The Microbe 4 (2024) 100103

Contents lists available at ScienceDirect

The Microbe

journal homepage: www.sciencedirect.com/journal/the-microbe

# Shifts in the structure and composition of root-associated bacterial communities caused by *Ceratocystis fimbriata* infection in eucalyptus☆

Anderson Ferreira [a,*], Esteban Roberto Gonzáles [b], Fernando Dini Andreote [c], João Lúcio de Azevedo [d], Welington Luiz Araújo [e]

[a] *Microbial Ecology and Genetics Lab, Brazilian Agricultural Research Corporation, Embrapa Wheat, Passo Fundo, Brazil*
[b] *FuturaGene Ltda, São Paulo, Brazil*
[c] *Department of Soil Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil*
[d] *Department of Genetics, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil*
[e] *Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*

ARTICLE INFO

*Keywords:*
16S rRNA
DGGE
Eucalyptus disease
Plant-bacteria-phytopathogen interaction

ABSTRACT

*Ceratocystis fimbriata* is the causal agent of Ceratocystis wilt in eucalypt plantations, resulting in lower volumetric growth, lower pulp yields and reduced timber. Therefore, the first hypothesis is these changes also could affect root-associated bacterial communities; and second hypothesis is that some bacteria associated with eucalyptus roots may act as a natural barrier against *C. fimbriata*. The structure and composition of bacterial communities associated to roots of eucalyptus plants presenting four levels of infection by *C. fimbriata* were evaluated. The abundance of rhizoplane community was higher than in endorhizosphere. On rhizoplane the abundance was higher in healthy than the infected plants, suggesting that these communities respond differentially to the *C. fimbriata* infection. *C. fimbriata* infection has effect on the endophytic bacterial community. The *Pseudomonas fluorescens*, *P veronii*, and *Rahnella aquatilis* were isolated only from roots of healthy plants. It was confirmed that *R. aquatilis* effectively controls *C. fimbriata* in vitro, strongly suggesting a negative association between the abundance of these bacterial species and the presence of *C. fimbriata* infection. In summary, data clearly suggest that sequential dynamics in shifting bacterial communities could be taken into account to increase understanding of the underlying mechanisms of *C. fimbriata* root infection.

## 1. Introduction

The genus *Eucalyptus* (family Myrtaceae) encompasses more than 700 species originally found in tropical and subtropical regions of the globe, being cultivated for pulp and cellulose production. The planted eucalypt forest had suffering by the presence of many phytopathogenic bacterial and fungi species. Among these diseases, the wilt is one of the most severe threats to planted forests. Wilt caused by the fungus *Ceratocystis fimbriata* Ellis; Halsted (ascomycete), a pathogen that starts infection mainly from the roots and exhibit symptoms of cankers, generalized wilt, root, and fruit decay, vase obstruction, and plant desiccation (Roux et al., 2004; Zauza et al., 2004; Ferreira et al., 2005). Halsted (1890) first described this fungus as a phytopathogenic agent, which, at that time, was attacking sweet potato tubers. In eucalyptus plants, the occurrence of *C. fimbriata* was first reported in 1998, when infected plants were observed simultaneously in Brazil and the Republic of Congo (Ferreira et al., 1999; Roux et al., 1999). The extension of affected areas and the consequent economic losses, the occurrence of this disease can compromise industrial processes and cellulose yield (Mafia et al., 2013).

The plant colonization by a phytopathogen is a result of a cascade of events, mediated by biotic and abiotic factors (Agrios, 2004). The genetic background of the host and pathogen, as well as the interaction between the phytopathogen and associated microbiota (endophytic, epiphytic, rhizoplane and rhizosphere) is a key factor that regulate the success of this interaction (Weller et al., 2002; Chandel et al., 2010; Hardoim et al., 2008; Mendes, 2013). In this sense, the composition of these non-pathogenic bacteria assembling at the plant roots is known to

☆ This article is available on the SSRN database without peer review. In the last two weeks it was among the 10 most accessed papers on the platform on the topics of i) Horticulture & Forestry eJournal Top Ten; ii) Botany eJournal; iii) Microbiology eJournal. ⟨https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4635944⟩.

* Correspondence to: Embrapa Wheat, Rodovia BR-285, Km 294, CP: 308, 99050-970, Passo Fundo, RS, Brazil.
*E-mail address:* anderson.ferreira@embrapa.br (A. Ferreira).

https://doi.org/10.1016/j.microb.2024.100103
Received 13 December 2023; Received in revised form 21 May 2024; Accepted 7 June 2024
Available online 13 June 2024

establish putative interactions with plants, resulting in ecologically important local communities, such as those occurring at the root surface (i.e., rhizoplane) and colonizing internal tissues (i.e., the endosphere). The root-associated microbiota has been studied during the past decades in many plant species, which has been reported to comprise members able to promote plant growth (Chanway, 1998; Azevedo et al., 2000) to protect plants against pest and diseases (Altindag et al., 2006) and even induce plant-defense against pathogens (Kavino et al., 2007; Mendes et al., 2011; Araujo et al., 2012) Ecological behavior of specific beneficial groups has been reported, such as their ability to penetrate plant roots and colonize internal tissues similarly to pathogens (Hardoim et al., 2008; Junker at al. 2012; Brader et al., 2017). This feature might allow the possible application of endophytic microorganisms in the bio-control of phytopathogens, mainly by antagonism (Silva et al., 2019), systemic resistance induction (Singh, 2017; Sahu, 2019).

Considering that surface colonization is the first step before endophytic colonization, the rhizoplane bacterial community could contribute to the inhibition of plant pathogens by competing with them to colonization sites, before during root establishment (Andreote et al., 2006; Fuqua, 2007).

Studying the composition and diversity of endophytic bacteria in Eucalyptus roots, Fonseca et al. (2018) found that various growing conditions influence and induce changes in the bacterial community structure. They suggest that bacterial community might enhance Eucalyptus fitness by increasing nitrogen availability through biological nitrogen fixation. In a similar way, Miguel et al. (2016) examined the diversity and distribution of endophytic bacterial communities in Eucalyptus. They observed higher diversity in field-grown plants compared to those cultivated in greenhouses.

Other researchers have explored the association between the endophytic community and phytopathogens in eucalyptus. Procópio et al. (2009) demonstrated the widespread presence of the genus *Pantoea* across different plant genotypes, correlating its occurrence with the die-back disease. Additionally, they identified several endophytic bacteria with the potential to control plant pathogens (Ferreira et al., 2008). In a related study, Paz et al. (2012) evaluated and characterized endophytic *Bacillus* strains from eucalyptus capable of enhancing plant growth in clones of the hybrid *Eucalyptus urophylla* × *E. grandis*.

Despite this study and a few others mentioned recently in the literature, relatively little is known about the main determinants of the eucalyptus root-associated microbes, and nothing has been reported on how these communities might interfere with the occurrence and success of infection events. In this context, the present study proposed two hypotheses: the first is that varying levels of *C. fimbriata* pathogen infection in eucalyptus could impact root-associated bacterial communities; and the second hypothesis suggests that certain bacteria associated with eucalyptus roots may serve as a natural barrier against *C. fimbriata*. To test these two hypotheses we defined the objectives of this study: i) assess the composition of antagonist bacteria in the rhizoplane and endosphere communities of eucalyptus plants exposed to different levels of *C. fimbriata* infestation, and ii) investigate how these varying disease levels may induces shifts in the structures of these associated bacterial communities.

