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Endophytic bacteria associated with tissue culture and leaves of *Plinia peruviana*

Abstract – The objective of this work was to isolate endophytic bacteria from tissue culture and leaves of jaboticaba (*Plinia peruviana*) and to evaluate their potential as plant growth-promoting bacteria. The bacteria were isolated from nodal segments grown in vitro and from leaves from a tree under natural conditions, totaling 11 and 54 isolates, respectively. The isolates were characterized by colony morphology. The indolic compounds produced by the isolates, in the presence or absence of 100 mg L^{-1} tryptophan, were quantified. The greatest producers of these compounds were identified by sequencing the *16S rRNA* gene and were inoculated on jaboticaba seeds, using *Azospirillum brasilense* (Ab-V6) as a positive control. The sensitivity of bacteria to eight antibiotics was also evaluated. All assessed bacteria produced indolic compounds, especially *Bacillus* sp., with a content of 27.41 µg mL^{-1}. The germination rate of the seeds inoculated with *Stenotrophomonas* sp. was high – 97.34% compared with that of 74.67% of the negative control. *Bacillus* sp. and *Stenotrophomonas* sp. also sped up germination. Chloramphenicol limited the growth of 82% of the isolates, followed by amoxicillin, gentamicin, levofloxacin, and tetracycline, which limited 70%; erythromycin was only effective against 35%. The endophytic bacteria isolated from jaboticaba show characteristics of plant growth-promoting bacteria, and *Bacillus* sp. and *Stenotrophomonas* sp., obtained from tissue culture, are capable of enhancing jaboticaba seed germination.

Index terms: *Azospirillum brasilense*, *Bacillus*, *Stenotrophomonas*, jaboticaba, micropropagation, plant growth-promoting bacteria.

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Bactérias endofíticas associadas à cultura de tecidos e às folhas de *Plinia peruviana*

Resumo – O objetivo deste trabalho foi isolar bactérias endofíticas de cultura de tecidos e de folhas de jabuticabeira (*Plinia peruviana*) e avaliar seu potencial como bactérias promotoras de crescimento de plantas. As bactérias foram isoladas a partir de segmentos nodais cultivados in vitro e de folhas de árvore sob condições naturais, tendo totalizado 11 e 54 isolados, respectivamente. Os isolados foram caracterizados pela morfologia da colônia. Foram quantificados os compostos indólicos produzidos pelos isolados, na presença ou na ausência de 100 mg L^{-1} de triptofano. Os maiores produtores destes compostos foram identificados pelo sequenciamento do gene *16S rRNA* e foram inoculados em sementes de jabuticaba, tendo-se utilizado *Azospirillum brasilense* (Ab-V6) como controle positivo. Também avaliou-se a sensibilidade das bactérias a oito antibióticos. Todas as bactérias avaliadas produziram compostos indólicos, especialmente *Bacillus* sp., com quantidade de 27,41 µg mL^{-1}. A taxa de germinação das sementes inoculadas com *Stenotrophomonas* sp. foi elevada – 97,34% em comparação à de 74,67% do controle negativo. *Bacillus* sp. e *Stenotrophomonas* sp. também

aumentaram a velocidade de germinação. O cloranfenicol limitou o crescimento de 82% dos isolados, seguido pelas amoxicilina, gentamicina, levofloxacina e tetraciclina, que limitaram 70%; a eritromicina foi eficaz apenas contra 35%. As bactérias endofíticas isoladas da jabuticaba apresentam características de bactérias promotoras de crescimento de plantas, e *Bacillus* sp. e *Stenotrophomonas* sp., provenientes da cultura de tecido, são capazes de aprimorar a germinação de sementes de jabuticaba.

Termos para indexação: *Azospirillum brasilense*, *Bacillus*, *Stenotrophomonas*, jabuticaba, micropropagação, bactérias promotoras de crescimento de plantas.

