PAB
Pesquisa Agropecuária Brasileira

Forestry/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Plant growth-promoting endophytic bacteria in peach palm seedlings

Abstract – The objective of this work was to isolate endophytic bacteria from peach palm (*Bactris gasipaes* var. *gasipaes*) plants and to evaluate the effects of their inoculation on the plant seedlings. Bacteria were isolated from the leaves and roots of the seedlings and from the meristems of peach palm plants in vitro. The isolates were characterized phenotypically and, then, 15 of them, representing different phenotypic groups, were selected and identified by partial sequencing of the 16S rRNA gene. Afterward, these isolates and two commercial strains of *Azospirillum brasilense* (Ab-V5 and Ab-V6) were inoculated in the peach palm seedlings. After 76 days, the seedlings were evaluated for plant development. The following six genera were identified based on the sequencing: *Pseudomonas, Enterobacter*, *Rhizobium*, *Stenotrophomonas*, *Klebsiella*, and *Erwinia*. Out of the 15 inoculated isolates, 9 had a positive effect on the root dry mass of palm peach, with CNPF 77 (*Enterobacter* sp.), CNPF 100 (*Rhizobium* sp.), and CNP 179 and CNPF 277 (*Stenotrophomonas* sp.) standing out. Peach palm seedlings harbor endophytic bacteria which are able to increase root dry matter.

Index terms: *Bactris gasipaes* var. *gasipaes*, *Enterobacter*, *Rhizobium*, *Stenotrophomonas*, bioinoculants, 16S rRNA.

Krisle da Silva[1] ✉, Regina Caetano Quisen[1], Juliana Degenhardt Goldbach[1], Kauanna Brok Ferreira Pepe[2] and Antônio Nascim Kalil Filho[1]

[1] Embrapa Florestas, Estrada da Ribeira, Km 111, Parque Monte Castelo, CEP 83411-000 Colombo, PR, Brazil.
E-mail: krisle.silva@embrapa.br, regina.quisen@embrapa.br, juliana.degenhardt@embrapa.br, antonio.kalil@embrapa.br

[2] Pontifícia Universidade Católica do Paraná, Escola de Ciências da Vida – Agronomia, Rua Imaculada Conceição, nº 1.155, Prado Velho, CEP 80215-901 Curitiba, PR, Brazil.
E-mail: kauanna.bfp@gmail.com

✉ Corresponding author

Received
April 20, 2022

Accepted
August 09, 2022

How to cite
SILVA, K. da; QUISEN, R.C.; GOLDBACH, J.D.; PEPE, K.B.F.; KALIL FILHO, A.N. Plant growth-promoting endophytic bacteria in peach palm seedlings. **Pesquisa Agropecuária Brasileira**, v.57, e02962, 2022. DOI: https://doi.org/10.1590/S1678-3921.pab2022.v57.02962.

Bactérias endofíticas promotoras de crescimento de plantas em mudas de pupunheira

Resumo – O objetivo deste trabalho foi isolar bactérias endofíticas de pupunheira (*Bactris gasipaes* var. *gasipaes*) e avaliar os efeitos da inoculação delas em mudas da planta. As bactérias foram isoladas de folhas e raízes das mudas e de meristemas de pupunheira in vitro. Os isolados foram caracterizados fenotipicamente, e, depois, 15 deles, representando grupos fenotípicos distintos, foram selecionados e identificados por meio do sequenciamento parcial do gene 16S rRNA. Em seguida, esses isolados e duas estirpes comerciais de *Azospirillum brasilense* (Ab-V5 e Ab-V6) foram inoculados em plântulas de pupunheira. Após 76 dias, as mudas foram avaliadas quanto ao desenvolvimento vegetal. Foram identificados os seis seguintes gêneros com base no sequenciamento: *Pseudomonas*, *Enterobacter*, *Rhizobium*, *Stenotrophomonas*, *Klebsiella* e *Erwinia*. Dos 15 isolados inoculados, 9 tiveram efeito positivo sobre a massa de matéria seca de raízes, com destaque para CNPF 77 (*Enterobacter* sp.), CNPF 100 (*Rhizobium* sp.), e CNP 179 e CNPF 277 (*Stenotrophomonas* sp.). Mudas de pupunheira abrigam bactérias endofíticas capazes de aumentar a matéria seca das raízes.

Termos para indexação: *Bactris gasipaes* var. *gasipaes*, *Enterobacter*, *Rhizobium*, *Stenotrophomonas*, bioinoculantes, 16S rRNA.

Introduction

Peach palm (*Bactris gasipaes* Kunth var. *gasipaes* Henderson) is considered the most important domesticated palm species in the Neotropics because of the diversity of its products, such as edible fruit rich in starch, flour, oil, and the production of palm hearts, (Graefe et al., 2013). Since the 1990s, this species has emerged as the main crop for supplying palm hearts in the Brazilian market; and the states of São Paulo, Bahia and Paraná are its main producers and consumers (Silva, 2017; Franchetti & Rozane, 2017).