## 2. Material and methods

### 2.1. Plant genotypes and sampling of roots

This work was performed using a eucalyptus hybrid (*Eucalyptus grandis* x *E. urophylla*), kindly supplied by the Suzano Pulp and Paper company (Suzano, Brazil). The trees were cultivated in an experimental field, with a Yellow Argisol (Ultisol) located in the city of Mucuri, Bahia, Brazil. Sampling was performed in areas exhibiting health three and others three different levels of infestation by *C. fimbriata*: *i*) non-*C. fimbriata* (NCF), healthy trees; *ii*) root *C. fimbriata* (RCF), *C. fimbriata* present only in the trees roots); *iii*) stem *C. fimbriata* (SCF), *C. fimbriata* present only in the trees stems; and *iv*) root and stem fungus (RSCF), *C. fimbriata* present in the trees roots and stems. To check that only the stem was infected and the roots were not, was carried out by visual observation of symptoms. Additionally, a specific methodology for isolating C. fimbriata was employed, as described by Alfenas et al. (2007). In total, five samples were collected from health trees and in each of the three levels of disease (20 total). Were choose lateral roots with a radius of 0.4 cm, in a depth of 10–15 cm. Samples were collected and microbial community isolated or total DNA extracted up to 24 h in the Laboratory of Microbial Genetics (Department of Genetics, ESALQ/University of São Paulo) in Piracicaba, Brazil.

### 2.2. Rhizoplane and endophytic bacterial isolation

Obtained root fragments were washed in sterile running tap water to remove the adhered soil particles. Soil-free roots were used to isolate the rhizoplane and endosphere bacterial communities. In each sample, five fragments were cut into pieces approximately 25 $cm^2$ in size. The extremities of the fragments were covered with paraffin to avoid potential escape of endophytic microorganisms. Fragments were placed in an Erlenmeyer flask containing 25 g of 0.1-cm diameter glass beads and 50 mL of sterile phosphate buffered saline (PBS) solution (containing 1.44 g $L^{-1}$ of $Na_2HPO_4$; 0.24 g $L^{-1}$ of $KH_2PO_4$; 0.20 g $L^{-1}$ of KCl; and 8.00 g $L^{-1}$ of NaCl at pH 7.4). Flasks were shaken at 150 rpm and 28 °C for 1 h. The resultant cell suspensions were used as the rhizoplane samples. Afterward, the same root fragments were removed from the flasks and used for bacterial endophytic isolation. Initially, root fragments from each sample were submitted to surface disinfestations according to the methodology previously described by Araújo et al. (2001). Briefly, a serial immersion of fragments was performed in 70 % ethanol for 1 min, sodium hypochlorite solution (2 % available $Cl^-$) for 2 min, 70 % ethanol for 1 min, and two washes in sterilized distilled water. After the surface disinfestations, 1 g of material was triturated in 10 mL of sterile PBS and maintained at 28 °C while shaking at 150 rpm for 1 h. The resulting cell suspension was used as a source to isolate the endophytic communities.

For the rhizoplane and endophytic communities, the cell suspensions were appropriately diluted and plated onto 10 % Trypticase Soy Agar plates (TSA-Merk) supplemented with 50 μg $mL^{-1}$ of benomyl fungicide to prevent fungal growth. Plates were incubated at 28 °C for 7 d, and the number of colony-forming units (CFU) was determined to estimate culturable population density. A random sample of approximately 20 % of the colonies was saved to represent the bacterial diversity in further analyzes. These colonies were purified, suspended in 30 % glycerol solution, and stored at − 70 °C.

### 2.3. Bacterial identification

The 16S rRNA gene amplification was performed using colony-PCRs according to methods described by Ferreira et al. (2008), in which isolates were grown in TSA and a single colony was transferred to a tube containing 200 μL of sterilized, ultra-pure water. The bacterial suspension was used as the source of DNA for PCR reactions. The primer sets used for 16S rRNA amplification were PO27F (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1387r (5'-CGGTGTGTACAAGGCCCGGGAACG-3'). The PCR reaction included 1 μL of bacterial suspension, 10 mM of Tris-HCl (pH 8.3), 3.75 mM of $MgCl_2$, 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), 200 mM of each primer, and 2.5 U of *Taq* DNA polymerase in a 50-μL final volume. A negative control (PCR mixture without DNA template) was included in all PCR amplifications. Amplifications were performed in a thermal cycler (PTC 200, MJ Research, USA) with the following PCR parameters: initial denaturation at 94 ºC for 5 min; 35 cycles of denaturation at 94 ºC for 1 min; primer annealing at 62.5 ºC for 1 min; and primer extension at 72 ºC for 1 min, followed by a final extension at 72 ºC for 7 min. PCR products were analyzed by electrophoresis in a 1.2 % (w/v) agarose gel

stained with ethidium bromide in 0.5x Tris-acetate-ethylene diamine tetra acetic acid (TAE) buffer.

Following PCR amplification, ARDRA analyses were used as a way to help in bacterial grouping and identification. These analyzes were carried out as follows: 1 μg of an amplified 16S rRNA gene was digested with 2 U of *Hha*I restriction enzyme (Fermentas Life Sciences, Brazil) according to the manufacturer's recommendations. The products were analyzed by electrophoresis in a 2.5 % (w/v) agarose gel and stained with ethidium bromide. The ribotypes obtained were separated in groups according to the obtained molecular fingerprints. The 16S rRNA gene PCR products were purified with polyethylene glycol (PEG) (20 % PEG 8000; 2.5 mM of NaCl) and sequenced at the Institute of the Human Genome (USP, São Paulo, Brazil). The taxonomical sequence affiliations were assessed using BLASTN (National Centre for Biotechnology Information website) against the non-redundant database of GenBank.

### 2.4. Antifungal action of *Pseudomonas fluorescens, P. veronii, and Rahnella aquatilis*

In this study, we utilized the fungal isolate Agn 7 (*Ceratocytis fimbriata*). The fungal isolate was cultured and maintained on BDA medium (Difco). Evaluations were conducted using the paired culture method, which involves direct confrontation on solid medium between the antagonist and the phytopathogenic fungus. For this purpose, bacterial isolates were inoculated on BDA culture medium (Difco) at 28 °C for 2 days. Subsequently, the fungal isolate was inoculated on the opposite part of the Petri dish, followed by a 20-day incubation. In control plates, only the fungus was inoculated. After cultivation, the presence of inhibition zones between the paired cultures and the radial growth of the phytopathogen in comparison to the control indicated pathogen inhibition by the bacterium. All analyzes were performed in triplicate.

