ACTA
AMAZONICA | http://dx.doi.org/10.1590/1809-4392201804831

ORIGINAL ARTICLE

Characterization of bacterial endophytes from the roots of native and cultivated Brazil nut trees (*Bertholletia excelsa*)

Patrícia Bombonati CHALITA[1], Eliane do Nascimento Cunha FARIAS[2], Ismaele Breckenfeld da COSTA[3], Brenda Ferreira SOUSA[4§], Marco Antônio Oliveira dos SANTOS[1], Teresinha Costa Silveira de ALBUQUERQUE[2], Marcos José Salgado VITAL[1], Krisle da SILVA[5*]

[1] Universidade Federal de Roraima (UFRR), Av. Enê Garcez, 2413, Aeroporto, 69.304-000 Boa Vista, RR, Brasil.
[2] Embrapa Roraima, Rodovia BR-174, Km 8, Distrito Industrial, 69301-970 Boa Vista, RR, Brasil
[3] Faculdade Roraimense de Ensino Superior, Av. Presidente Juscelino Kubitscheck 300, Canarinho, 69306-535 Boa Vista, RR, Brasil
[4] Instituro Federal de Roraima, Av. Glaycon de Paiva 2496, Pricumã, 69.303-340 Boa Vista, RR, Brasil
[5] Embrapa Florestas, Estrada da Ribeira, Km 111, Parque Monte Castelo, 83411-000 Colombo, PR, Brasil
[§] *In memorian*
* Corresponding author: krisle.silva@embrapa.br; https://orcid.org/0000-0002-2512-9519

ABSTRACT

Brazil nut is a very important nontimber forest product in the Amazon region. Propagation of this tree still represents a challenge due to slow and uneven seed germination. In this context, plant growth-promoting bacteria can facilitate the process of propagation. The aims of this study were to isolate and characterize endophytic bacteria from the roots of Brazil nut trees in native *terra firme* forest and cultivation areas in northern Brazil, and to identify mechanisms by which bacteria act in plant growth promotion. Overall, 90 bacterial isolates were obtained from the roots of Brazil nut trees in monoculture, agroforestry and native forest areas by using different semisolid media. The isolates were characterized by sequencing the 16S rRNA gene. Plant growth-promoting characteristics were evaluated by the presence of the *nif*H gene, aluminum phosphate solubilization and the production of indole compounds. The isolates were affiliated with 18 genera belonging to 5 different classes (α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Bacilli and Actinobacteria). The genus *Bacillus* was predominant in the forest and monoculture areas. Fourteen isolates presented the *nif*H gene. Most of the bacteria were able to solubilize aluminum phosphate and synthetize indole compounds. The results indicated high diversity of endophytic bacteria present among the roots of Brazil nut trees, mainly in the agroforestry area, which could be related to soil attributes. Among the 90 isolates, the 22 that presented the best results regarding plant growth promotion traits were good candidates for testing in seedling production of Brazil nut trees.

KEYWORDS: 16S rRNA, phosphate solubilization, indole compounds, biological nitrogen fixation, plant growth-promotion bacteria, Amazon

Caracterização de bactérias endofíticas de raízes de castanha-do-Brasil (*Bertholletia excelsa*) em habitats nativos e cultivados

RESUMO

A castanha-do-brasil é um produto florestal não madeireiro muito importante na região amazônica. A propagação desta árvore ainda representa um desafio, devido ao lento e irregular processo de germinação das sementes. Neste contexto, bactérias promotoras do crescimento vegetal podem facilitar o processo de propagação. O objetivo deste estudo foi isolar e caracterizar bactérias endofíticas em raízes de castanha-do-Brasil em floresta de terra firme e em áreas cultivadas no norte do Brasil, e identificar alguns mecanismos de promoção do crescimento vegetal executados por essas bactérias. No total, 90 isolados bacterianos foram obtidos de raízes de castanha-do-Brasil em monocultura, agrofloresta e floresta nativa, usando diferentes meios de cultivo semi-sólidos. Os isolados foram caracterizados pelo sequenciamento do gene 16S rRNA. As características de promoção do crescimento vegetal foram avaliadas através da presença do gene *nif*H, solubilização de fosfato de alumínio e produção de compostos indólicos. Os isolados foram afiliados a 18 gêneros, pertencentes a cinco diferentes classes (α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Bacilli e Actinobacteria). O gênero *Bacillus* foi predominante, principalmente nas áreas de floresta e monocultura. Quatorze isolados apresentaram o gene *nif*H. A maioria dos isolados foi capaz de solubilizar fosfato de alumínio e sintetizar compostos indólicos. Os resultados indicam uma elevada diversidade de bactérias endofíticas presente em raízes de castanha-do-Brasil, principalmente em área de agrofloresta, que pode estar relacionado aos atributos do solo. Entre os 90 isolados, 22 apresentaram os melhores resultados relacionados às características de promoção do crescimento vegetal, e são bons candidatos para testes em produção de mudas de castanha-do-Brasil.