The expansion of peach palm cultivation area brought new demands. The provision of sufficient quantities of seed and seedlings of genetic and sanitary quality are some of the bottlenecks in this production system of this crop (Yokomizo & Kalil Filho, 2020). For this reason, studies involving different cloning techniques have been reported, such as the rooting of basal offshoots (Isaid et al., 2018). However, few significant advances are reported for the rooting percentage and field survival of this type of propagule that could result in a technique applicable on a larger scale (Yokomizo & Kalil Filho, 2020).

Plant growth-promoting bacteria (PGPB) can act on plant growth either through direct mechanisms (biological nitrogen fixation, phytohormone productions, phosphorus solubilization, and iron sequestration by siderophore producers) or indirect mechanisms (induction of systemic resistance and competition and production of antibiotics, among others) (Olanrewaju et al., 2017; Afzal et al., 2019). Several studies for the isolation and inoculation of bacteria have shown a large number of endophytic bacteria colonizing specific niches inside plants with different responses for plant growth (Jha et al., 2013; Brusamarello-Santos et al., 2017).

Considering the PGPB benefits, their inoculation in some agricultural crops and the use of biofertilizers obtained from bacteria, when applied at early stages of plant development and in vegetative propagules, can positively influence the initiation and growth of roots and stems (Mariosa et al., 2017; Cipriano et al., 2021). For instance, promising results were obtained from bacterial isolates in the induction and formation of adventitious roots in cuttings of woody species of eucalyptus, in which *Azospirillum* spp. strains were inoculated (Raasch et al., 2013). These bacterial groups have the ability to produce phytohormones, such as indole acetic acid, and they can induce further development of the root system (Lana et al., 2017). Costa et al. (2019) isolated endophytic fungi and bacteria from peach palm fruit with phytopathogen inhibition properties; however, the identification of the isolates was not carried out in their study. The isolation and selection of PGPB in peach palm are of great interest to increase the survival rate of seedlings, rooting of basal offshoots, pathogen control, and productivity.

The objective of this work was to isolate endophytic bacteria from peach palm plants, and to evaluate their effects on peach palm seedlings.

Materials and Methods

Ten grams of root and leaf tissues obtained from four peach palm seedlings approximately 8 months of age were used for the evaluation of the density and isolation of bacteria. These seedlings were produced from seed in a commercial nursery (MM Mudas, Eldorado, SP, Brazil) in plastic bags containing substrate. The roots and leaves were collected, and they were first washed in running tap water and superficially disinfected with alcohol 70% for 30 s, followed by sodium hypochlorite 6% (active chlorine) for 2 min and 30 s, and six washes in sterile deionized water. To ensure the isolation of only endophytes, 100 µL water from the last wash was plated to verify the absence of external bacteria. The plant material was placed in 90 mL of sterile saline solution (NaCl) at 0.85%, and ground in a blender at full power for 1 min, representing a 10^{-1} dilution. An aliquot of 500 µL was taken from each sample, which was then added to a test tube containing 4.5 mL of sterile saline solution, representing a dilution of 10^{-2}, followed by the same procedure until a 10^{-7} dilution was attained. In Petri dishes containing solid culture medium (DYGS) with dextrose, yeast, and glutamate (Rodrigues Neto et al., 1986), 100 µL of each dilution was inoculated with three replicates each. The plates were incubated at 28°C for 10 days. Different colonies (approximately 10 colonies per plate) diluted from 10^{-4} to 10^{-7} were then selected and subcultured until purification. In addition to the isolates obtained from roots and leaves, 11 isolates obtained from meristems of peach palm grown in vitro, maintained at the laboratory of tissue culture and transformation, at Embrapa Florestas, were also included. All isolates were morphologically characterized in the DYGS medium.

For that, the morphology of at least three colonies isolated from each isolate was evaluated for the growth time (very fast, fast, intermediate, slow or very slow), form (punctiform, circular, or irregular), elevation (flat, convex, raised, pulvinate, or umbonate), margin (smooth, undulate, lobate, erose, or filamentous), surface (smooth, or rough), mucus production (sparse, little, moderate, or abundant), mucus transparency (opaque, transparent, or translucent), homogeneity, and color (Hungria & Silva, 2011). The similarity of the bacterial isolates was calculated using the Jaccard coefficient with NTSYS-pc software, version 2.1t (Rohlf, 2000). A dendrogram grouping the bacteria from roots, leaves, and meristems was generated using the UPGMA method. Bacterial isolates representative of the different phenotypic groups, with at least 75% dissimilarity, were selected with three isolates obtained from meristems (Table 1); then, they were subjected to partial sequencing of the 16S rRNA gene and to inoculation in seedlings. All isolates were stored in glycerol at -20°C and in mineral oil, at room temperature, in the laboratory of soil microbiology of Embrapa Florestas.