### 2.5. Identification of the unculturable bacterial communities

The total DNA extraction was performed using the same bacterial cell suspensions obtained in the bacterial community isolation process. The DNA was extracted using 0.5 mL of initial material for each sample and carried out with a MoBio UltraClean™ soil DNA kit (MoBio Laboratories, EUA) following the manufacturer's instructions. The DNA obtained from rhizoplane and endosphere samples was checked in a 1 % (w/v) agarose gel.

The structure of total bacterial communities associated with eucalyptus roots was assessed by PCR-DGGE. The amplification of 16S rRNA gene fragments from total DNA extracted from rhizoplane and endosphere samples was performed using semi-nested PCR. The first PCR was performed using primers 799F (5'-AACMGGATTAGATACCCKG-3') (CHELIUS; TRIPLETT, 2001) e 1492R (5'-TACGGYTACCTTGTTACGACT-3'), using 35 cycles of 94 °C for 20 s, 53 °C for 40 s, and 72°C for 40 s. The reactions were performed in a final volume of 25 μL using 5–10 ng of initial DNA, 3.75 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primer, 2.5 U of *Taq* DNA polymerase, 1 × buffer, and 0.2 mg of bovine serum albumin (BSA). The second PCR was performed using 1 μL (20 ng) of PCR product from the first PCR. It consisted of a 50-μL reaction mixture containing 0.4 μM of universal primers U968GC (5'-Grampo de CG-AACGCGAAGAACCTTAC–3') and R1387 (5'–CGGTGTGTACAAGGCCCGGGAACG-3') for the 16 S rRNA gene and 1 % of formamide under similar conditions as the first PCR, with the exception that the annealing temperature was 56 °C for 1 min and the extension was 72 °C for 2 min. All amplicons were checked in 1 % (w/v) electrophoresis agarose gels stained with ethidium bromide prior to the DGGE analysis.

The DGGE analyses were performed as described previously (Heuer et al., 1997) in a PhorU-2 gradient system (Ingeny International, The Netherlands). The obtained 16S rRNA gene amplicons were loaded onto 6 % (w/v) polyacrylamide gels in 0.5 × TAE buffer. The polyacrylamide gels were made with denaturing gradients ranging from 45 % to 65 % (where the 100 % denaturant contained 7 M of urea and 40 % formamide). The gels ran for 16 h at 100 V and 60 °C, after which they were soaked for 1 h in SYBR Green nucleic acid stain (1:10,000 dilution, Invitrogen) and immediately photographed under ultraviolet light.

### 2.6. Statistical analysis

The statistical analysis for the isolation data was performed using the SAS software package (The SAS Institute, USA). Bacterial counts were transformed using the $\log_{10}$ of x + 1 prior to implementing analysis of variance (ANOVA). An *a priori* 0.05 level of significance was established for data analysis. In addition, a Tukey's least-significant-difference test was applied for paired comparison of means. The obtained community fingerprints from DGGE were analyzed using the GelComparII software package. Initially, the images were normalized, and standardization methods that were applied to mark the presence of bands were carefully checked manually; corrections were made when deemed necessary. Relative intensity of each band was calculated, and a table containing band positions and their relative intensities was exported for further analysis. Interpretation of the results obtained by PCR-DGGE was performed via principal component analysis (PCA) using Canoco 4.5 software (Microcomputer Power, USA). The table containing band positions was used as species data, and the same table was used to estimate bacterial community structures in all evaluated samples.

## 3. Results

### 3.1. Rhizoplane culturable bacterial community composition

The isolation method allows obtaining culturable bacterial strains encompassing distinct morphological groups in the four rhizoplane treatments (NCF, RCF, SCF, and RSCF). The total culturable densities among treatments ranged from 12.78 to 20.66 × $10^4$ CFU $cm^{-2}$ of root tissue (Table 1). Differences were observed ($F = 47.62$ and $p < 0.0001$) in the densities of CFU among treatments by analysis of CFU cross-comparison averages (Table 1). Remarkably, the number of CFU obtained for the NF treatment exhibited statistically higher CFU than those observed in the RF, SF, and RSF treatments, which did not differ among each other in CFU density (Table 1).

Based on ARDRA technique 160 isolates were evaluated, resulting in 13 ribotypes belonging to 12 bacterial families (Table 2). The most frequent ribotypes were identified as B (Bacillaceae) and E (Bacillaceae and Paenibacillaceae) (Table 2). The remaining ribotypes were identified as follows: G (Micrococcaceae); O (Microbacteriaceae); T (Microbacteriaceae); Z (Nocardiaceae); F (Rhizobiaceae); J (Comamonadaceae

**Table 1**
Bacterial densities in the rhizoplane of the eucalyptus roots with different infestation levels by *Ceratocystis fimbriata* phytopathogen. In each column, values followed by same letter are statically similar according to the Tukey test, (p value < 0.05).

| Treatment* | Rhizoplane bacteria ($10^4$ CFU $cm^{2\ -1}$ of root)** | |
|---|---|---|
| NF*** | 20,66 | a |
| RF | 14,19 | b |
| SF | 12,78 | b |
| RSF | 17,00 | b |

* Samples of 40 π $cm^2$ of eucalyptus roots.
** Data mean the average of three replicates and five isolation procedure.
*** *i*) non-fungus (NF), with healthy trees but without external symptoms of the *C. fimbriata* and fungus in stems and roots; *ii*) root fungus (RF), with healthy trees but without external symptoms of the *C. fimbriata* presence and fungus in stems (but presence in roots); *iii*) stem fungus (SF), with affected trees, external symptoms of the *C. fimbriata* presence, and fungus in stems but without fungus in roots; and *iv*) root and stem fungus (RSF), with affected trees, external symptoms of the *C. fimbriata* presence, and fungus in stems and roots.

**Table 2**
ARDRA riboptypes distribution in each rhizoplane treatment (*i*, *ii*, *iii* e *iv*), with different infestation level by *Ceratocystis fimbriata* phytopathogen.

| Ribotypes | Family | Percentage in each treatment | | | | Total percentage |
|---|---|---|---|---|---|---|
| | | NF | RF | SF | RSF | |
| A | Xanthomonadaceae | 2,3 | 3,8 | 4,5 | 8,7 | 4,7 |
| B | Bacillaceae | 36,5 | 23,2 | 52,3 | 40,0 | 39,6 |
| C | Microbacteriaceae | 4,5 | NO* | 2,3 | 5,8 | 3,4 |
| E | Bacillaceae e Paenibacillaceae | 22,8 | 34,7 | 36,3 | 31,5 | 30,8 |
| F | Rhizobiaceae | 9,0 | 11,5 | NO | 2,8 | 5,4 |
| G | Micrococcaceae | 9,0 | 3,8 | NO | 2.8 | 4,0 |
| H | Pseudomonadaceae | NO | 3,8 | NO | 2.8 | 1,3 |
| J | Comamonadaceae e Burkholderiaceae | 6,9 | NO | 2,3 | NO | 2,7 |
| O | Microbacteriaceae | 4,5 | NO | NO | 2.8 | 2,0 |
| R | Enterobacteriaceae | NO | 7,7 | NO | NO | 1,3 |
| T | Microbacteriaceae | 2,3 | 7,7 | 2,3 | 2,8 | 3,4 |
| Z | Nocardiaceae | 2,3 | NO | NO | NO | 0,7 |
| W | Pseudomonadaceae | NO | 3,8 | NO | NO | 0,7 |

* Not observed.

and Burkholderiaceae); A (Xanthomonadaceae); C (Microbacteriaceae); H and W (Pseudomonadaceae); and R (Enterobacteriaceae) (Table 2). Ribotypes H, W, and R were observed only in the RF and RSCF treatments (with fungus infesting roots), while ribotype J was observed exclusively in the NCF and SCF treatments (without fungus infesting roots). The families Xanthomonadaceae (Ribotype A), Bacillaceae (Ribotype B and E), and Microbacteriaceae (Ribotype T) were isolated from all four treatments, while the other families were observed throughout treatments (Table 2).