Introduction

Jaboticaba [*Plinia peruviana* (Poir.) Govaerts] is a fruit tree naturally distributed in Brazil, belonging to the Myrtaceae family. Its fruits are the most popular among the native species of the country and are internationally known as "Brazilian grape" due to their sweet flesh and dark-purple skin (Alezandro et al., 2013). Its propagation is mainly by seeds, which are recalcitrant and can only be stored for a short period (Danner et al., 2011). Therefore, it is necessary to develop new strategies to improve the propagation of jaboticaba. An alternative is the micropropagation of this species, which would be extremely important for the clonal production of selected matrices, but has not yet been reported. Other species of Myrtaceae, such as *Campomanesia xanthocarpa* O. Berg and *Psidium cattleianum* Sabine, have already been successfully micropropagated. However, as in most reports on the in vitro tissue culture of woody species, bacterial growth was observed in the explants (Freire et al., 2018; Machado et al., 2020).

Although the presence of microorganisms in tissue culture is considered one of the greatest problems of micropropagation, it is almost impossible to maintain axenic cultures (Esposito-Polesi, 2011). Among the present bacteria, these authors highlighted the endophytes, which are usually latent even if they show no growth in the medium for a long period. Consequently, their functions in the tissue culture are still little known. One hypothesis is that they can promote plant growth. Therefore, further studies to explore the potential use of these bacteria in tissue culture would be of great importance.

Endophytic bacteria live within plant tissues and cause no visible symptoms in the host (Wang & Dai, 2011). According to these authors, these bacteria interact in different ways with the plant – some are tightly bound to the host by a coevolution process, while others are considered opportunistic, entering the roots and settling on stem and leaves. Many endophytes are also beneficial to the host, being classified as plant growth-promoting bacteria since they produce indolic compounds with auxinic action that can be used by the host to promote its own growth (Santoyo et al., 2016). It should be noted that almost all of those bacteria are resistant to some antibiotics for some unknown reason (Ramakrishna et al., 2019).

Until now, there has been a lack of knowledge of endophytic bacteria associated with jaboticaba tree species, both in situ and in vitro environments. However, these plants are likely to be colonized by plant growth-promoting bacteria, as has been found in studies on other Myrtaceae species (Paz et al., 2012).

The objective of this work was to isolate endophytic bacteria from the tissue culture and leaves of jaboticaba and to evaluate their potential as plant growth-promoting bacteria.

Materials and Methods

For the experiment, bacteria were isolated from tissues grown in vitro and from leaves from a tree under natural conditions. Then, the obtained isolates were characterized and evaluated as to their effects on the in vitro germination of jaboticaba seeds.

The explants used for in vitro cultivation were nodal segments of three-month-old in vitro cultivated seedlings of jaboticaba, without apparent contamination, provided by the Plant Micropropagation Laboratory of the Department of Botany of Universidade Federal do Paraná, located in the municipality of Curitiba, in the state of Paraná, Brazil (25°23'53.4"S, 49°19'04.5"W, at 974 m altitude). Cultures were maintained in a woody plant medium (Lloyd & McCown, 1980) supplemented with 0.1% Plant Preservative Mixture (PPM, Plant Cell Technology, Washington, DC, USA), a broad-spectrum commercial biocide.

The leaves, mature and healthy in appearance, were collected from the outermost branches (cut with 30 cm length) from the lowest region of the canopy of a tree of approximately 45 years of age.

The bacteria were isolated at the Soil Microbiology Laboratory of Embrapa Florestas, located in the

municipality of Colombo, also in the state of Paraná. Nodal segments and leaves were superficially disinfected in a laminar flow hood, immersed in 70% ethyl alcohol for 1 min, then immersed in a 5% sodium hypochlorite solution for 5 and 10 min, respectively, followed by six washes in sterile distilled water. To isolate bacteria from the in vitro cultures, 1 g nodal segments was macerated in 9 mL sterile saline solution (NaCl 0.85%). From this solution, a serial dilution (factor 10) of 10^{-2} to 10^{7} was obtained. Aliquots of 100 µL of each dilution were inoculated in triplicate in Petri dishes, with 9.5 cm in diameter, containing 30 mL dextrose, yeast, and glutamate (DYGS) solid culture medium (Rodrigues Neto et al., 1986). The plates were incubated at 28±2ºC for seven days in the dark for bacterial growth.