PALAVRAS-CHAVE: 16S rRNA, solubilização de fosfatos, compostos indólicos, fixação biológica de nitrogênio, bactéria promotora do crescimento vegetal, Amazônia

CITE AS: 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. 2019. Characterization of bacterial endophytes from the roots of native and cultivated Brazil nut trees (*Bertholletia excelsa*). *Acta Amazonica* 49: 257-267.

INTRODUCTION

Brazil nut (*Bertholletia excelsa* H.B.K.) is native to Brazil, Bolivia, Venezuela, Colombia, Peru and the Guianas (Mori 1992; Lorenzi 2002) and represents the most important commercial nontimber forest product in the Amazon region. Brazil nut trees are long-living organisms, and large trees have been carbon dated with ages of more than 300 years (Vieira *et al.* 2005). As the nuts are solely harvested in the wild, the sustainable exploitation of Brazil nut contributes to promote Amazonian forest conservation and has enjoyed widespread and longstanding economic success in the international market (Wadt *et al.* 2008). However, the species also suffers from increasing habitat loss due to deforestation for illegal timber trade, opening of pasture areas, and forest fires. Studies on the introduction of cultivated Brazil nut trees are needed (Camargo *et al.* 2010), as habitat loss puts the species at risk due to loss of pollinators and seed-dispersing agents, and increased pollination distances (Ortiz 2002). A problem with Brazil nut cultivation is the time required for seed germination (20-60 days) and the uneven time germination of its seeds (Müller *et al.* 1995).

An alternative for improving the production of Brazil nut seedlings could be the use of plant growth-promoting bacteria (PGPB). These bacteria are able to improve the growth of plants and protect them from disease and abiotic stress (Glick 2012; Souza *et al.* 2015). PGPB can be found in the rhizosphere, on the root surfaces, or in the internal tissues of plants. PGPB that colonize internal tissues are known as endophytes and are found in nearly every plant worldwide (Santoyo *et al.* 2016) and do not harm the plant (Hallman *et al.* 1997). Endophytic bacteria can accelerate seedling emergence and increase plant growth (Chanway 1997). The mechanisms involved in plant growth promotion by these bacteria are similar to those observed among rhizobacteria, such as nitrogen fixation, phytohormone production, phosphate solubilization, and the production of antifungal compounds, induction of systemic resistance and production of siderophores (Vessey 2003; Compant *et al.* 2010; Glick 2012; Chanway 1997), which are bioactive compounds produced by bacteria that are readily available to the plant (Afzal *et al.* 2019). To date, there is no information about the isolation or characterization of endophytic PGPB from Brazil nut trees. The isolation and characterization of endophytic plant growth-promoting bacteria from Brazil nut roots would be important for the identification of new biological inoculants which may provide an alternative approach for the improvement of seed germination and seedling development.

Bacterial diversity in Amazon soils is higher in association with more intensive land uses than in undisturbed primary forest (Carvalho *et al.* 2016). Land use and changes in soil fertility, especially pH, alter the bacterial community composition (Jesus *et al.* 2009, Carvalho *et al.* 2016). Therefore, the main hypothesis of our study was that cultivated Brazil nut trees may harbor a greater diversity of endophytic bacteria than those in native forest areas. An additional hypothesis was that endophytic bacteria associated with Brazil nut roots exhibit mechanisms for promoting plant growth, such as the ability to solubilize phosphates and produce phytohormones. To test these hypotheses, we isolated and characterized endophytic bacteria from Brazil nut trees in an area of native *terra firme* forest and two experimental cultivation areas in the state of Roraima (northern Brazilian Amazon region), one containing only a monoculture of Brazil nut trees, and an agroforestry system containing Brazil nut trees in combination with other agricultural activities. The isolates were then tested to identify the mechanisms of plant growth promotion.