Representative isolates of these ribotypes were taxonomically characterized by sequencing and analysis of the 16S rRNA gene (Table 3). The microbial community associated to eucalyptus rhizoplane are composed by members of the genera *Arthrobacter*, *Bacillus*, *Brevibacillus*, *Burkholderia*, *Curtobacterium*, *Dyella*, *Leifsonia*, *Luteibacter*, *Lysinibacillus*, *Microbacterium*, *Nocardia*, *Pseudomonas*, *Rahnella*, *Rhizobium*, *Rhodanobacter*, *Stenotrophomonas*, and *Variovorax* (Table 3).

**Table 3**
Bacterial identification of isolates corresponding to ARDRA rhizoplane ribotypes. The molecular identification of species was carried out by partial 16S rDNA sequencing and BLASTn analysis (*National Center for Biotechnology information website*).

| Ribotype | Phylum | Specie (Blast-NCBI) | %* | Reference strain |
|---|---|---|---|---|
| B | Firmicutes | *Bacillus cereus* | 100 | EU104731.1 |
| E | | *Bacillus fusiformis* | 96 | AB271743.1 |
| E | | *Brevibacillus brevis* | 98 | EF173465.1 |
| E | | *Lysinibacillus sphaericus* | 96 | EF690426.1 |
| G | Actinobacteria | *Arthrobacter* sp. | 100 | EU034524.1 |
| O | | *Leifsonia xyli* | 97 | DQ232616.2 |
| T | | *Microbacterium phyllosphaerae* | 100 | DQ365561.1 |
| T | | *Microbacterium oxydans* | 98 | EU249583.1 |
| C | | *Curtobacterium flaccumfaciens* | 81 | EU102272.1 |
| Z | | *Nocardia terpenica* | 92 | AB201298.2 |
| F | Alphaproteobacteria | *Rhizobium tropici* | 99 | EU074181.1 |
| J | Betaproteobacteria | *Burkholderia kururiensis* | 99 | AJ300686.1 |
| J | | *Variovorax paradoxus* | 95 | EU169184.1 |
| A | Gamaproteobacteria | *Dyella koreensis* | 91 | AB272381.1 |
| A | | *Luteibacter rhizovicinus* | 97 | EU022023.1 |
| A | | *Rhodanobacter thiooxydans* | 98 | AB286179.1 |
| A | | *Stenotrophomonas maltophilia* | 99 | EU221397.1 |
| H | | *Pseudomonas fluorescens* | 98 | EF552157.1 |
| W | | *Pseudomonas veronii* | 99 | AB334768.1 |
| R | | *Rahnella aquatilis* | 86 | X79939.1 |

* Similarity with the reference strain.

### 3.2. Endophytic culturable bacterial community composition

The culturable fraction of eucalyptus root-associated microbiota also revealed bacterial isolates exhibiting distinct morphological groups in four endophytic root treatments (NCF, RCF, SCF, and RSCF). It is important to note that no bacterial colonies were observed in the negative control of surface-disinfected plates, which ultimately guaranteed that obtained colonies were undoubtedly endophytic. Total culturable densities of endophytic communities among treatments ranged from 9.20 to 25.13 $\times$ $10^4$ CFU $g^{-1}$ of root tissue (Table 4). Differences were observed ($F = 44.44$ and $p < 0.0001$) in the densities of CFU among treatments by analysis of CFU cross-comparison averages (Table 4). Remarkably, the number of CFU obtained for the RSCF treatment exhibited statistically higher CFU than those observed in the NCF, RCF, and SCF treatments, which did not differ among each other in CFU density (Table 4).

Based on ARDRA technique 160 isolates were evaluated, resulting in 8 ribotypes belonging to 5 bacterial families (Table 5).

From these totals, three ribotypes (B, E, and F) were present in all treatments, and the other five (A, D, P, R, and X) were observed to differ among treatments (Table 5).

Through sequencing and analysis of 16S rRNA, representative isolated ribotypes revealed isolates belonging mainly to the phylum Firmicutes and classes Alphaproteobacteria and Gamaproteobacteria

**Table 4**
Endophytic bacterial densities in the eucalyptus roots with different infestation levels by *Ceratocystis fimbriata* phytopathogen. In each column, values followed by same letter are statically similar according to the Tukey test, (p value $< 0.05$).

| Treatment* | Endophytic bacteria ($10^4$ CFU $g^{-1}$ of root)** | |
|---|---|---|
| NF*** | 19,60 | b |
| RF | 9,20 | b |
| SF | 15,73 | b |
| RSF | 25,13 | a |

* 1 g of eucalyptus root samples.
** Data mean the average of three replicates and five isolation procedure.
*** *i*) non-fungus (NF), with healthy trees but without external symptoms of the *C. fimbriata* and fungus in stems and roots; *ii*) root fungus (RF), with healthy trees but without external symptoms of the *C. fimbriata* presence and fungus in stems (but presence in roots); *iii*) stem fungus (SF), with affected trees, external symptoms of the *C. fimbriata* presence, and fungus in stems but without fungus in roots; and *iv*) root and stem fungus (RSF), with affected trees, external symptoms of the *C. fimbriata* presence, and fungus in stems and roots.

**Table 5**
ARDRA Riboptypes distribution in each endophytic treatment (*i*, *ii*, *iii* e *iv*), with different infestation level by *Ceratocystis fimbriata* phytopathogen.

| Ribotype | Family | Percentage in each treatment | | | | Total percentage |
|---|---|---|---|---|---|---|
| | | NF | RF | SF | RSF | |
| A | Xanthomonadaceae | NO* | 3,1 | 2,2 | 2,4 | 2,2 |
| B | Bacillaceae | 60,0 | 43,8 | 39,0 | 71,0 | 52,9 |
| D | Bacillaceae | NO | 18,7 | 15,2 | NO | 9,4 |
| E | Bacillaceae | 15,0 | 3,1 | 8,8 | 14,5 | 10,1 |
| F | Rhizobiaceae | 20,0 | 3,1 | 6,5 | 4,8 | 7,3 |
| P | Moraxellaceae | NO | 9,4 | 24,0 | NO | 10,1 |
| R | Enterobacteriaceae | NO | 12,5 | NO | NO | 2,9 |
| X | Enterobacteriaceae | NO | 6,3 | 4,4 | 7,3 | 5,1 |

* Not observed.