The isolated bacteria were cultured in solid DYGS medium for 48 hours, and the DNA from isolated colonies was extracted using the PureLink Genomic DNA mini kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA), following the manufacturer's instructions. The amplification of the 16S rRNA gene was performed using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991), and the partial sequencing was performed using the primer 27F. Sequencing was performed by Macrogen Inc., located in Seoul, South Korea, on an Applied Biosystems 3730xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The ends showing low quality were manually edited using the MEGA version X software (Kumar et al., 2018). Then, the sequences were subjected to the basic local alignment search tool (BLAST) in NCBI (2022a), using only alignment with type strains of the databank. The 16S rRNA gene sequences were deposited in the GenBank under the accession number OM912645-OM912659.

The nursery experiment was installed in February 2020 using peach palm seedlings provided by the company MM Mudas (Eldorado, SP, Brazil) of about 60 cm height and approximately 8 months of age. The seedlings were transplanted into 3.8 L pots containing a mixture of nonsterile soil and expanded vermiculite of medium texture at 1:1 (v/v). The soil chemical characteristics were the following: pH 6.29 in $CaCl_2$; 0.0 cmol dm^{-3} Al^{+3}; 3.13 cmol dm^{-3} $H+Al^{+3}$; 8.26 $cmol_c$ dm^{-3} Ca^{+2}; 5.85 $cmol_c$ dm^{-3} Mg^{+2}; 0.21 $cmol_c$ dm^{-3} K^{+}; 2.1 mg dm^{-3} P; and 2.85% total orgnic carbon. The seedlings were subjected to the inoculation of 15 bacterial isolates obtained from peach palm tissues and two strains of *Azospirillum brasilense* (Ab-V5 and Ab-V6). Bacteria were previously cultured in liquid DYGS medium for 48 hours, in an incubator at 28°C, under 150 rpm agitation. For that, 5 mL of each isolate was inoculated at the base of the seedlings (treatment), and also in the control treatment (5 mL of water). The strains of *A. brasilense* were inoculated at a proportion of 1:1 (v/v) both together and individually.

The statistical design was completely randomized with 5 replicates, and each one represented by a pot with one seedling. Fertilization was applied fortnightly following the recommendation of Morsbach et al. (1998). Intermittent nebulization was activated every 3 hours for 3 min. At 76 days after transplanting, the plant height (PH) and the number of leaves (NL) were evaluated. Aerial parts and roots were separated and dried at 60°C until they reached a constant weight. Shoot dry matter (SDM), root dry matter (RDM), and the root dry matter/shoot dry matter ratio (RDM/SDM) were evaluated. For all variables, the Shapiro-Wilk's normality test was performed, at 5% probability. The NL data did not show a normal distribution and were transformed to $(x+1)^{0.5}$ before the analysis of variance. The means were compared using the Scott-Knott's test (1974), at 5% probability. Statistical analyses were performed by the R software version 3.2.2 (R Core Team, 2015), using the statistical package ExpDes.pt version 1.1.2 (Ferreira et al., 2013).

Results and Discussion

Colony-forming units (CFU) from roots and leaves of the palm peach seedlings were estimated between 30 and 300 colonies. The bacterial densities were 3.40 x 106 CFU g^{-1} and 1.14 x 108 CFU g^{-1} for leaves and roots, respectively. After the purification, 207 bacterial isolates were obtained, including 58 from leaves and 138 from roots. There was no bacterial growth after roots and leaves surface washing, which suggests

that the isolated bacteria are endophytic. A group of 5 isolates from roots and 7 isolates from leaves were selected based on their phenotypic similarities, and these isolates together with 3 isolates from meristems were grouped into 15 phenotypic groups (Table 1).

The partial sequencing of the 16S rRNA gene allowed of the identification of the following bacterial genera in peach palm roots: *Enterobacter* (CNPF 77 and CNPF 108), *Pseudomonas* (CNPF 99), *Rhizobium* (CNPF 152), and *Stenotrophomonas* (CNPF 179). As to leaves, the same genera found in roots were identified: *Rhizobium* (CNPF 94 and CNPF 100), *Pseudomonas* (CNPF 118 and CNPF 154), *Enterobacter* (CNPF 235 and CNPF 248) and *Stenotrophomonas* (CNPF 277). From meristems, two genera distinct from those found in roots and leaves were identified: *Klebsiella* (CNPF 90 and CNPF 105) and *Erwinia* (CNPF 110).

There were no significant differences between the inoculation treatments and the control without inoculation for SDM in seedlings (Table 2). Because the experiment was carried out using seedlings, there was genetic variability between them, which may have resulted in the absence of a difference between inoculation and noninoculation treatments. Statistical differences among the treatments were observed only for RDM. Higher RDM values were observed for CNPF 77 (*Enterobacter* sp.), CNPF 100 (*Rhizobium* sp.), CNPF 179 (*Stenotrophomonas* sp.) and CNPF 277 (*Stenotrophomonas* sp.). The RDMs were also higher for five isolates – CNPF 90 (*Klebsiella* sp.), CNPF 94 (*Rhizobium* sp.), CNPF 105 (*Klebsiella* sp.), CNPF 118

Table 1. Bacterial isolates obtained from leaves, meristems, and roots of peach palm (*Bactris gasipaes* Kunth var. *gasipaes* Henderson), selected by the morphology of their colonies in DYGS medium: origin, morphology, identification, and number of access in the Genbank (NCBI, 2022b).