For bacterial isolation from leaves, 10 g tissues were crushed in 90 mL saline solution in a sterilized blender for 1 min. Then, the same procedure described for the nodal segments was followed. Different colonies were chosen at each dilution where bacterial growth was observed. The colonies were then purified by streaking in a culture medium with the same composition, and pure isolates were obtained.

The bacteria were characterized by the morphology of their colonies in DYGS medium and separated into different phenotypic groups. Three isolated colonies from each isolated bacterium were evaluated for: growth (classified as very fast, 1 day; fast, 2 to 3 days; intermediate, 4 to 5 days; slow, 6 to 9 days; and very slow; more than 10 days); diameter (mm); shape (punctiform, circular, or irregular); elevation (flat, convex, concave, raised, and protruding); border (whole or irregular); surface (smooth or rough); mucus production (sparse, poor, moderate, and abundant); and transparency (opaque, transparent, and translucent) (Hungria & Silva, 2011).

The similarity of the isolates was calculated using the Jaccard coefficient with the NTSYS-pc, version 2.1t, software (Rohlf, 2000). Then, a dendrogram grouping the isolates from the cultures in vitro and leaves was generated using the unweighted pair group method with arithmetic averages. Bacteria representative of the different phenotypic groups – with at least 80% dissimilarity – were selected to test antibiotic sensitivity and measure the production of indolic compounds.

To evaluate indolic compound production, isolated bacteria were cultured in DYGS liquid medium for 24 hours until reaching an optical density between 0.6 and 0.8 at 630 nm. The control was the Ab-V6 *Azospirillum brasilense* strain – widely used to inoculate seeds of commercial grasses and crops in Brazil –, which was supplied by Embrapa Soja, located in the municipality of Londrina, in the state of Paraná. Aliquots of 500 µL of each cell inoculum were inoculated in triplicate in test tubes containing 5 mL DYGS liquid medium (supplemented or not with 100 mg L^{-1} L-tryptophan) and then were maintained for 48 hours in an incubator in the dark, at 28°C and under agitation at 150 rpm. The solutions were centrifuged at 11,963 *g* for 10 min, and 2 mL supernatant were mixed with 1 mL Salkowski's reagent (Sarwar & Kremer, 1995) and kept in the dark for 30 min for staining. The optical density at 535 nm was recorded in the UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan), and indolic compounds were quantified based on a standard indole-3-acetic acid (Sigma-Aldrich, Saint Louis, MO, USA) curve within a range from 0 to 100 µg L^{-1} ($y = 0.0028x + 0.1178$, $R^2 = 0.9977$). Data were analyzed using the R 3.6.0 environment (R Core Team, 2013). To meet the assumptions of the analysis of variance (Anova), the data were transformed to $(x+1)^{0.5}$. The Anova and Tukey's test to compare means were used at 5% probability.

The five isolates that presented the highest production of indolic compounds – M4 and M7 from the in vitro cultures, and F6, F31, and F45 from the leaves – were selected to be tested separately as jaboticaba seed inoculants. The Ab-V6 strain was used as a positive control, and the DYGS liquid medium (without bacterial cells) as a negative one. The seeds of jaboticaba were taken from mature fruits acquired at a market and superficially disinfected by the same protocol used for the leaves. The bacteria were cultured in DYGS liquid medium under constant agitation, at 150 rpm, for 24 hours, and cell concentration was adjusted to approximately 10^{7} bacterial cells per milliliter. For inoculation, the seeds were placed in plastic bags, separated by treatment for each isolate, to which 300 µL aliquots of bacterial suspension were added. Each bag was closed, keeping a certain volume of air inside, and shaken vigorously for homogenization. The inoculated seeds were placed in a previously sterilized Gerbox, on autoclaved

germitest paper, and kept moist throughout the test. A completely randomized design was used, with five replicates of 15 seeds in each treatment. After 30 days, the germination rate was evaluated (the seeds that emitted roots were considered germinated), as well as the germination speed according to Maguire (1962). The percentage of seeds that emitted the aerial part, the mean number of roots emitted per seed (jaboticaba seeds are polyembryonic), and mean root size (cm) were also evaluated. The Anova and Scott-Knott's test to compare means were used at 5% probability.