(Table 5). The most frequent ribotype was identified as B (Bacillaceae). Other ribotypes were identified as follows: A (Xanthomonadaceae); E and D (Bacillaceae); F (Rhizobiaceae); P (Moraxellaceae); and R and X (Enterobacteriaceae) (Table 5). Representative isolates of these ribotypes were taxonomically characterized by sequencing and analysis of the 16S rRNA gene (Table 6). The microbial community associated to endophytic eucalyptus are composed by members of the genera *Bacillus, Erwinia, Lysinibacillus, Psychrobacter, Rahnella, Rhizobium* and *Stenotrophomonas* (Table 6).

### 3.3. Antifungal test against Ceratocystis fimbriata

To verify whether the bacteria found in non infected root eucalyptus were able to control *Ceratocystis fimbriata*. We evaluated the in vitro capacity of *Pseudomonas fluorescens*, *P. veronii*, and *Rahnella aquatilis* to control the fungus *Ceratocystis fimbriata*. The paired culture methodology allowed us to assess the bacterial isolates, and it revealed that *Rahnella aquatilis* could inhibit the in vitro growth of *C. fimbriata* (Fig. 1). On the other hand, the two Pseudomonas species did not control *C. fimbriata*.

### 3.4. Analysis of bacterial community associated to eucalyptus plants

The unculturable microbial communities of rhizoplane and inner root tissues of Eucalyptus plants with different levels of *C. fimbriata* infection were evaluated by PCR-DGGE. The generated profiles were used for assess the structure of these microbial communities by Principal Component Analysis (Fig. 2).

On the x-axis, which accounted for 16.9 % of the total variance, it was evident that one cluster of NCF and RSCF treatments was distinct from the RCF and SCF treatments (Fig. 2). This suggests that there is no correlation between infected and non-infected plants by the *C. fimbriata*.

**Table 6**
Bacterial identification of isolates corresponding to ARDRA endophytic ribotypes. The molecular identification of species was carried out by partial 16S rDNA sequencing and BLASTn analysis (*National Center for Biotechnology information website*).

| Ribotype | Phylum | Specie (Blast-NCBI) | %* | Reference strain |
|---|---|---|---|---|
| P | Firmicutes | *Psychrobacter* sp. | 97 | EU075122.1 |
| B | | *Bacillus cereus* | 100 | EU104731.1 |
| D | | *Bacillus pumilus* | 94 | AB244297.1 |
| E | | *Lysinibacillus sphaericus* | 96 | EF563985.1 |
| F | Alphaproteobacteria | *Rhizobium tropici* | 99 | EU074181.1 |
| R | Gamaproteobacteria | *Rahnella aquatilis* | 97 | DQ862543.1 |
| X | | *Erwinia persicina* | 97 | AM294946.1 |
| A | | *Stenotrophomonas maltophilia* | 99 | AJ293467.1 |

* Similarity with the reference strain.

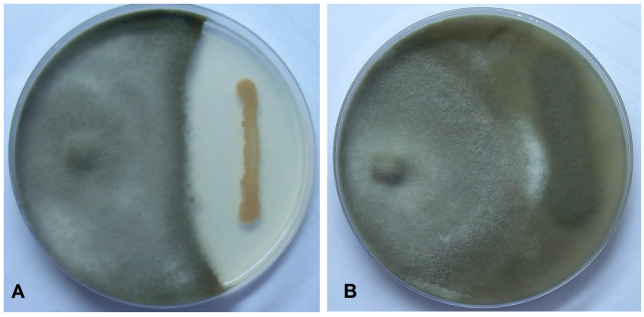


**Fig. 1.** Fotos de placas de Petri contendo meio de cultura BDA onde foram inoculados isolados bacterianos em uma das margens e o fungo *Ceratocystis fimbriata* na outra. A - *Rahnella aquatilis* com capacidade de inibir o fungo *C. fimbriata*; B – isolado bacteriano sem a capacidade de inibir o fungo *C. fimbriata.*

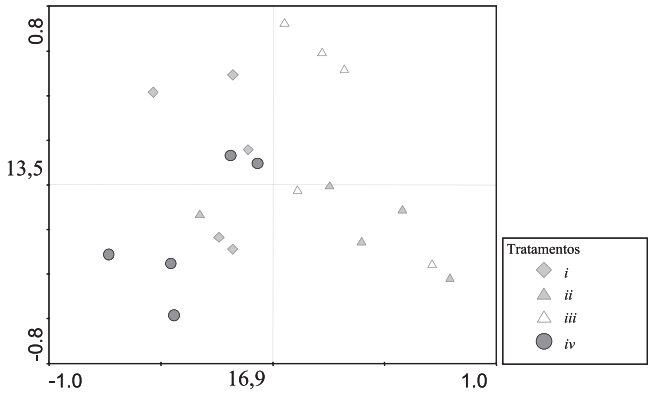


**Fig. 2.** Principal component analysis (PCA) based on PCR-DGGE profiles obtained with universal 16S rDNA primers in rhizoplane samples. *i*) non-fungus (NF), with healthy trees but without external symptoms of the *C. fimbriata* and fungus in stems and roots; *ii*) root fungus (RF), with healthy trees but without external symptoms of the *C. fimbriata* presence and fungus in stems (but presence in roots); *iii*) stem fungus (SF), with affected trees, external symptoms of the *C. fimbriata* presence, and fungus in stems but without fungus in roots; and *iv*) root and stem fungus (RSF), with affected trees, external symptoms of the *C. fimbriata* presence, and fungus in stems and roots. Data mean the average of Were assessed three replicates and five isolation procedure for each treatment. The values for the axes show the percentage of explained variance.

On the other hand, the y-axis, representing 13.5 % of the total variance, indicated that the cluster of NCF and SCF treatments was separate from the RCF and RSCF treatments (Fig. 2). This demonstrates a grouping of samples from roots with the presence of *C. fimbriata* separately from non-infected roots. With a lower, but detectable degree of correlation, the bacterial communities in the rhizoplane are modulated when they are infected by *C. fimbriata*.

For root endophytic communities, all treatments exhibited high similarity with each other, with the exception of the RSCF treatment (Fig. 3), which was the highest infested level by *C. fimbriata*. This treatment appeared to be separate from the others in the PCA, with the *x* axis explaining 20.3 % of the total variance (Fig. 3). This demonstrates that endophytic bacterial communities are strongly modulated when they are infected by C. fimbriata.