Isolate	Origin	Morphological characteristics	Genus	Genbank number
CNPF 77	Roots	Very fast growth, circular colony, convex, entire margin, rugose surface, moderate mucus, translucent, cream color, and homogenous colonies.	*Enterobacter* sp.	OM912659
CNPF 90	Meristems	Fast growth, circular colony, convex, entire margin, smooth surface, moderate mucus, translucent, white color, and homogenous colonies.	*Klebsiella* sp.	OM912658
CNPF 94	Leaves	Fast growth, circular colony, raised, entire margin, smooth surface, abundant mucus, translucent, cream color, and homogenous colonies.	*Rhizobium* sp.	OM912657
CNPF 99	Roots	Very fast growth, circular colony, flat, erose margin, rugose surface, little mucus, transparent, cream color, and heterogenous colonies.	*Pseudomonas* sp.	OM912656
CNPF 100	Leaves	Fast growth, circular colonies, raised, entire margin, smooth surface, moderate mucus, translucent, cream color and homogenous colonies.	*Rhizobium* sp.	OM912655
CNPF 105	Meristems	Intermediary growth, circular colonies, convex, entire margin, smooth surface, moderate mucus, opaque, black color, and homogenous colonies.	*Klebsiella* sp.	OM912654
CNPF 108	Roots	Fast growth, circular colonies, convex, entire margin, smooth surface, moderate mucus, translucent, cream color, and heterogenous colonies.	*Enterobacter* sp.	OM912653
CNPF 110	Meristems	Fast growth, circular colonies, convex, entire margin, smooth surface, abundant mucus, opaque, cream color, and homogenous colonies.	*Erwinia* sp.	OM912652
CNPF 118	Leaves	Very fast growth, circular colonies, flat, erose margin, rugose surface, moderate mucus transparent, cream color, and homogenous colonies.	*Pseudomonas* sp.	OM912651
CNPF 152	Roots	Fast growth, circular colonies, raised, undulate margin, smooth surface, moderate mucus, translucent, cream color, and homogenous colonies.	*Rhizobium* sp.	OM912650
CNPF 154	Leaves	Fast growth, irregular colonies, flat, lobate margin, rugose surface, moderate mucus, translucent, yellow and cream colors, and heterogenous colonies.	*Pseudomonas* sp.	OM912649
CNPF 179	Roots	Fast growth, circular colonies, flat, entire margin, smooth surface, sparse mucus, translucent, cream color, and homogenous colonies.	*Stenotrophomonas* sp.	OM912648
CNPF 235	Leaves	Fast growth, circular colonies, convex, erose margin, smooth surface, moderate mucus, transparent, yellow and cream colors, and heterogenous colonies.	*Enterobacter* sp.	OM912647
CNPF 248	Leaves	Fast growth, circular colonies, convex, entire margin, smooth surface, abundant mucus, translucent, cream color, and homogenous colonies.	*Enterobacter* sp.	OM912646
CNPF 277	Leaves	Fast growth, irregular colonies, flat, lobate margin, smooth surface, moderate mucus, translucent, yellow and cream colors, and heterogenous colonies.	*Stenotrophomonas* sp.	OM912645

(*Pseudomonas* sp.), and CNPF 152 (*Rhizobium* sp.) – and the combination of two commercial *Azospirillum brasilense* strains, Ab-V5 + Ab-V6, in comparison with the control without inoculation. The other isolates and the inoculation of *A. brasilense* strains Ab-V5 or Ab-V6 alone did not differ from the control without inoculation.

Among the genera isolated from meristems, *Erwinia* is a genus among plant pathogens (Hauben et al., 1998) that cause a great damage to important crops; however, a recently identified strain as *E. gerundensis* showed evidence of plant growth promotion, especially regarding the acquisition of nutrients (Saldierna Guzmán et al., 2021). In our work, the isolate CNPF 110 (*Erwinia* sp.) did not cause any damage to seedlings. Increases of the RDM were observed when isolates belonging to the genus *Klebsiella* sp.– CNPF 90 and CNPF 105 – were inoculated in the seedlings. Bacteria belonging to the genus *Klebsiella* are commonly associated with plants and show growth-promoting characteristics and pathogen control (Dey et al., 2019), siderophore (Mowafy et al., 2021), phosphorus solubilization, and indolic acid compound production (Chalita et al., 2019) properties, among others. Bacteria of the *Enterobacter* genus, such as *E. cloacae*, have been reported to increase the germination rate, seed vigor, and shoot and root dry mass in rice (*Oryza sativa*), peanut (*Arachis hypogaea*), black bean (*Vigna mung*o) and canola (Yaish et al., 2015). Among the four bacterial isolates identified as *Enterobacter*, only CNPF 77 increased the root system in seedlings of peach palm. Isolates of the genus *Pseudomonas* were also found to be associated with peach palm in this work. Although bacteria of the *Pseudomonas* genus are plant growth promoters (Sah et al., 2021), among the three bacteria used in this study, only one promoted the increase of the root system, in comparison with the control, although at lower rates than those of the other isolates. Plants subjected to the inoculation of *Stenotrophomonas*, represented by the CNPF 179 and 277 isolates, also showed higher RDMs. The genus *Stenotrophomonas* comprises species which have been also reported to have growth-promoting abilities. When inoculated in tomato plants, bacteria of this

Table 2. Peach palm response to the inoculation of endophytic bacterial isolates under nursery conditions[(1)].