MATERIAL AND METHODS

Origin of bacteria

Root samples were collected between April and October 2013 in three areas in Roraima State, Brazil (Figure 1): (a) from 11 trees in a monoculture of Brazil nut trees planted in 2007 in the Serra da Prata experimental field of Embrapa Roraima in the city of Mucajaí (02º22'28.2"N, 60º59'46.8"W) [the Brazil nut seedlings received 100 g of triple superphosphate at the tips at planting, and, after two years, 30 kg ha^{-1} of N (ammonium sulfate) and 30 kg ha^{-1} of K_2O (potassium chloride) in the crown projection area]; (b) from 10 trees in an agroforestry system planted in 1995 in the Confiança experimental field of Embrapa Roraima, in the city of Cantá (02º15'00"N, 60º39'54"W) [the area was plowed and received 2000 kg ha^{-1} liming (PRNT 100%), 40 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} of FTE BR12 Nutriplant (7.1% Ca, 5.7% S, 1.8% B, 0.8% Cu, 2.0% Mn, 0.1% Mo, and 9.0% Zn) as a source of micronutrients]. Apart from Brazil nut, other species planted were cupiúba (*Goupia glabra* Aubl.), peach palm (*Bactris gasipae*s Kunth.), cupuassu (*Theobroma grandiflorum* Schum.), coffee (*Coffea canephora* Pierre.), rain tree (*Samanea saman* (Jacq.) Merr.) and andiroba (*Carapa guianensis* Aubl.)]; and (c) from seven trees in a dense ombrophilous forest in a private estate located in São João da Baliza (0º57'024"N, 59º54'41"W).

The climate in the region of the monoculture and agroforestry areas is Ami (Köppen system), with mean annual temperatures ranging from 26 to 29 °C, and rainfall from around 1800 to 2400 (Oliveira Junior *et al.* 2003), and the soil type is a red-yellow ultisol, representing an area of savanna-forest transition on a flat relief. In the forest area the relief is flat to wavy, with climate of type Awi (Köppen system), with mean annual temperature of 27 ºC and rainfall range from 1700 to 2000 mm; the soils were not classified, but yellow ultisols and oxisols are predominant in the region (Femact 1993; Seplan 2010). Soil samples from the three sampling areas were chemically and physically characterized by Embrapa (1997) (parameters are presented in Table 1).