Four isolates – M4, M7, F6, and F31 – were selected from the largest producers of indolic compounds in the absence of tryptophan and cultured in a DYGS solid medium in Petri dishes for 24 hours. DNA was extracted from isolated colonies using a DNA extraction kit (Norgen Biotek Corp., Thorold, ON, Canada), following the manufacturer's instructions. For amplification and sequencing, primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) were used (Lane, 1991). An aliquot of 2.5 µL of the extracted DNA was used in the polymerase chain reaction (PCR) for a final volume of 50 µL per reaction. Reagent concentrations per reaction were 0.2 µmol L^{-1} of each primer, 2.5 µmol L^{-1} magnesium chloride, 1X PCR buffer, 0.2 µmol L^{-1} of each dNTP, and 0.02 U Taq DNA polymerase. The conditions for amplification were: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 40 s, annealing at 55°C for 40 s, extension at 72°C for 1.5 min, and final extension at 72°C for 7 min. Amplification products were separated on 1% agarose gel, supplemented with 3 µL ethidium bromide (10 mg mL^{-1}), and visualized under UV light. Sequencing was performed by Macrogen Inc., located in Seoul, South Korea, on the Applied Biosystems 3730xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The ends of the sequences were cut, and the contig was formed using the ChromasPro, version 2.1.8, software (Technelysium Pty Ltd., South Brisbane, Australia). The sequences were compared with those of the GenBank database using the basic local alignment search tool (Blast, 2020) and then aligned with ClustalW (Tamura et al., 2007). The phylogenetic tree was constructed using the neighbor-joining method and Kimura's two-parameter model (Kimura, 1980) with the Mega, version 10.0.5, software (Kumar et al., 2018), by performing 1,000 replicates. The *16S rRNA* gene sequences were deposited in the GenBank under accession numbers MN315373–MN315376.

The sensitivity of the endophytic isolates and *A. brasilense* to antibiotics was tested by the agar diffusion method (Bauer et al., 1966) adapted to the inoculum state and culture medium. Antibiogram disks (Laborclin, Pinhais, PR, Brazil) were used, containing the following antibiotics: 30 µg amoxicillin, 30 µg cefotaxime, 30 µg tetracycline, 30 µg chloramphenicol, 10 µg streptomycin, 10 µg gentamicin, 15 µg erythromycin, and 5 µg levofloxacin. Bacteria were cultured in a DYGS solid medium for 24 hours, then a smear from an isolated colony was made on the plates with the same medium. Four distinct antibiotic disks were inserted in each plate. The assay was performed in duplicate, under incubation at 28±2ºC for 24 hours, and then the diameter of the growth inhibition halo was measured (mm) with the MTX 150 mm digital pachymeter (Mundo das Ferramentas do Brasil Ltda., Guarulhos, SP, Brazil).

Results and Discussion

Sixty-five endophytic microorganisms were isolated, 11 from the in vitro cultures of the nodal segments of micropropagated plants (M1 to M11) and 54 from the jaboticaba leaves (F1 to F54). The endophytes predominated in the leaves from the tree, which was expected since a lower amount of bacteria is usually found in tissue cultures due to the in vitro environment and the use of substances to inhibit their growth. In the in vitro cultures from which the shoots were obtained, the endophytic bacteria were probably latent since no contamination symptoms were observed. In a study on *Eucalyptus benthamii* Maiden & Cambage subjected to a micropropagation process, also without an apparent contamination of the explants, Esposito-Polesi et al. (2015) reported that the composition of the endophytic community varied between explants (branches and trunk) and within the same explants in different subcultures.