Treatment	SDM	RDM	RDM/SDM	Plant height	Number of leaves
	----------(g per plant)----------			(cm per plant)	(no. per plant)
CNPF 77 (*Enterobacter* sp.)	5.68	6.57a	1.18a	13.60	4.60
CNPF 90 (*Klebsiella* sp.)	5.44	5.53b	1.02b	14.40	4.20
CNPF 94 (*Rhizobium* sp.)	4.94	5.63b	1.15a	12.75	4.25
CNPF 99 (*Pseudomonas* sp.)	5.40	5.07c	0.93b	11.75	3.75
CNPF 100 (*Rhizobium* sp.)	5.45	6.73a	1.25a	13.60	3.80
CNPF 105 (*Klebsiella* sp.)	5.15	5.51b	1.06a	14.00	3.75
CNPF 108 (*Enterobacter* sp.)	4.58	4.36c	0.95b	13.00	4.25
CNPF 110 (*Erwinia* sp.)	5.61	4.94c	0.90b	12.20	4.20
CNPF 118 (*Pseudomonas* sp.)	5.06	5.50b	1.10a	13.00	4.50
CNPF 152 (*Rhizobium* sp.)	4.43	5.68b	1.29a	13.25	4.25
CNPF 154 (*Pseudomonas* sp.)	4.08	4.22c	1.05a	13.00	4.00
CNPF 179 (*Stenotrophomonas* sp.)	6.31	7.03a	1.18a	12.40	4.20
CNPF 235 (*Enterobacter* sp.)	4.50	4.26c	0.94b	13.40	4.20
CNPF 248 (*Enterobacter* sp.)	5.40	4.17c	0.77b	13.60	4.40
CNPF 277 (*Stenotrophomonas* sp.)	5.23	6.43a	1.25a	13.00	4.20
Ab-V5 (*Azospirillum brasilense*)	5.05	4.71c	0.93b	13.25	4.34
Abv-V6 (*Azospirillum brasilense*)	5.29	4.81c	0.91b	12.80	4.20
Ab-V5 + Abv-V6	5.17	5.42b	1.09a	12.60	4.00
Control without inoculation	4.81	4.52c	0.91b	13.00	4.00
Coefficient of variation (%)	17.85	20.97	5.14	14.16	5.86

[(1)]Means followed by different letters in the columns are different, by the Scott-Knott's test, at 5% probability. SDM, shoot dry matter; RDM, root dry matter; and RDM/SDM, root/shoot dry matter ratio.

genus have also enhanced the tolerance to biotic stress, besides increasing the growth of the root system, (Alijani et al., 2020). *Mucuna utilis* seedlings subjected to the inoculation of *Stenotrophomonas maltophilia* also showed an increased tolerance to the biotic stress caused by *Fusarium*, besides a 30% increase of plant growth (Aeron et al., 2020). *Stenotrophomonas* isolates also incremented the *Populus* plants tolerance to abiotic stresses, besides increasing the growth of aerial parts and roots of in vitro cultivated plants and promoting the adventitious rooting of cuttings (Ulrich et al., 2021). The beneficial effects observed by the inoculation of *Stenotrophomonas* sp. in plants are attributed to rather direct effects, such as the production of AIA and phosphate solubilization, or indirect effects, such as the production of the enzyme ACC deaminase and siderophores (Aeron et al., 2020). These results are corroborated by the findings obtained in the present work, in which the inoculation of peach palm seedlings with two *Stenotrophomonas* isolates resulted in the increase of the root system, in comparison with the control treatment. Regarding the RDM/SDM ratio (Table 2), there were significant differences among treatments. However, for all treatments, the values were balanced and most were close to 1, indicating that the seedlings were healthy, although imbalanced values of the RDM/SDM ratio can occur under stress (Agathokleous et al., 2019).

In peach palm, this is the first report on the occurrence of *Rhizobium* as an endophytic microorganism, and it was observed both in roots and leaves. However, the function of the *Rhizobium* genus in this culture still needs to be investigated, and further studies are required to understand the mechanisms involved in the plant growth promotion. Despite this lack of information, a positive effect of *Rhizobium* inoculation was observed on the production of root dry matter in peach palm. This fact becomes quite interesting, since this genus has long been characterized as being capable of establishing symbiosis with legumes, with which it is involved in the biological nitrogen fixation. Until recently, it was believed that the *Rhizobium* life cycle involved growth in the legume nodule as a symbiont, and in the soil as a saprophyte (Yanni et al., 1997). However, the genus *Rhizobium* has also been associated with various nonleguminous plants (Díez-Méndez et al., 2021). In our work, it increased the RDM of peach palm.