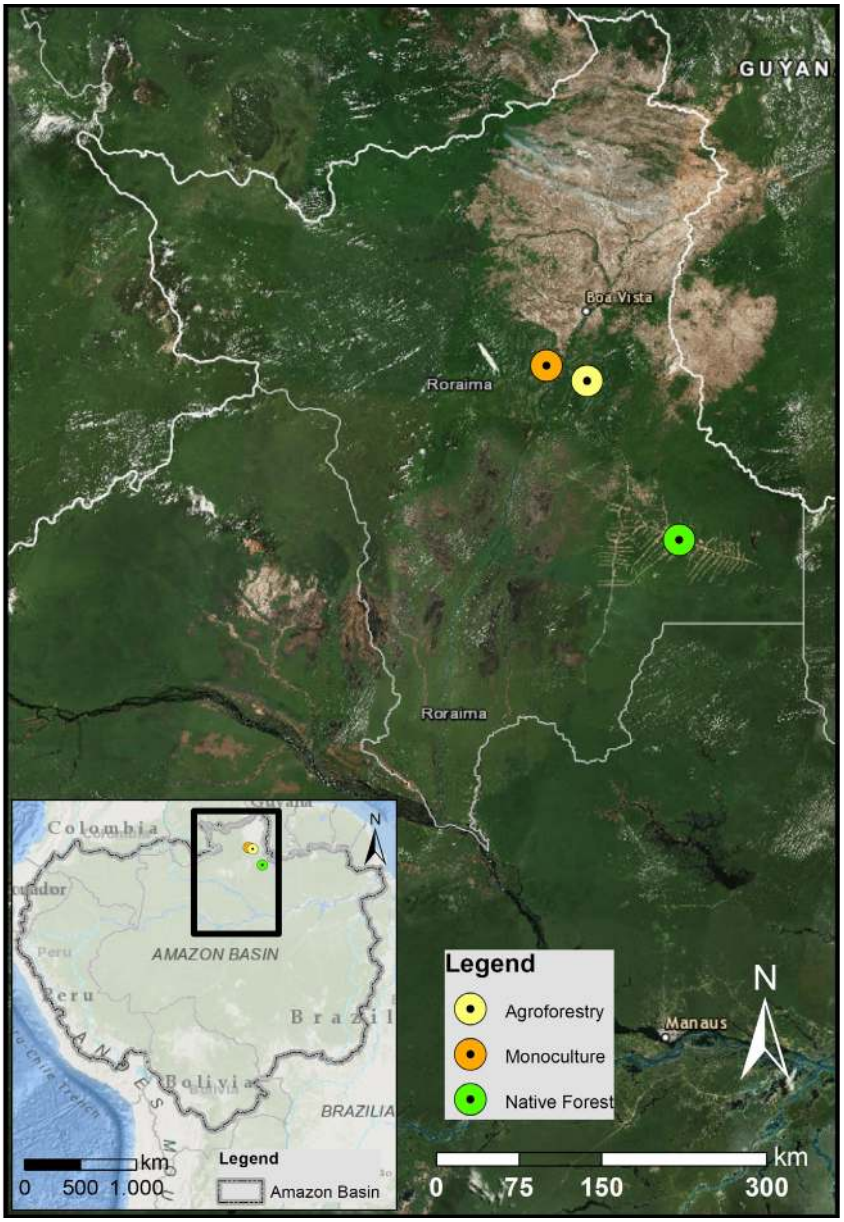

Figure 1. Location of the three sampling areas for bacterial isolates from roots of Brazil nut (*Bertholletia excelsa*) trees in the state of Roraima, in the northern Brazilian Amazon. This figure is in color in the electronic version.

Bacteria were isolated from nonwoody roots in different semisolid culture media that included NFb, LGI (both of which favor the growth of *Azospirillum* spp.) (Döbereiner *et al.* 1995), JMV (*Burkholderia* spp.) (Estrada De Los Santos *et al.* 2001) and DYG's (nonselective) (Rodrigues Neto *et al.* 1986). The roots were washed with water and cut into fragments of approximately 1 cm in length. then subjected to surface disinfection with sodium hypochlorite (2%) for 2 min and then hydrogen peroxide (5%) for 1 min, after which they were washed six times with sterilized water. The media with roots (5 replicates) were incubated for 10 days in a growth chamber at 28 °C. The isolation was performed from all the cultures that presented growth, i.e., the formation of a pellicle in the medium. After confirmation of bacterial growth, a loopful of the pellicle was streaked onto the corresponding solid medium to isolate the bacterium based on the phenotypic characteristics of the colonies (Döbereiner *et al.* 1995). After isolation, the following colony characteristics were evaluated: growth time, average diameter, shape, margin, surface, elevation, color and gum production. Ninety bacteria were selected from 303 obtained isolates (41 isolates from the agroforestry system, 30 from the monoculture system and, 19 from the native *terra firme* forest). All isolates obtained were stored at -80 °C in 20% glycerol.

Table 1. Chemical and physical attributes of the soil in areas where roots of Brazil nut (*Bertholletia excelsa*) trees were sampled in Roraima state (northern Brazil). Data from Embrapa (1997).

Areas	Measure unit	Agroforestry	Monoculture	Native *terra firme* Forest
Chemical attributes				
pH (water)		5.9	4.8	4.5
Ca^{2+}	$cmol_c\ dm^{-3}$	3.5	1.2	0.33
Mg^{2+}	$cmol_c\ dm^{-3}$	0.7	0.4	0.19
K^{+}	$cmol_c\ dm^{-3}$	0.2	0.04	0.19
Al^{3+}	$cmol_c\ dm^{-3}$	0.1	1	0.82
H+Al	$cmol_c\ dm^{-3}$	5.12	2.3	5.77
P	$mg\ dm^{-3}$	19.33	3	1.95
V	%	44.9	41	11
O.M.	$g\ dm^{-3}$	41.4	7	23
Physical attributes				
Argila	%	23	28	28
Silte	%	8	15	15
Areia	%	69	57	57

V (base saturation), O. M (organic matter)

16S rRNA partial sequencing

Bacteria were grown in DYG's liquid medium (Rodrigues Neto *et al.* 1986) for 24 h, after which their DNA was extracted from 1 ml of cell suspension with an RBC extraction kit (cat. YGB300, Taiwan), following the instructions provided by the manufacturer. Amplification of the 16S rRNA gene was performed using the 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers (Lane 1991), and partial sequencing was performed using the 27F primer. Sequencing was performed using a 3730xl DNA sequencer (Applied Biosystems, Foster City, CA, USA). The sequences were aligned using the ClustalW program (Thompson *et al.* 1994) in MEGA 5.01 software (Tamura *et al.* 2011). A phylogenetic tree was constructed using the "neighbor-joining" method and Kimura's 2-parameter model (Kimura 1980) with MEGA 5.01 software, performing 1000 repetitions. The 16S rRNA gene sequences were deposited in the GenBank database under accession numbers MF442264–MF442353.

*nif*H gene amplification

The *nif*H gene was amplified using the primers 19F (5'-CCI WTYTAYGGIAARGGIGG-3') and 407R (5'-AAICCRCCRCAIACIACRTC-3') (Ueda *et al.* 1995).