## 4. Discussion

The colonization of plants by microorganisms might occur in a pyramidal profile, where higher diversity and density levels may occur on the rhizoplanes and/or in the roots, stems, and leaves (Elvira-Recuenco and van Vuurde, 2000; Mendes et al., 2007). Similar results were

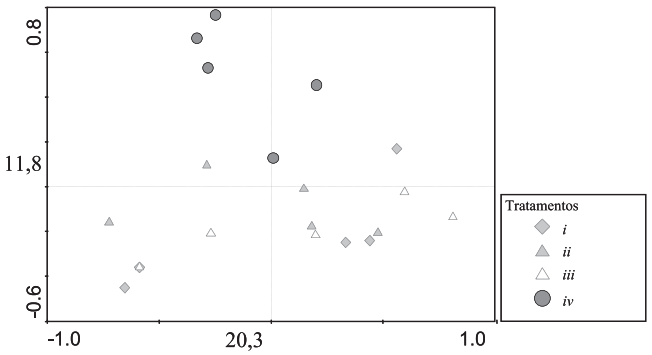


**Fig. 3.** Principal component analysis (PCA) based on PCR-DGGE profiles obtained with universal 16S rDNA primers in endophytic samples. *i*) non-fungus (NF), with healthy trees but without external symptoms of the *C. fimbriata* and fungus in stems and roots; *ii*) root fungus (RF), with healthy trees but without external symptoms of the *C. fimbriata* presence and fungus in stems (but presence in roots); *iii*) stem fungus (SF), with affected trees, external symptoms of the *C. fimbriata* presence, and fungus in stems but without fungus in roots; and *iv*) root and stem fungus (RSF), with affected trees, external symptoms of the *C. fimbriata* presence, and fungus in stems and roots. Data mean the average of Were assessed three replicates and five isolation procedure for each treatment. The values for the axes show the percentage of explained variance.

observed in this study, where the bacterial densities and complexity of community composition on the rhizoplane were higher than those observed for endophytic communities. The samples assessed were collected in a highly infested area by *C. fimbriata*, and the eucalyptus root-associated communities (rhizoplane and endophytic) were composed of the following: *Arthrobacter* sp.; *B. cereus*; *Bacillus fusiformis*; *B. pumilus*; *Brevibacillus brevis*; *Burkholderia kururiensis*; *Curtobacterium flaccumfaciens*; *Dyella koreensis*; *E. persicina*; *Lysinibacillus sphaericus*; *Leifsonia xyli*; *Luteibacter rhizovicinus*; *Microbacterium phyllosphaerae*; *Microbacterium oxydans*; *Nocardia terpenica*; *P. fluorescens*; *P. veronii*; *Psychrobacter* spp.; *R. aquatilis*; *R. tropici*; *Rhizobium tropici*; *Rhodanobacter thiooxydans*; *S. maltophilia*; and *Variovorax paradoxus*. It is possible to highlight that within this group of species, some successfully have been reported to be important plant-associated organisms. For instance, some genera can be involved in host plant protection against pathogens, such as *Pseudomonas*, which has been largely used in phytopathogenic fungi biological control, including against *Rhizoctonia solani* (Nagarajkumar et al., 2004), *Botrytis mali* (Mikani et al., 2008), and *Microdochium nivale* (Amein et al., 2008). The genus *Rhanella* also has been described as associated with fungi biological control of species, including *Penicillium expansum*, *Botrytis cinerea* (Calvo et al., 2007), and *Fusarium oxysporum* (Hassni et al., 2007).

It is also interesting that most of these genera also have been described as exhibiting beneficial host-associated characteristics and biotechnologically interesting capabilities. For instance, *Bacillus* and *Stenotrophomonas* produce indole-3-acetic acid (IAA) (Park et al., 2005), *Stenotrophomonas* contains biodegradators (Qureshi et al., 2007), and *Arthrobacter*, *Bacillus*, and *Microbacterium* act as plant growth promoters (Karlidag et al., 2007; Tsavkelova et al., 2007; Paz et al., 2012), and *Bacillus* is a well-known biological control agent (Ryu et al., 2006; Senthilkumar et al., 2007). These occurrences of bacterial species with different beneficial characteristics can be related to the survival of plants under high pathogen infestation levels.

An interesting feature observed was that of the 18 endophytic bacterial species found, five were also present on rhizoplanes (Tables 3 and 6). This suggests that a possible interwoven system of interactions is occurring in such systems. In addition, since not all species matched each other in both 'niches', it can be assumed that not all bacteria species exhibit such a capability to successfully occupy and thrive in both 'niches'. Another important feature is that the species *B. pumilus* and *E. persicina* were only observed as endophytes, suggesting a fast transient state from soil to endophytic colonization occurs, in addition to their capacity to be transmitted internally by seeds (Ferreira et al., 2008).

To a large extent, plant-associated microbiota are sensitive to environmental alterations, mainly because these communities are present during the entire plant development cycle and respond directly to the biotic and abiotic variations (Moreira and Siqueira, 2006). In this sense, shifts in the occurrence and distribution of root-assembled microbial communities have been reported to differ during yearly stages of plant development (Andreote et al., 2004, 2006) and due to the presence of phytopathogens (Araújo et al., 2002). In this study, we report that *P. veronii* (W) and *R. aquatilis* (R) were observed exclusively on the rhizoplanes of non-infected roots by *C. fimbriata* (Table 2), which possibly could be associated with plant protection. Hence, *R. aquatilis* (R) was also isolated only as an endophyte in the NCF treatment (healthy plants). In the present study, we assessed and demonstrated that *R. aquatilis* was capable of controlling *C. fimbriata* in vitro, corroborating findings from other authors who have shown that these bacterial species act as efficient bio-control agents against fungi (Calvo et al., 2007; Mikani et al., 2008). In the case of eucalyptus and its interaction with *C. fimbriata*, *R. aquatilis* appears to have a strong connection with root plant protection.

We also observed that in the second treatment, in which plants were protected from colonization by microorganisms, there was the occurrence of three specific species: *B. cereus*, *L. sphaericus*, *and R. tropici*. Also, in the second treatment, plants exhibited a larger abundance of species in their tissues. According to Sturz et al. (1999), the microorganisms are able to form barriers on root surfaces, reducing possibility of phytopathogen infection. In this study, we suggest that *P. veronii* and *R. aquatilis* are strongly correlated with the biological control of *C. fimbriata*, protecting the rhizoplanes and endospheres of the eucalyptus plants. However, the plant-bacteria phytopathogen interactions should be better studied to confirm these antagonistic interactions.

Analysis of total community structure showed that *C. fimbriata* present in eucalyptus roots can interfere with the structures of both the rhizoplane and endosphere communities. In the rhizoplane samples, differences in bacterial diversity between roots infected or not infected by *C. fimbriata* were observed. It is likely that during initial stages of plant infection, the plant defense system is activated and able to alter the bacterial community of the rhizoplane (i.e., secreting antagonistic chemical compounds). However, when the plant is completely colonized by *C. fimbriata*, the rhizoplane environment behaves like that of a healthy plant (i.e., without activation of the host defense system). Other studies have shown effects of root-secreted compounds on bacterial community selection. *Pseudomonas* communities of many plant species are modulated by root-secreted compounds (Costa et al., 2006). By assessing infection effects on bacterial communities, it is possible to show differences among the highly affected plants compared to others when the plant system defense activation is unable to interfere with these communities because these communities already naturally colonize internal host plant tissues.

The endophytic microorganisms are also able to deceive the plant host defense systems to effectively colonize their internal tissues. In similar fashion, our analysis showed no distinction between root communities present in healthy plants or those subjected to medium infestation levels by *C. fimbriata*.