The lower amount of the endophytic bacteria from the nodal segments is probably also related to the fact that the explants were isolated from seedlings grown in vitro and not exposed to the endophytic community of the environment, mainly originated from the soil. In addition, the use of PPM (Plant Cell Technology,

Washington, DC, USA) in the culture medium may have contributed to reducing the endophytic community in the shoots. The activity of PPM is effective in areas of high absorption and transportation by the plant, especially in the xylem. However, endophytic bacteria are also distributed in intercellular spaces and in regions with low transport and absorption, where the reach and effect of the biocide is reduced.

All isolates produced indolic compounds in the media supplemented or not with 100 mg mL^{-1} L-tryptophan (Table 1). In the absence of tryptophan, a significant difference was observed in the amount of indolic compounds produced by the isolates, which ranged from 1.54 to 27.41 μg mL^{1} for M10 and M4, respectively; in its presence, the differences were nonsignificant, ranging from 1.25 to 22.01 μg mL^{-1} for M7 and M8.

Table 1. Production of indolic compounds by endophytic bacteria isolated from jaboticaba (*Plinia peruviana*) tissues in the absence or presence of 100 mg L^{-1} L-tryptophan in dextrose, yeast, and glutamate (DYGS) liquid medium[(1)].

Isolate[(2)]	Indolic compounds (μg mL^{-1})	
	Without tryptophan	With tryptophan
Ab-V6	9.35c	6.77[ns]
M4 (*Bacillus* sp.)	27.41a	5.91
M7 (*Stenotrophomonas* sp.)	10.75c	1.25
M8	6.55c	22.01
M9	4.48c	14.62
M10	1.54c	2.75
F1	8.17c	5.91
F6 (*Pseudomonas* sp.)	12.79b	10.01
F10	3.97c	12.58
F11	3.18c	6.56
F13	6.45c	3.87
F21	5.26c	4.62
F29	3.51c	1.57
F31 (*Stenotrophomonas* sp.)	9.89c	8.38
F33	3.44c	9.03
F40	7.63c	8.71
F45	16.45b	8.49
Coefficient of variation (%)	28.84	42.71

[(1)]Means followed by equal letters, in the columns, do not differ significantly by Scott-Knott's test, at 5% probability. [(2)]Ab-V6, *Azospirillum brasilense* (control); initial M, isolates from nodal segments grown in vitro; and initial F, isolates from leaves of a jaboticaba tree grown under natural conditions. The isolates without a name between parenthesis were not identified. [ns]Nonsignificant.

Isolate M4 (*Bacillus* sp.) showed a high production of indolic compounds in the absence of tryptophan, almost three times higher than that of *A. brasilense*, which is a reference among bacteria classified as plant growth-promoting bacteria (PGPB) due to its phytohormone production. Among 16 *Bacillus* strains, Ali et al. (2009) found an auxin production ranging from 1.7 to 22.2 μg mL^{-1} without tryptophan, lower than that observed for M4. It is possible that the presence of M4 in in vitro cultures could have influenced the development of the explants, because compounds with auxinic action can induce, for example, the rooting of nodal segments. Therefore, these bacteria would probably be beneficial during the rooting and acclimatization step of jaboticaba seedlings. Isolates F6 (*Pseudomonas* sp.) and F45 (not identified) showed a production higher than 12 μg mL^{-1} without tryptophan.

The M8, M9, F10, and F33 isolates increased more than twice their production of indolic compounds in the presence of tryptophan. These bacteria probably used the tryptophan-dependent pathway for the synthesis of indolic compounds. However, the independent method of indolic compound biosynthesis is desirable as it allows the selection of bacteria with potential for plant growth promotion without the need of using tryptophan, which is relatively scarce in the environment (Porto et al., 2017).