Aluminum phosphate solubilization assays

An initial qualitative estimation of the P-solubilizing activity of the bacteria was carried out on modified National Botanical Research Institute Phosphorus (NBRIP) agar (Nautiyal 1999). Tricalcium phosphate was substituted with aluminum phosphate ($AlPO_4$, 0.236%), and the pH of the medium was adjusted to 4.5. All isolates were cultured in DYG'S liquid medium for 24 h at 28 °C until the cell concentration measured via the optical density at 630 nm (OD_{630}) reached 0.5–0.7. Ten-microliter samples of the bacterial cultures were inoculated into NBPRIP medium at three equidistant points on a plate, which was subsequently incubated for 18 days at 28 °C.

A quantitative assay was also performed using NBPRIP liquid medium. One milligram of cells from the isolates cultured in DYG'S solid medium was inoculated into 30 mL of NBPRIP with $AlPO_4$ at a concentration of 12 mg P l^{-1}. These cultures were incubated at 28 °C for 4 days under 150 rpm agitation. At the end of this period, the pH was determined; each sample was centrifuged (10 000 rpm for 5 min), and the levels of soluble P in the supernatant were quantified using the phosphomolybdate method (Murphy and Riley 1962). The concentration of P was estimated using a standard curve previously prepared with 0, 0.1, 0.5, 0.75, 1, 2, 3, 4, 4.5, 5 and 6 mg l^{-1} P in the form of KH_2PO_4.

In all tests, BR 11001^T (*Azospirillum brasiliense*), BR 11340 (*Burkholderia* sp.), BR11175^T (*Herbaspirillum seropedicae*), BR 11790^T (*Herbaspirillum frisigense*), and ERR 532 (*Bacillus* sp. isolated from the roots of *Brachiaria* sp.) were included for comparison. The experiments were completely randomized with three replications. The data were statistically analyzed using the SISVAR program, version 4.3 (Ferreira 2011), with the effects from the treatments evaluated by the Scott-Knott test (Scott and Knott 1974) at a 5% level of significance.

Production of indole compounds

The isolates and the type and reference strains, as well as *Azospirillum brasilense* BR 11001^T (Radwan *et al.* 2002), *Herbaspirillum seropedicae* BR11175^T (Baldani *et al.* 1986), *Burkholderia* sp. BR11340, *Herbaspirillum frisigense* BR11790^T, and ERR 532 (*Bacillus* sp. isolate from roots of *Brachiaria* sp.), were cultured in DYG'S liquid medium (Rodrigues Neto *et al.* 1986) for 24 h. After growth, the cultures were adjusted to an OD_{630} ranging from 0.6–0.8. Then, 500 µl aliquots of the bacterial cultures were inoculated into 6 ml of DYG's medium (without $_L$-tryptophan or supplemented with 100 mg L^{-1} $_L$-tryptophan), followed by incubation for 48 h at 28 °C under constant stirring at 150 rpm. The cultures were recovered after centrifugation at 10.000 rpm g for 5 min, and 3 ml of the recovered supernatant and 2 ml of Salkowski reagent were then mixed together (Sarwar and Kremer 1995). This mixture was incubated in the dark for 20 min, until a pink color developed that is indicative of indole production. The color intensity was measured in a spectrophotometer at 535 nm. The concentration of indoles was estimated using a standard curve prepared with 0, 10, 25, 50, 75 and 100 µg AIA ml^{-1} (Sigma-Aldrich, cod. I3750). The experiments were completely randomized with three replications. The data

were then statistically analyzed as described for the phosphate solubilization assays.

Selection of bacteria

For the selection of bacteria exhibiting growth-promoting characteristics, histograms were constructed to visualize the data and group the isolates (data not shown). Bacteria associated with relatively higher phosphate solubilization and indole compound production were selected.

RESULTS

16S rRNA partial sequencing

The closest matches for the 90 bacterial isolates within the NCBI database are presented in Table 2. The 90 endophytic bacteria were affiliated with 18 genera belonging to 5 different classes: α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Bacilli and Actinobacteria. Members of the classes Bacilli (43.3%) and γ-Proteobacteria (32.2%) were predominant followed by β-Proteobacteria (12.2%), α-Proteobacteria (6.7%) and Actinobacteria (5.6%). In the class Bacilli, the genus *Bacillus* was predominant, with 30 isolates. *Stenotrophomonas* (10 isolates) was predominant in the class γ-Proteobacteria, and *Burkholderia* and *Achromobacter*, both with four isolates, in the class β-Proteobacteria. Members of the predominantly occurring genus *Bacillus* were obtained mainly from the native–forest and monoculture samples. The greatest diversity of bacterial genera was isolated from the agroforestry samples.