When a plant is highly infected, the community is directly affected, perhaps because some bacteria are able to colonize host plants only in compromising conditions, like during infection by *C. fimbriata*. Similar results were observed by Araújo et al. (2002), where the endophytic bacterial communities of citrus plants infected by *Xylella fastidiosa* were significantly different than those of healthy plants. In another case, Araújo et al. (2001) observed that *Guignardia citricarpa* exhibits a synergistic effect with the endophytic citrus bacterium *Pantoea agglomerans* and an antagonistic one with *B. pumilus*. The present work showed that interaction between *C. fimbriata* and the bacterial communities of the rhizoplane and endosphere in eucalyptus roots can occur, suggesting

that the presence of *R. aquatilis* could be associated with protection against *C. fimbriata* attack. Furthermore, it was observed that *C. fimbriata* penetration in the host plant was associated with changes in the root endophytic community, which can directly or indirectly benefit the pathogenic process.

In this context, the present study confirmed our first hypothesis, revealing that different levels of *C. fimbriata* infection modulate the endophytic and rhizoplane bacterial communities of eucalyptus. Additionally, the second hypothesis was also confirmed, demonstrating that specific bacteria, like *R. aquatilis*, associated with eucalyptus roots can act as a natural barrier against *C. fimbriata*. Future research should prioritize the identification of antagonistic bacterial species against *C. fimbriata* capable of inducing systemic resistance in host plants. Furthermore, a deeper understanding of the interaction mechanisms is needed for practical application.

## Funding

This work was supported by CNPq (Brazilian National Counsel of Technological), CAPES (Foundation Coordination for the Improvement of Higher Education Personnel).

## CRediT authorship contribution statement

**Anderson Ferreira:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Esteban Roberto Gonzáles:** Writing – review & editing, Writing – original draft, Funding acquisition, Data curation, Conceptualization. **Fernando Dini Andreote:** Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Conceptualization. **João Lúcio Azevedo:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Welington Luiz Araújo:** Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Anderson Ferreira reports financial support was provided by CNPq (Brazilian National Counsel of Technological), CAPES (Foundation Coordination for the Improvement of Higher Education Personnel). Anderson Ferreira reports a relationship with Brazilian Agricultural Research Corporation that includes: employment.

## Data availability

Data will be made available on request.

## Acknowledgements

This work was supported by CNPq (Brazilian National Counsel of Technological), CAPES (Foundation Coordination for the Improvement of Higher Education Personnel), Brazilian Agricultural Research Corporation - Embrapa.

*Competing Interests*

The authors declare no financial and non-financial competing interests under the heading paper.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2024.100103.

## References

Agrios, G.N., 2004. Plant Pathology. Elsevier, San Diego.

Alfenas, A.C., Ferreira, F.A., Maffia, R.G., Gonçalves, R.C., 2007. Isolamento de fungos fitopatogênicos. In: Alfenas, A.C., Mafia, R.G., Métodos em fitopatologia. Editora UFV, Viçosa. cap. 2, p. 53–90.

Altindag, M., Sahin, M., Esitken, A., Ercisli, S., Guleryuz, M., Donmez, M.F., Sahin, F., 2006. Biological control of brown rot (*Moniliana laxa* Ehr.) on apricot (*Prunus armeniaca* L. cv. Hacıhaliloglu) by *Bacillus*, *Burkholdria*, and *Pseudomonas* application under *in vitro* and *in vivo* conditions. Biol. Control 38, 369–372.

Amein, T., Omer, Z., Welch, C., 2008. Application and evaluation of *Pseudomonas* strains for biocontrol of wheat seedling blight. Crop Prot. 27, 532–536.

Andreote, F.D., Gullo, M.J.M., Lima, A.O.S., Maccheroni, W., Azevedo, J.L., Araújo, W.L., 2004. Impact of genetically modified *Enterobacter cloacae* on indigenous endophytic community of *Citrus sinensis* seedlings. J. Microbiol. 42, 169–173.

Andreote, F.D., Lacava, P.T., Gai, C.S., Araújo, W.L., Maccheroni, W., Van Overbeek, L.S., Van Elsas, J.D., Azevedo, J.L., 2006. Model plants for studying the interaction between *Methylobacterium mesophilicum* and *Xylella fastidiosa*. Can. J. Microbiol. 52, 419–426.

Araujo, F., Guabert, L.M., Silva, I.F., 2012. Bioprospecção de rizobactérias promotoras de crescimento em *Brachiaria brizantha*. Rev. Bras. Zootec. 41, 521–527.

Araújo, W.L., Maccheroni, W.J.R., Aguilar-Vildoso, C.I., Barroso, P.A.V., Saridakis, H.O., Azevedo, J.L., 2001. Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Can. J. Microbiol. 47, 229–236.

Araújo, W.L., Marcon, J., Maccheroni, W., Van Elsas, J.D., Van Vuurde, J.W.L., Azevedo, J.L., 2002. Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl. Environ. Microbiol. 68, 4906–4914.

Azevedo, J.L., Maccheroni, W.J.R., Pereira, J.O., Araújo, W.L., 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. EJB - Electron. J. Biotechnol. 3, 40–65.

Brader G, Compant S, Vescio K, Mitter B, Trognitz F, Ma LJ, Sessitsch A. Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. Annu Rev Phytopathol. 2017 Aug 4;55:61-83. doi: 10.1146/annurev-phyto-080516-035641. Epub 2017 May 10. PMID: 28489497.

Calvo, J., Calvente, V., Orellano, M.E., Benuzzi, D., Tosetti, M.I.S., 2007. Biological control of postharvest spoilage caused by *Penicillium expansum* and *Botrytis cinerea* in apple by using the bacterium *Rahnella aquatilis*. Int. J. Food Microbiol. 113, 251–257, 2007.

CHANDEL, S., ALLAN, E. J. WOODWARD, S. Biological Control of *Fusarium oxysporum* f. sp. *lycopersici* on Tomato by *Brevibacillus brevis*. Journal of Phytopathology, Berlin, v. 158, p. 470–478, 2010.

Chanway, C.P., 1998. Bacterial endophytes: ecological and practical implications. Sydowia 50, 149–170.

Costa, R., Gomes, N.C.M., Peixoto, R.S., Rumjanek, N., Berg, G., Mendonca-Hagler, L.C. S., Smalla, K., 2006. Diversity and antagonistic potential of *Pseudomonas* spp. associated to the rhizosphere of maize grown in a subtropical organic farm. Soil Biol. Biochem. 38, 2434–2447.

Elvira-Recuenco, M., van Vuurde, J.W.L., 2000. Natural incidence of endophytic bacteria in pea cultivars under field conditions. Can. J. Microbiol. 46, 1036–1041.

Ferreira, F.A., Demuner, A.M., Demuner, N.L., Pigatto, S., 1999. Murcha-de-*Ceratocystis* em eucalipto no Brasil (Abstr.). Fitopatol. Bras. 24, 284.

Ferreira, F.A., Maffia, L.A., Ferreira, E.A., 2005. Detecção rápida de *Ceratocystis fimbriata* em lenho infetado de eucalipto, mangueira e outros hospedeiros lenhosos. Fitopatol. Bras. 30, 543–545.

