISCUSSION

Some microorganisms identified by our metabarcoding analysis were similar to those reported in different citrus species while others had not been described before. Such variability in the rhizospheric microbiota may be associated to geographic location and plant genotype.[31,32] Furthermore, the microbial structure of the rhizosphere could be modulated due to the plant health status.[24]

Proteobacteria are the most abundant and important phylum within the microbial communities found in the citrus rhizosphere compared to those microbes reported in the bulk soil.[33] Proteobacterial species have been described as dominant in the rhizosphere of citrus.[23,24,34,35] and of other plant species of economic importance such as grapevine, cocoa, rice, etc. However, we could appreciate some differences in microbiological abundance according to plant health status. Proteobacteria phylum was decreased in the rhizosphere of *C. aurantifolia*/*C. jambhiri* healthy tree compared to those that showed decline symptoms. This relative difference could explain the modulation of the rhizospheric microbiota due to the presence of diseases in citrus.[24] A high diversity of bacterial phyla in the rhizosphere of *C. aurantifolia*/*C. jambhiri* healthy tree could suggest that the microbiota may be regulated by the balance of interactions that occur at the root and upper part of plant, which reflects its healthy status (Figure 2a). Proteobacteria, Cyanobacteria, Bacteroidota, Actinobacteriota, Firmicutes, Planctomycetota, Verrucomicrobiota and other phyla were detected in healthy plants, and many species belonging to those taxa are known to be beneficial microorganisms. Otherwise, the abundances of the phyla for *C. jambhiri* and *C. volkameriana* showed differences. Although both species did not show decline symptoms, the low diversity of *C. volkameriana* may be associated with genotype or location. Furthermore, the cultivation conditions for this species (rootstocks) could influence its microbiological diversity, since the type of irrigation, fertilization and pest management differ from those of *C. aurantifolia/C. jambhiri* healthy trees.

The main rhizobacteria genera identified in *C. aurantifolia*/*C. jambhiri*, *C. jambhiri* and *C. volkameriana* healthy trees have been reported as endophytic microorganisms, growth promoters, inducers of systemic resistance bioremediators. Some endophytes from the genus *Sphingomonas* have been described in citrus plants in China.[36] The genus *Paenibacillus* harbors some species described as growth suppressants of citrus pathogenic fungi.[37]

Interestingly, three genera of the Enterobacteriaceae family were prominent in the rhizosphere of *C. aurantifolia*/*C. jambhiri* (05ML) decline symptoms trees evaluated by metabarcoding analysis. *Cronobacter*, *Klebsiella*, and *Kosakonia* can behave as pathogens in humans and be food contaminants. However, some *Cronobacter* strains are capable of endophytically colonizing tomato and corn roots. In addition, this genus comprises species with phosphate solubilizing properties and producers of 3-IAA.[38] While some *Klebsiella* strains have been isolated from the rhizospheres of different crops and show endophytic properties, tolerance to salt stress and promote growth in sugarcane and wheat.[39,40] *Kosakonia* species have shown endophytic capacity in rice roots.[41]

Cronobacter and *Klebsiella* have been found in the rhizosphere of citrus,[42,43] while *Kosakonia* has been isolated from citrus leaves and branches.[44] There is no evidence that *Cronobacter* and *Klebsiella* are associated with the development of decline symptoms in citrus trees, however, these bacteria appears to have an environmental role and have been found in water, soil and plant material.[45] Although some species of *Kosakonia* are benefical, *K. cowanii* has been described as pathogen in soybean leaves.[46] Also, a *K. radicincitans* strain has been associated with bacterial wilt in banana plants,[47] but no evidence showing the same action in citrus. Studies that include the isolation, characterization and analysis are required to evaluate the pathogenic effect that these Enterobacteriaceae species could develop when interacting with *C. aurantifolia* trees.

Pseudomonas, *Bacillus*, and *Acinetobacter* are cosmopolitan microbes acting as endophytes in roots, stems, leaves, flowers, and fruits. Furthermore, those microorganisms have been proven to be promoters of plant growth, phytohormones producers and inducers of systemic resistance.[48,49] These genera were

identified in healthy and sick rhizospheres in our study. *Pseudomonas* and *Acinetobacter* were predominant in the rhizosphere of *C. volkameriana* healthy trees, whereas a lower abundance was founded in trees with decline symptoms (Figure 2b). Moreover, the genus *Bacillus* was present in decline symptoms trees according to our study. However, *Bacillus* strains were isolated from roots of healthy trees (data not shown). In the other hand, different species of *Pseudomonas* have pathogenic effects on citrus leaves and fruits.[50,51] Interestingly, we found that the relative abundance of the genus *Pseudomonas* was abundant in diseased plants (Figure 2b) and this could be a key factor associated with the decline symptoms.