*nif*H gene amplification

Fourteen bacterial isolates were positive for the amplification of DNA with primers 19F and 407R, corresponding to a region of the *nif*H gene (approximately 400 bp). These bacteria were identified as belonging to *Bacillus* (ERR 667, ERR 679, ERR 785, ERR 819, ERR 821 and ERR 830), *Paenibacillus* (ERR 684), *Enterobacter* (ERR 604 and ERR 838), *Klebsiella* (ERR 602), *Stenotrophomonas* (ERR 640), *Pseudomonas* (ERR 711), *Delftia* (ERR 762) and *Microbacterium* (ERR 773) (Table 2). Seven of these isolates were from the agroforestry system; four from the forest, and three from the monoculture. Among the 14 isolates, five were obtained from media selective for *Azospirillum* spp. (NFb and LGI) (ERR 602, ERR 604, ERR 640, ERR 830, ERR 838) and nine from nonselective medium (DYG's medium) (ERR 667, ERR 679, ERR 684, ERR 711, ERR 762, ERR 773, ERR 785, ERR 819, ERR 821).

Aluminum phosphate solubilization assays

None of the bacteria or type strains showed a halo of solubilization on solid medium. In liquid medium, however, 88.9% of the isolates were able to solubilize aluminum phosphate (Table 3). Some isolates, such as ERR 587, ERR 589, ERR 733, ERR 719 and ERR 584, had significantly higher values and made available up to 14% of the P added to the medium (Table 3).

Table 2. Identification of 90 bacterial isolates sampled from roots of Brazil nut (*Bertholletia excelsa*) trees in native and cultivated habitats in Roraima (northern Brazil). The closest match in the NCBI database of the partial sequencing of the 16S rRNA gene is indicated, as well as the origin and medium of isolation of the isolates.

Isolates	Closest match	Origin	Medium
Class α -Proteobacteria			
ERR 739	*Ochrobactrum*	Agroforestry	DYG'S
ERR 745	*Ochrobactrum*	Agroforestry	DYG'S
ERR 792	*Ochrobactrum*	Forest	DYG'S
ERR 858	*Agrobacterium/ Rhizobium*	Agroforestry	NFb
ERR 865	*Agrobacterium/Rhizobium*	Agroforestry	NFb
ERR 870	*Agrobacterium/Rhizobium*	Agroforestry	NFb
Class β - Proteobacteria			
ERR 584	*Burkholderia*	Agroforestry	JMV
ERR 587	*Burkholderia*	Forest	JMV
ERR 589	*Burkholderia*	Monoculture	JMV
ERR 594	*Burkholderia*	Monoculture	JMV
ERR 689	*Achromobacter/ Uncultured*	Monoculture	DYG'S
ERR 737	*Achromobacter/ Uncultured*	Agroforestry	DYG'S
ERR 738	*Achromobacter*	Agroforestry	DYG'S
ERR 744	*Uncultured*	Agroforestry	DYG'S
ERR 763	Achromobacter/ Uncultured	Agroforestry	DYG'S
ERR 772	*Delftia*	Agroforestry	DYG'S
ERR 762	*Delftia*	Agroforestry	DYG'S
Class γ - Proteobacteria			
ERR 596	Pantoea	Monoculture	LGI
ERR 602	*Klebsiella*	Agroforestry	LGI
ERR 604	*Enterobacter*	Agroforestry	LGI
ERR 626	*Klebsiella*	Agroforestry	LGI
ERR 640	*Stenotrophomonas*	Agroforestry	LGI
ERR 652	*Enterobacter*	Monoculture	DYG'S
ERR 692	*Enterobacter*	Monoculture	DYG'S
ERR 710	*Pseudomonas*	Agroforestry	DYG'S
ERR 711	*Pseudomonas*	Agroforestry	DYG'S
ERR 719	*Serratia*	Agroforestry	DYG'S
ERR 720	Pseudomonas	Agroforestry	DYG'S
ERR 721	*Pseudomonas*	Agroforestry	DYG'S
ERR 723	*Pseudomonas*	Agroforestry	DYG'S
ERR 727	*Enterobacter*	Agroforestry	DYG'S
ERR 750	*Uncultured*	Agroforestry	DYG'S
ERR 752	*Pseudomonas*	Agroforestry	DYG'S
ERR 761	*Uncultured/ Stenotrophomonas*	Agroforestry	DYG'S
ERR 765	*Pseudomonas*	Agroforestry	DYG'S
ERR 770	*Stenotrophomonas/Uncultured*	Agroforestry	DYG'S
ERR 771	*Stenotrophomonas*	Agroforestry	DYG'S
ERR 779	*Pseudomonas*	Forest	DYG'S
ERR 782	*Uncultured*	Forest	DYG'S
ERR 828	*Stenotrophomonas*	Agroforestry	NFb
ERR 833	*Enterobacter*	Forest	NFb
ERR 838	*Uncultured*	Agroforestry	NFb
ERR 843	*Stenotrophomonas/ Uncultured*	Agroforestry	NFb
ERR 849	*Uncultured*	Agroforestry	NFb
ERR 859	*Stenotrophomonas*	Agroforestry	NFb
ERR 873	*Uncultured*	Agroforestry	NFb