Some isolates that showed the ability to increase the RDM were identified within genera with species that can cause damage to human and animal health (*Enterobacter*, *Stenotrophomonas*, *Klebsiella*, and *Pseudomonas*) (Martin & Bachman, 2018; Keswani et al., 2019; Ambreetha et al., 2022) and which should be carefully evaluated. These isolates need a complete characterization, at species and strain levels, by using polyphasic approaches for microbial classification prior to bioinoculant development and environmental release (Keswani et al., 2019). However, two isolates, identified as *Rhizobium*, are more viable candidates for short-term development because this genus shows low risk and is widely used in agriculture in Brazil.

Conclusion

Peach palm (*Bactris gasipaes*) seedlings harbor endophytic bacteria that are able to increase root dry matter, with four isolates belonging to the genera *Enterobacter*, *Rhizobium*, and *Stenotrophomonas* standing out.

Acknowledgments

To Empresa Brasileira de Pesquisa Agropecuária (Embrapa, project number 22.16.05.002.00.00), for providing the financial support; and to Márcio Franchetti (Vivetech) for providing the peach palm seedlings.

References

AERON, A.; DUBEY, R.C.; MAHESHWARI, D.K. Characterization of a plant-growth-promoting non-nodulating endophytic bacterium (*Stenotrophomonas maltophilia*) from the root nodules of *Mucuna utilis* var. *capitata* L. (Safed Kaunch). **Canadian Journal of Microbiology**, v.66, p.670-677, 2020. DOI: https://doi.org10.1139/cjm-2020-0196.

AFZAL, I.; SHINWARI, Z.K.; SIKANDAR, S.; SHAHZAD, S. Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. **Microbiological Research**, v.221, p.36-49, 2019. DOI: https://doi.org/10.1016/j.micres.2019.02.001.

AGATHOKLEOUS, E.; BELZ, R.G.; KITAO, M.; KOIKE, T.; CALABRESE, E.J. Does the root to shoot ratio show a hormetic response to stress? An ecological and environmental perspective. **Journal of Forestry Research**, v.30, p.1569-1580, 2019. DOI: https://doi.org/10.1007/s11676-018-0863-7.

ALIJANI, Z.; AMINI, J.; ASHENGROPH, M.; BAHRAMNEJAD, B. Volatile compounds mediated effects of *Stenotrophomonas maltophilia* strain UN1512 in plant growth promotion and its

potential for the biocontrol of *Colletotrichum nymphaeae*. **Physiological and Molecular Plant Pathology**, v.112, art.101555, 2020. DOI: https://doi.org/10.1016/j.pmpp.2020.101555.

AMBREETHA, S.; MARIMUTHU, P.; MATHEE, K.; BALACHANDAR, D. Rhizospheric and endophytic *Pseudomonas aeruginosa* in edible vegetable plants share molecular and metabolic traits with clinical isolates. **Journal of Applied Microbiology**, v.132, p.3226-3248, 2022. DOI: https://doi.org/10.1111/jam.15317.

BRUSAMARELLO-SANTOS, L.C.; GILARD, F.; BRULÉ, L.; QUILLERÉ, I.; GOURION, B.; RATET, P.; SOUZA, E.M. de; LEA, P.J.; HIREL, B. Metabolic profiling of two maize (*Zea mays* L.) inbred lines inoculated with the nitrogen fixing plant-interacting bacteria *Herbaspirillum seropedicae* and *Azospirillum brasilense*. **Plos One**, v.12, e0174576, 2017. DOI: https://doi.org/10.1371/journal.pone.0174576.

CHALITA, P.B.; FARIAS, E. do N.C.; COSTA, I.B. da; SOUSA, B.F.; SANTOS, M.A.O. dos; ALBUQUERQUE, T.C.S. de; VITAL, M.J.S.; SILVA, K. da. Characterization of bacterial endophytes from the roots of native and cultivated Brazil nut trees (*Bertholletia excelsa*). **Acta Amazonica**, v.49, p.257-267, 2019. DOI: https://doi.org/10.1590/1809-4392201804831.

CIPRIANO, M.A.P.; FREITAS-IÓRIO, R. de P.; DIMITROV, M.R.; ANDRADE, S.A.L. de; KURAMAE, E.E.; SILVEIRA, A.P.D. da. Plant-growth endophytic bacteria improve nutrient use efficiency and modulate foliar N-metabolites in sugarcane seedling. **Microorganisms,** v.9, art.479, 2021. DOI: https://doi.org/10.3390/microorganisms9030479.

COSTA, G.V. da; ROCHA, W.C.; FREITAS, A.D.G. de. Microrganismos endofíticos encontrados no fruto da pupunheira (*Bactris gasipaes* Kunth) e seu potencial antimicrobiano. **Scientia Amazonia**, v.8, p.CB23-CB27, 2019.