Ferreira, A., Quecine, M.C., Lacava, P.T., Oda, S., Azevedo, J.L., Araújo, W.L., 2008. Diversity of endophytic bacteria from eucalyptus species seeds and colonization of seedlings by pantoea aglomerans. Fems Microbiol. Lett. 287, 8–14.

Fonseca, E.S., Peixoto, R.S., Rosado, A.S., et al., 2018. The Microbiome of Eucalyptus Roots under Different Management Conditions and Its Potential for Biological Nitrogen Fixation. Microb Ecol 75, 183–191. https://doi.org/10.1007/s00248-017-1014-y.

HASSNI, M.E.; HADRAMI, A.E.; DAAYF, F.; CHÉRIF, M.; BARKA, E.A.; HADRAMI, I.E. Biological control of bayoud disease in date palm: Selection of microorganisms inhibiting the causal agent and inducing defense reactions. **Environmental and Experimental Botany**, Elmsford, v. 59, p. 224–234, 2007.

Heuer, H., Krsek, M., Baker, P., Smalla, K., Wellington, E.M.H., 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl. Environ. Microbiol. 63, 3233–3241.

Junker, C, Draeger, S, Schulz, B, 2012. A fine line e endophytes or pathogens in Arabidopsisthaliana. Fungal Ecol 5 (3), 657–662.

Karlidag, H., Esitken, A., Turan, M., Sahin, F., 2007. Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. Sci. Hortic. 114, 6–20.

Kavino, M., Harish, S., Kumar, N., Saravanakumar, D., Damodaran, T., Soorianathasundaram, K., Samiyappan, R., 2007. Rhizosphere and endophytic bacteria for induction of systemic resistance of banana plantlets against bunchy top virus. Soil Biol. Biochem. 39, 1087–1098.

Mafia, R.G., Ferreira, M.A., Zauza, E.A.V., Silva, J.F., Colodette, J.L., &Alfenas, A.C., 2013. Impact of ceratocystis wilt on eucalyptus treegrowth and cellulose pulp yield. Forest Pathology 43, 379–385.

Mendes, R., Pizzirani-Kleiner, A.A., Araújo, W.L., Raaijmakers, J.M., 2007. Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. Appl. Environ. Microbiol. 73, 7259–7267.

Miguel PS, de Oliveira MN, Delvaux JC, de Jesus GL, Borges AC, Tótola MR, Neves JC, Costa MD. Diversity and distribution of the endophytic bacterial community at different stages of Eucalyptus growth. Antonie Van Leeuwenhoek. 2016 Jun;109(6): 755-71. doi: 10.1007/s10482-016-0676-7. Epub 2016 Mar 24. PMID: 27010209.

Mikani, A., Etebarian, H.R., Sholberg, P.L., O'gorman, D.T., Stokes, S., Alizadeh, A., 2008. Biological control of apple gray mold caused by *Botrytis mali* with *Pseudomonas fluorescens* strains. Postharvest Biol. Technol. 48, 107–112.

Moreira, F.M.S., Siqueira, J.O., 2006. Microbiologia e bioquímica do solo. 2 ed. Editora UFLA, Lavras, 729 p.

Nagarajkumar, M., Bhaskaran, R., Velazhahan, R., 2004. Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol. Res. 159, 73–81.

Nimali I. De Silva, Siraprapa Brooks, Saisamorn Lumyong, Kevin D. Hyde, Use of endophytes as biocontrol agents, Fungal Biology Reviews, Volume 33, Issue 2, 2019, Pages 133-148, doi.org/10.1016/j.fbr.2018.10.001.

Park, M., Kim, C., Yang, J., Lee, H., Shin, W., Kim, S., Sa, T., 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiol. Res. 160, 127–133.

Paz, I.C.P., Santin, R.C.M., Guimarães, A.M., Rosa, O.P.P., Dias, A.C.F., Quecine, M.C., Azevedo, J.L., Matsumura, A.T.S., 2012. Eucalyptus growth promotion by endophytic *Bacillus* spp. Genet. Mol. Res. 11, 3711–3720.

Pramod Kumar Sahu, Shailendra Singh, Amrita Gupta, Udai B. Singh, G.P. Brahmaprakash, Anil K. Saxena, Antagonistic potential of bacterial endophytes and induction of systemic resistance against collar rot pathogen Sclerotium rolfsii in tomato, Biological Control, Volume 137, 2019, doi.org/10.1016/j.biocontrol.2019.104014.

Procópio, R.E.L., Araújo, W.L., Maccheroni, W.Jr, Azevedo, J.L., 2009. Characterization of an endophytic bacterial community associated with *Eucalyptus* spp. Genet. Mol. Res. 8, 1408–1422.

Qureshi, A., Verma, V., Kapley, A., Purohit, H.J., 2007. Degradation of 4-nitroaniline by *Stenotrophomonas* strain HPC 135. Int. Biodeterior. Biodegrad. 60, 215–218.

Rodrigo Mendes, Paolina Garbeva, Jos M. Raaijmakers, The rhizosphere microbiome: significance of planMendes, 2013 ic, and human pathogenic microorganisms, FEMS Microbiology Reviews, Volume 37, Issue 5, September 2013, Pages 634–663, https://doi.org/10.1111/1574-6976.12028.

Roux, J., Van Wyk, M., Hatting, H., Wingfield, M.J., 2004. *Ceratocystis* species infecting stem wounds on *Eucalyptus* grandis in South Africa. Plant Pathol. 53, 414–421.

Roux, J., Wingfield, M.J., Wingfield, E.D., Bouillett, J.P., Alfenas, A.C., 1999. A serious new disease of *Eucalyptus* caused by *Ceratocystis frimbriata* in Central Africa. For. Pathol. 30, 175–184.

Ryu, C.M., Kim, J., Choi, O., Kim, S.H., Park, C.S., 2006. Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. Biol. Control 39, 282–289.

Senthilkumar, M., Govindasamy, V., Annapurna, K., 2007. Role of antibiosis in suppression of charcoal rot disease by soybean endophyte *Paenibacillus* sp. HKA-15. Curr. Microbiol. 55, 25–29.

Singh, H., ESITKEN, A., TURAN, M., SAHIN, F., 2007. Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. Scientia Horticulturae, Amsterdam 114, 6–20.

Sturz, A.V., Christieb, B.R., Mathesona, B.G., Arsenaultb, W.J., Buchanana, N.Ak, 1999. Endophytic bacterial communities in the periderm of potato tubers and their potential to improve resistance to soil-borne plant pathogens. Plant Pathol. 48, 360–369.

Tsavkelova, E.A., Cherdyntseva, T.A., Botina, S.G., Netrsov, A.I., 2007. Bacteria associated with orchid roots and microbial production of auxin. Microbiol. Res. 162, 69–76.

WELLER, D.M.; RAAIJMAKERS, J.M.; McSPADDEN GARDENER, B.B.; THOMASHOW, L. S. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology, Palo Alto, v, 40, p. 309-348, 2002.

Zauza, E.A.V., Alfenas, A.C., Harrington, T.C., Mizubuti, E.S., Silva, J.F., 2004. Resistance to *Eucalyptus* clones to *Ceratocystis frimbiata*. Plant Dis. 88, 758–760.