Table 2. Continued.

Isolates	Closest match	Origin	Medium
Class Bacilli			
ERR 575	*Paenibacillus*	Agroforestry	JMV
ERR 651	*Bacillus*	Monoculture	DYG'S
ERR 654	*Bacillus*	Monocuture	DYG'S
ERR 656	*Lysinibacillus*	Monoculture	DYG'S
ERR 657	*Bacillus*	Monoculture	DYG'S
ERR 660	*Bacillus*	Monoculture	DYG'S
ERR 665	*Bacillus*	Monoculture	DYG'S
ERR 667	*Bacillus*	Monoculture	DYG'S
ERR 673	*Bacillus*	Monoculture	DYG'S
ERR 676	*Bacillus*	Monoculture	DYG'S
ERR 677	*Paenibacillus*	Monoculture	DYG'S
ERR 678	*Paenibacillus*	Monoculture	DYG'S
ERR 679	*Bacillus*	Monoculture	DYG'S
ERR 680	*Bacillus*	Monoculture	DYG'S
ERR 684	*Paenibacillus*	Monoculture	DYG'S
ERR 691	*Bacillus*	Monoculture	DYG'S
ERR 694	*Bacillus*	Monoculture	DYG'S
ERR 701	*Bacillus*	Monoculture	DYG'S
ERR 703	*Lysimibacillus*	Monoculture	DYG'S
ERR 705	*Bacillus*	Monoculture	DYG'S
ERR 706	*Bacillus*	Monoculture	DYG'S
ERR 708	*Bacillus*	Monoculture	DYG'S
ERR 709	*Bacillus*	Monoculture	DYG'S
ERR 717	*Lysinibacillus*	Agroforestry	DYG'S
ERR 741	*Bacillus*	Agroforestry	DYG'S
ERR 785	*Bacillus*	Forest	DYG'S
ERR 794	*Lysinibacillus*	Forest	DYG'S
ERR 795	*Bacillus*	Forest	DYG'S
ERR 797	*Bacillus*	Forest	DYG'S
ERR 799	*Bacillus*	Forest	DYG'S
ERR 800	*Paenibacillus*	Forest	DYG'S
ERR 803	*Bacillus cereus*	Forest	DYG'S
ERR 805	*Bacillus*	Forest	DYG'S
ERR 807	*Bacillus*	Forest	DYG'S
ERR 809	Bacillus	Forest	DYG'S
ERR 813	*Bacillus*	Forest	DYG'S
ERR 819	*Bacillus*	Forest	DYG'S
ERR 821	*Bacillus*	Forest	DYG'S
ERR 830	*Bacillus*	Forest	NFb
Class Actinobacteria			
ERR 644	*Curtobacterium*	Forest	LGI
ERR 733	*Microbacterium*	Agroforestry	DYG'S
ERR 753	*Microbacterium*	Agroforestry	DYG'S
ERR 773	*Microbacterium*	Agroforestry	DYG'S
ERR 822	*Curtobacterium*	Forest	DYG'S

Table 3. Soluble phosphate after solubilization by bacterial isolates sampled from roots of Brazil nut (*Bertholletia excelsa*) trees in native and cultivated habitats in Roraima (northern Brazil), and reference strains in liquid media. Different letters in columns indicate statistical differences ($p < 0.05$) according to the Skott-Knott test. The coefficient of variation was 35.9%. Isolates in bold were used as controls.