DEY, S.; DUTTA, P.; MAJUMDAR, S. Biological control of *Macrophomina phaseolina* in *Vigna mungo* L. by endophytic *Klebsiella pneumoniae* HR1. **Jordan Journal of Biological Sciences**, v.12, p.219-227, 2019.

DÍEZ-MÉNDEZ, A.; MENÉNDEZ, E. *Rhizobium* presence and functions in microbiomes of non-leguminous plants. In: SHRIVASTAVA, N.; MAHAJAN, S.; VARMA, A. (Ed.). **Symbiotic soil microorganisms**. Cham: Springer, 2021. p.241-266. DOI: https://doi.org/10.1007/978-3-030-51916-2_16.

FERREIRA, E.B.; CAVALCANTI, P.P.; NOGUEIRA, D.A. **ExpDes**: experimental designs package. R package version 1.1.2, 2013. Available at: <http://CRAN.R-project.org/package=ExpDes>. Accessed on: July 28 2022.

FRANCHETTI, M.; ROZANE, D.E. Produção de mudas de palmito de pupunha. In: ROZANE, D.E.; SILVA, C. de A. e; FRANCHETTI, M. (Ed.). **Palmito pupunha**: do plantio à colheita. Registro: Unesp, 2017. p.33-50.

GRAEFE, S.; DUFOUR, D.; VAN ZONNEVELD, M.; RODRIGUEZ, F.; GONZALEZ, A. Peach palm (*Bactris gasipaes*) in tropical Latin America: implications for biodiversity conservation, natural resource management and human nutrition. **Biodiversity and Conservation**, v.22, p.269-300, 2013. DOI: https://doi.org/10.1007/s10531-012-0402-3.

HAUBEN, L.; MOORE, E.R.B.; VAUTERIN, L.; STEENACKERS, M.; MERGAERT, J.; VERDONCK, L.; SWINGS, J. Phylogenetic position of phytopathogens within the *Enterobacteriaceae*. **Systematic and Applied Microbiology**, v.21, p.384-397, 1998. DOI: https://doi.org/10.1016/S0723-2020(98)80048-9.

HUNGRIA, M.; SILVA, K. da. **Manual de curadores de germoplasma – micro-organismos**: rizóbios e bactérias promotoras do crescimento vegetal. Brasília: Embrapa Recursos Genéticos e Biotecnologia, 2011. 21p. (Embrapa Recursos Genéticos e Biotecnologia. Documentos, 333; Embrapa Soja. Documentos, 332). Available at: <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/933488/1/doc333332.pdf>. Accessed on: July 28 2022.

ISAID, H.M.A.; ARAR, A.S.M.; ABU BAKER, T.A.S. Evaluation of aerial offshoots rooting of three international date palm varieties. **Journal of Al-Quds Open University for Research and Studies**, v.43, p.29- 34, 2018. DOI: https://doi.org/10.33977/0507-000-043-050.

JHA, P.N.; GUPTA, G.; JHA, P.; MEHROTRA, R. Association of rhizospheric/endophytic bacteria with plants: a potential gateway to sustainable agriculture. **Greener Journal of Agricultural Sciences**, v.3, p.73-84, 2013.

KESWANI, C.; PRAKASH, O.; BHARTI, N.; VÍLCHEZ, JI.; SANSINENEA, E.; LALLY, R.D.; BORRIS, R.; SINGH, S.P.; GUPTA, V.K.; FRACETO, L.F.; LIMA, R. de; SINGH, H.B. Re-addressing the biosafety issues of plant growth promoting rhizobacteria. **Science of the Total Environment**, v.690, p.841-852, 2019 DOI: https://doi.org/10.1016/j.scitotenv.2019.07.046.

KUMAR, S.; STECHER, G.; LI, M.; KNYAZ, C.; TAMURA, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. **Molecular Biology and Evolution**, v.35, p.1547-1549, 2018. DOI: https://doi.org/10.1093/molbev/msy096.

LANA, U.G. de P.; RIBEIRO, V.P.; GOMES, E.A.; OLIVEIRA-PAIVA, C.A. de. **Seleção em larga escala de bactérias produtoras do hormônio ácido indolacético (AIA), auxina associada à promoção de crescimento em plantas**. Sete Lagoas: Embrapa Milho e Sorgo, 2017. (Embrapa Milho e Sorgo. Documentos, 218). Available at: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/172785/1/doc-218.pdf>. Accessed on: Jan. 4 2022.

LANE, D.J. 16S/23S rRNA sequencing. In: STACKEBRANDT, E.; GOODFELLOW, M. (Ed). **Nucleic acid techniques in bacterial systematics**. New York: Wiley, 1991. p.115-175.

MARIOSA, T. de N.; MELLONI, E.G.P.; MELLONI, R.; FERREIRA, G.M. dos R.; SOUZA, S.M.P. de; SILVA, L.F. de O. da. Rizobactérias e desenvolvimento de mudas a partir de estacas semilenhosas de oliveira (*Olea europeae* L.). **Revista de Ciências Agrárias**, v.60, p.302-306, 2017. DOI: https://doi.org/10.4322/rca.2447.