Our metabarcoding analysis showed that Ascomycota was the predominant fungal phylum in citrus rhizospheres. The *Rhizophagus* and *Glomus* genera, known as arbuscular mycorrhizal fungi, were unique to healthy trees, and the genus *Entoloma*, a beneficial ectomycorrhizal basidiomycete, was also found in the rhizosphere of healthy trees (Figure 4b). Some species of *Rhizophagus* and *Glomus* would have beneficial effects on citrus. A study evaluated the effect *R. intraradices* co-inoculated with a rhizobacteria on seeds and seedlings of citrus *Poncirus trifoliata*, found that both microorganisms improved the performance in the uptake of phosphorus and nitrogen, in addition, the physiological characteristics were improved, compared to control.[52] Watanarojanaporn et al.[53] isolated thirteen mycorrhizae from the rhizosphere of citrus orchards in Thailand, seven of them belonged to the genus *Glomus*. *G. etunicatum* was found to have a better colonization capacity in citrus roots and antagonism to *Phytophthora nicotianae*. This shows that arbuscular mycorrhizal fungi detected in rhizospheres, could be associated to healthy physiological status.

Some *Trichoderma* strains have shown positive effects to control *Penicilium digitatum*, *Alternaria alternata* and *Colletotrichum gloeosporioides* by producing antifungal enzymes in sweet orange fruits.[54,55] *Clonostachys*, *Cladosporium*, *Aporospora*, *Acrocalymma*, and *Acremonium* are fungal genera found in citrus plant with beneficial features. *C. rosea* is an entomopathogen of citrus-pests.[56] Bioactive metabolites from *C. cladosporoides*, isolated from citrus orchards in Florida, inhibited the growth *in vitro* of *Liberibacter crescens* [44]. *Acrocalymma* has been found to inhabit citrus tree roots with symptoms of HLB.[57] Although there is no evidence that *Aporospora* and *Acremonium* inhabit citrus roots, these fungi behave as endophytes in other plants. Whereas, *Kazachstania*, found both in the rhizospheres of healthy and sick trees, comprises yeasts that control pathogens in lemon fruits.[58]

Certain species of the genus *Aspergillus* have been associated with postharvest diseases affecting *Citrus limón* and *Citrus sinensis* fruits.[59,60] However, it is unknown whether microorganisms of this genera cause roots rot. A study found that *Curvularia spicifera*, causes fruit rot of *Citrus reticulata*[61] and *Gibberella* species have not been reported to affect citrus, but they could be pathogens of some grasses.[62-64]

Many species of the genus *Fusarium* cause root rot, blight and dieback in citrus.[7] *F. solani* causes dry rot in *C. aurantifolia* in India,[65] while *F. sarcochroum*, *F. oxysporum*, *F. citricola*, *F. salinense*, *F. ensiforme* and *F. siculi* affect lemon trees in Europe.[7] *Fusarium* is a relevant pathogen in top harmful fungi of citrus. Our results showed a high abundance of the genus *Fusarium* in healthy trees and those with decline symptoms. A high number of *Fusarium* (50.6%) in trees with decline symptoms (Figure 4b) is an interesting characteristic to consider when evaluating the role of those microbes in the disease. Javier-Alva[5] carried out pathogenicity studies where *Phytophthora parasitica* was found to cause a primary infection triggering decline symptoms in *C. aurantifolia* seedlings. Javier-Alva[5] suggests that *Fusarium* species and another fungi subsequently would promote the development of the decline. Besides, Handique et al.[66] suggests that *Phytophthora* alters microbial community structure in rhizosphere of citrus. In our study, the genus *Fusarium* was present in different citrus species with and without decline symptoms. Furthermore, we isolated species of *F. solani* and *P. parasitica* in healthy trees (data not shown), which would indicate that the presence of both pathogens in the citrus production fields in Piura is still an agricultural challenge that needs attention.

CONCLUSION

This is the first study that describes the diversity of the microbiota associated with the rhizospheres of *C. aurantifolia*, *C. jambhiri* and *C. volkameriana* in Piura by using culture-independent technique. Over 600 ASVs bacterial and 200 ASVs fungal were identified by metabarcoding analysis. In addition, we were able to establish the differences between the microbiota associated with healthy trees and those with decline symptoms, a problem that has affected "*limón sutil*" crops in Piura for more twenty years. The diversity of bacterial and fungal identified allowed us to have insight into the structure and behavior of the microbiota that interact with citrus rhizospheres and how they differ according to the health status of host. The use of native bacterial and fungal strains isolated from healthy trees could be evaluated in future to find out their capabilities in improving the health status of the *Citrus aurantifolia* trees in crops of Piura.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.18.3.49

Additional file: Additional Table S1 and Figure S1.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

ENM and EM conceived and designed the experiments. ENM and MSB carried out the sampling. ENM and MSB carried out the lab experiments. CCM led the processing of sequencing data and bioinformatic analysis. MSB and ENM wrote the manuscript with contributions from CCM and EM. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or in the supplementary files.

ETHICS STATEMENT

Not applicable.

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