After the inoculation of the seeds with each isolate, a significant effect was observed for germination rate and speed, as well as for the average root size of the seedlings (Table 2). However, the other studied variables were not affected by the bacteria. Seeds inoculated with isolate M7 (*Stenotrophomonas* sp.) germinated faster and in a greater quantity than those of the negative control. In addition, this isolate caused an increase of more than 20% in germinated seeds when compared with this same control. However, there are still few reports in the literature on the use of *Stenotrophomonas* as an inoculant. Schmidt et al. (2012) verified positive effects of *Stenotrophomonas rhizophila* on the germination of tomato (*Solanum lycopersicum* L.), cotton (*Gossypium hirsutum* L.), and sweet pepper (*Capsicum annuum* L.) seeds. The reported benefits were mainly due to the antagonism of *S. rhizophila* to pathogenic and deleterious microorganisms. However, as the seeds were disinfected prior to bacterial inoculation, it is possible that the observed improvement was, at least in part, due

to the high production of 10.75 μg mL^{-1} phytohormones by *Stenotrophomonas* sp. The treatment with isolate M4 also sped up germination, when compared with the negative control (Table 2), but it did not alter the germination rate. Likewise, Mia et al. (2012) found a higher growth of rice (*Oryza sativa* L.) in the presence of *Bacillus sphaericus*; however, as in the present study, the germination rate did not vary.

Root size increased due to the inoculation of the Ab-V6 strain of *A. brasilense* compared with the negative control (Table 2), possibly because of the production of plant-growth regulators, particularly indolic compounds. Similarly, in seed inoculation, Hungria et al. (2010) verified the positive influence of an *A. brasilense* Ab-V6 strain on corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) production, which increased in 28 and 8%, respectively. However, the inoculation with isolates F6, F31 (*Stenotrophomonas* sp.), and F45 did not affect the different variables, also in comparison with the negative control.

The partial sequencing of the *16S rRNA* gene of four isolates indicated a similarity with the genera *Bacillus*, *Pseudomonas*, and *Stenotrophomonas* (Figure 1), which have species classified as PGPB. The M4 isolate is close to *Bacillus nealsonii*, a species able to produce spores resistant to UV light, γ radiation, low humidity, and high temperature (Zammuto et al., 2018). Bacteria of this genus are often used in commercial inoculants, and, in agriculture, they have advantages over other PGPB due to the long viability of their spores (Sansinenea & Ortiz, 2011). When associated with the host, they act in the biological control of pathogens (Souza Júnior et al., 2010).

Isolate F6 was characterized as *Pseudomonas lutea*. Kwak et al. (2016) showed the ability of this species to solubilize phosphate and produce a yellow pigment, as well as the presence of genes related to auxin synthesis in its genome. However, no report of the association of the isolate with leaf tissues was found in the literature. *Pseudomonas* is one of the most complex and abundant genera of bacteria, whose members are among the most common endophytes in the rhizosphere (Santoyo et al., 2016).

Isolates M7 and F31 clustered near *Stenotrophomonas pavanii* and *Stenotrophomonas maltophilia*. Bacteria of the genus *Stenotrophomonas* are found in soil and mainly associated with plants, showing potential to be used in agriculture due to their ability to synthesize indolic compounds, fix nitrogen, and produce antibiotics (Ryan et al., 2009). In most researches, the endophytic strains of *S. maltophilia* were isolated from plant roots, as those of cucumber (*Cucumis sativus* L.) (Islam et al., 2016); however, in the present study, they were also found in stem and leaves.

Of the 17 isolates evaluated, only F45 was not susceptible to any of the eight tested antibiotics (Table 3). Regarding the isolates from the nodal segments grown in vitro, all were affected at different levels by amoxicillin, chloramphenicol, streptomycin, and levofloxacin. Erythromycin was effective against only 35% of the isolates. However, chloramphenicol had the largest coverage, limiting the growth of 82% of them, followed by amoxicillin, gentamicin, levofloxacin and

Table 2. In vitro germination and seedling development of jaboticaba (*Plinia peruviana*) seeds inoculated with endophytic bacteria isolated from the plant[1].