MARTIN, R.M.; BACHMAN, M.A. Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*. **Frontiers in Cellular and Infection Microbiology**, v.8, art.4, 2018. DOI: https://doi.org/10.3389/fcimb.2018.00004.

MORSBACH, N.; RODRIGUES, A. dos S.; CHAIMSOHN, F.P.; TREITNY, M.R. **Pupunha para palmito**: cultivo no Paraná. Londrina: IAPAR, 1998. 56p. (IAPAR. Circular, 103).

MOWAFY, A.M.; FAWZY, M.M.; GEBREIL, A.; ELSAYED, A. Endophytic *Bacillus*, *Enterobacter*, and *Klebsiella* enhance the growth and yield of maize. **Acta Agriculturae Scandinavica, Section B — Soil & Plant Science**, v.71, p.237-246, 2021. DOI: https://doi.org/10.1080/09064710.2021.1880621.

NCBI. National Center for Biotechnology Information. **BLAST**. Available at: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. Accessed on: July 28 2022a.

NCBI. National Center for Biotechnology Information. **GenBank**. Available at: https://www.ncbi.nlm.nih.gov/genbank/>. Accessed on: July 28 2022b.

OLANREWAJU, O.S.; GLICK, B.R.; BABALOLA, O.O. Mechanisms of action of plant growth promoting bacteria. **World Journal of Microbiology and Biotechnology**, v.33, art.197, 2017. DOI: https://doi.org/10.1007/s11274-017-2364-9.

R CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2015.

RAASCH, L.D.; BONALDO, S.M.; OLIVEIRA, A.A.F. de. *Bacillus subtilis*: enraizamento e crescimento de miniestacas de eucalipto em Sinop, norte de Mato Grosso, Brasil. **Bioscience Journal**, v.29, p.1446-1457, 2013. Suppl.1.

RODRIGUES NETO, J.; MALAVOLTA JÚNIOR, V.A.; VICTOR, O. Meio simples para o isolamento e cultivo de *Xanthomonas campestris* pv. *citri* tipo B. **Summa Phytopathologica**, v.12, p.16, 1986.

ROHLF, F.J. **NTSYSpc**: numerical taxonomy and multivariate analysis system. Version 2.1. Owner manual. New York: Exeter Publishing Setauket, 2000. Available at: <http://www.exetersoftware.com/downloads/ntsysguide21.pdf>. Accessed on: Jan. 22 2022.

SAH, S.; KRISHNANI, S.; SINGH, R. *Pseudomonas* mediated nutritional and growth promotional activities for sustainable food security. **Current Research in Microbial Sciences**, v.2, art.100084, 2021. DOI: https://doi.org/10.1016/j.crmicr.2021.100084.

SALDIERNA GUZMÁN, J.P.; REYES-PRIETO, M.; HART, S.C. Characterization of *Erwinia gerundensis* A4, an almond-derived plant growth-promoting endophyte. **Frontiers in Microbiology**, v.12, art.687971, 2021. DOI: https://doi.org/10.3389/fmicb.2021.687971.

SCOTT, A.J.; KNOTT, M. A cluster analysis method for grouping means in the analysis of variance. **Biometrics**, v.30, p.507-512, 1974. DOI: https://doi.org/10.2307/2529204.

SILVA, C. de A. A cultura do palmito pupunha e o mercado. In: ROZANE, D.E.; SILVA, C. de A. e; FRANCHETTI, M. (Ed.). **Palmito pupunha**: do plantio à colheita. Registro: Unesp, 2017. p.1-12.

ULRICH, K.; KUBE, M.; BECKER, R.; SCHNECK, V.; ULRICH, A. Genomic analysis of the endophytic *Stenotrophomonas* strain 169 reveals features related to plant-growth promotion and stress tolerance. **Frontiers in Microbiology**, v.16, art.687463, 2021. DOI: https://doi.org/10.3389/fmicb.2021.687463.

YAISH, M.W.; ANTONY, I.; GLICK, B.R. Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera* L.) and their potential role in salinity tolerance. **Antonie Van Leeuwenhoek**, v.107, p.1519-1532, 2015. DOI: https://doi.org/10.1007/s10482-015-0445-z.

YANNI, Y.G.; RIZK, R.Y.; CORICH, V.; SQUARTINI, A.; NINKE, K.; PHILIP-HOLLINGSWORTH, S.; ORGAMBIDE, G.; DE BRUIJN, F. de; STOLTZFUS, J.; BUCKLEY, D.; SCHMIDT, T.M.; MATEOS, P.F.; LADHA, J.K.; DAZZO, F.B. Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. **Plant and Soil**, v.194, p.99-114, 1997. DOI: https://doi.org/10.1023/A:1004269902246.

YOKOMIZO, G.K.I.; KALIL FILHO, A.N. **Propagação clonal por perfilhos em pupunheira**. 2020. Colombo: Embrapa Florestas, 2020. 10p. (Embrapa Florestas. Comunicado técnico, 456). Available at: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/216747/1/CT-456-1789-final-3.pdf>. Accessed on: July 22 2022.