Bacteria (300 μL)[2]	Germination (%)	GS[3]	PE (%)[4]	MNR[5]	ARS (cm)[6]
DYGS liquid medium[7]	74.67b	0.86c	50.44[ns]	1.60[ns]	2.12b
Ab-V6 (*Azospirillum brasilense*)	85.34ab	1.06abc	53.51	1.83	3.28a
M4 (*Bacillus* sp.)	85.34ab	1.21ab	51.93	1.70	2.59ab
M7 (*Stenotrophomonas* sp.)	97.34a	1.35a	53.05	1.75	2.67ab
F6 (*Pseudomonas* sp.)	96.67ab	1.04bc	40.42	1.72	2.02b
F31 (*Stenotrophomonas* sp.)	85.34ab	1.03bc	39.10	1.94	2.29ab
F45 (Not identified)	94.67ab	1.10abc	52.57	1.77	2.39ab
Coefficient of variation (%)	12.04	13.50	31.85	17.78	23.06

[1]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability. [2]Bacteria were cultured in dextrose, yeast, and glutamate (DYGS) liquid medium under 150 rpm agitation for 24 hours and at 10^7 cells per milliliter. [3]Germination speed. [4]Plumule emission in germinated seed. [5]Mean number of roots. [6]Average root size. [7]DYGS without bacterial cells (control). [ns]Nonsignificant.

tetracycline, which limited 70%. The resistance to some antibiotics by isolated bacteria was already expected, since the presence of genes that enable resistance is a common feature in PGPB. According to Ramakrishna et al. (2019), the biological significance of almost all PGPB showing antibiotic resistance is still unknown, but may be related to the fact that the proteins involved in resistance are essential for plant growth promotion. Therefore, these authors highlighted that knowing which antibiotics do not affect the PGPB of interest can be useful to control contamination by unwanted bacteria in micropropagation without affecting growth promotion.

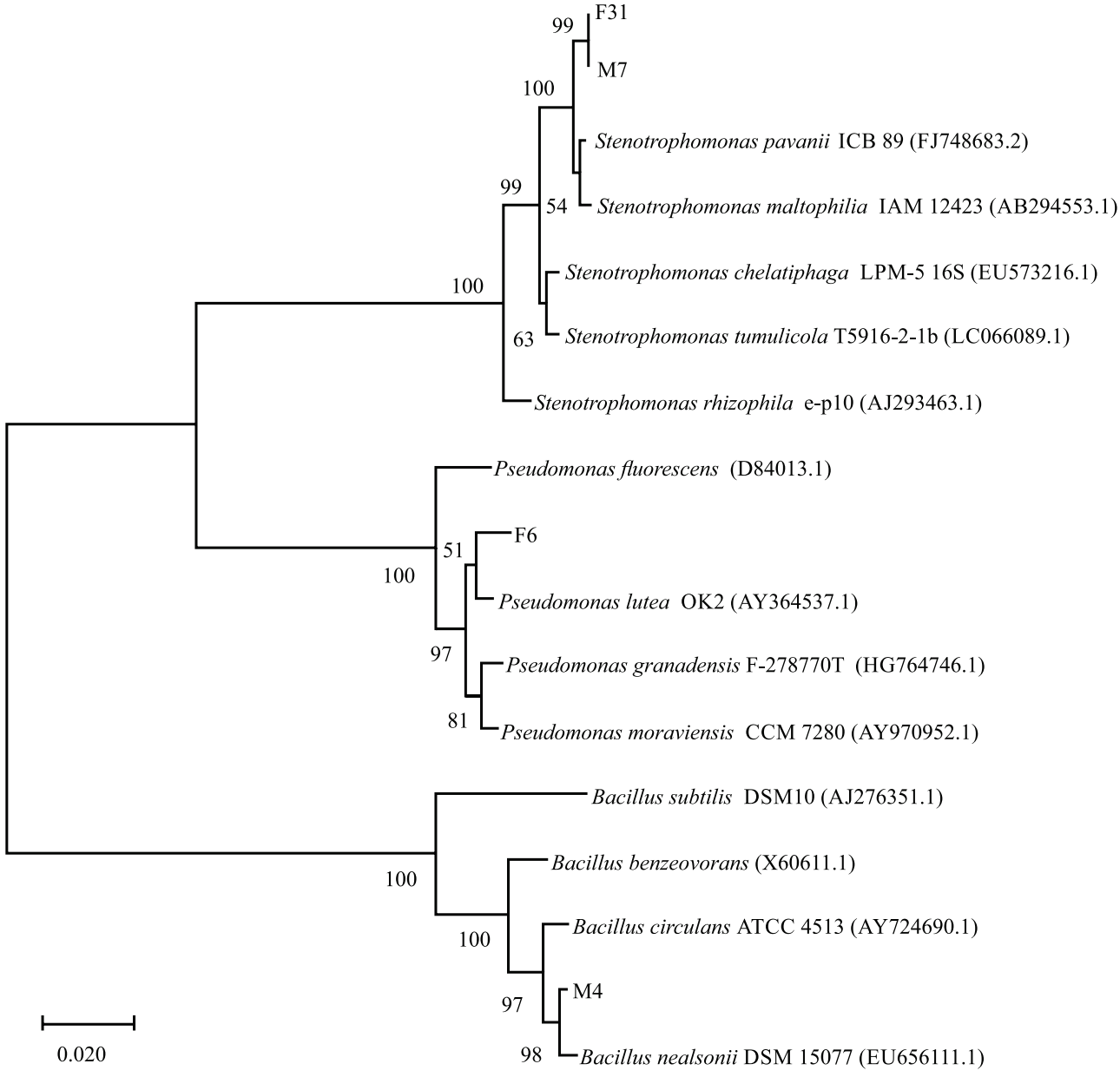

Figure 1. Phylogenetic tree based on *16s rRNA* gene sequences relating bacteria isolated from jaboticaba (*Plinia peruviana*) tissues to reference strains. M4 and M7, isolated from nodal segments grown in vitro; and F6 and F31, isolated from leaves of a jaboticaba tree grown under natural conditions. Bootstrap values greater than 50% are indicated on nodes. Scale: two substitutions per 100 nucleotide positions.

Table 3. Sensitivity of endophytic bacteria isolated from jaboticaba (*Plinia peruviana*) to antibiotics, when assessed by inhibition halo diameter (mm) around disks impregnated with antibiotics in dextrose, yeast, and glutamate (DYGS) solid medium.

Isolate[1]	Antibiotic (µg)[2]							
	AMC(30)	CTX (30)	CLO (30)	ERY (15)	STR (10)	GEN (10)	LVX (5)	TET (30)
Ab-V6	18	14	12	23	-	26	21	24
M4 (*Bacillus* sp.)	43	32	41	26	18	11	21	34
M7 (*Stenotrophomonas* sp.)	11	-	13	-	17	9	32	-
M8	18	8	12	9	11	9	7	16
M9	32	25	35	14	8	-	18	17
F1	9	7	11	7	7	11	-	9
F5	9	7	15	13	-	9	10	25
F6 (*Pseudomonas* sp.)	9	7	-	-	7	12	-	14
F10	8	13	16	-	20	35	43	16
F11	8	17	20	-	8	10	32	18
F13	11	-	8	-	-	7	11	11
F21	8	19	23	-	9	11	-	10
F29	-	-	18	-	-	-	27	9
F31 (*Stenotrophomonas* sp.)	-	-	18	-	-	-	16	-
F33	-	-	13	-	-	-	22	-
F40	-	-	-	-	-	13	-	-
F45	-	-	-	-	-	-	-	-

[1]Ab-V6, *Azospirillum brasilense*; initial M, isolates from nodal segments grown in vitro; and initial F, isolates from leaves of a jaboticaba tree grown under natural conditions. [2]AMC, amoxicillin; CTX, cefotaxime; CLO, chloramphenicol; ERY, erythromycin; STR, streptomycin; GEN, gentamicin; LVX, levofloxacin; and TET, tetracycline. -, absence of inhibition halo. The isolates without a name between parenthesis were not identified.

Conclusions

1. Endophytic bacteria isolated from the tissue culture and leaves of jaboticaba (*Plinia peruviana*) have characteristics of plant growth-promoting bacteria, such as the ability to produce indolic compounds.

2. The *Bacillus* and *Stenotrophomonas* bacteria isolated from the tissue culture of jaboticaba are the greatest producers of indolic compounds, and their separate inoculation on seeds of this tree has a positive effect on germination rate and speed, as well as on the average root size of the seedlings, compared with the uninoculated seeds.

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