Isolate	Soluble P (mg l^{-1})	pH	Soluble P (%)	Isolate	Soluble P (mg l^{-1})	pH	Soluble P (%)
ERR 587	1.69 a	3.7	14.1	ERR 805	0.30 e	3.7	2.5
ERR 589	1.54 a	3.0	12.8	ERR 677	0.25 e	3.9	2.1
ERR 733	1.38 a	3.0	11.5	ERR 828	0.24 e	4.4	2.0
ERR 719	1.23 b	3.1	10.2	ERR 640	0.24 e	3.8	2.0
ERR 584	1.14 b	3.3	9.5	ERR 779	0.23 e	4.0	1.9
ERR 723	0.99 c	3.3	8.3	ERR 799	0.23 e	4.4	1.9
ERR 833	0.98 c	3.4	8.1	ERR 809	0.22 e	4.7	1.9
ERR 727	0.96 c	3.4	8.0	ERR 813	0.22 e	4.4	1.9
ERR 773	0.93 c	4.1	7.8	ERR 738	0.22 e	4.7	1.8
ERR 753	0.84 c	4.2	7.0	ERR 800	0.20 f	4.1	1.7
ERR 765	0.83 c	4.1	6.9	ERR 706	0.20 f	4.3	1.6
ERR 721	0.78 c	4.1	6.5	ERR 772	0.20 f	4.2	1.6
ERR 694	0.78 c	3.9	6.5	ERR 858	0.20 f	4.3	1.6
ERR 752	0.76 c	4.0	6.3	**BR 11001**	0.19 f	3.8	1.6
ERR 678	0.72 d	4.0	6.0	ERR 657	0.19 f	3.5	1.6
ERR 594	0.72 d	3.4	6.0	ERR 744	0.19 f	4.0	1.6
ERR 602	0.69 d	3.3	5.8	ERR 692	0.15 f	3.4	1.3
ERR 821	0.69 d	3.7	5.8	ERR 830	0.15 f	4.2	1.3
ERR 708	0.68 d	3.6	5.7	ERR 575	0.14 f	3.7	1.2
BR 11340	0.63 d	3.6	5.3	ERR 673	0.14 f	3.0	1.2
ERR 604	0.62 d	3.9	5.2	ERR 782	0.14 f	4.5	1.1
ERR 596	0.61 d	3.4	5.1	ERR 849	0.14 f	4.4	1.1
ERR 626	0.61 d	3.3	5.1	ERR 803	0.13 f	4.5	1.1
ERR 652	0.60 d	3.4	5.0	ERR 865	0.13 f	4.4	1.1
ERR 711	0.58 d	3.9	4.8	ERR 651	0.12 f	4.2	1.0
ERR 720	0.58 d	4.1	4.8	ERR 870	0.12 f	4.6	1.0
ERR 838	0.58 d	3.5	4.8	ERR 795	0.11 f	4.4	0.9
ERR 739	057 d	3.8	4.8	ERR 761	0.10 f	4.6	0.9
ERR 771	0.57 d	3.7	4.7	ERR 656	0.10 f	4.3	0.8
ERR 785	0.55 d	4.1	4.6	ERR 750	0.09 f	4.3	0.8
ERR 684	0.53 d	3.8	4.4	ERR 807	0.09 f	4.5	0.8
ERR 667	0.52 d	3.6	4.3	ERR 859	0.09 f	4.3	0.8
ERR 680	0.48 e	3.7	4.0	ERR 770	0.09 f	4.5	0.7
ERR 745	0.45 e	3.9	3.7	ERR 665	0.08 f	4.2	0.7
ERR 763	0.45 e	3.7	3.7	ERR 737	0.08 f	4.4	0.7
BR 11790	0.44 e	4.1	3.6	ERR 822	0.08 f	3.9	0.7
ERR 532	0.42 e	3.6	3.5	ERR 843	0.08 f	4.2	0.7
ERR 741	0.40 e	3.9	3.3	ERR 703	0.07 f	4.0	0.6
ERR 717	0.39 e	4.4	3.2	ERR 644	0.07 f	3.9	0.6
ERR 710	0.37 e	4.0	3.1	ERR 660	0.06 f	4.2	0.5
ERR 762	0.37 e	4.1	3.1	ERR 679	0.06 f	4.3	0.5
BR 11175	0.36 e	4.2	3.0	ERR 654	0.05 f	4.4	0.4
ERR 819	0.35 e	4.3	2.9	ERR 691	0.01 f	4.4	0.1
ERR 792	0.35 e	3.9	2.9	ERR 701	0.01 f	4.4	0.1
ERR 794	0.34 e	4.2	2.8	ERR 705	0.01 f	4.3	0.1
ERR 797	0.34 e	4.5	2.8	ERR 873	0.01 f	4.4	0.1
ERR 689	0.30 e	3.9	2.5	ERR 676	0.01 f	4.4	0.1

Type or reference strains such as BR 11001^T (*A. brasilense*), BR 11340 (*Burkholderia* sp.), BR 11790^T (*H. frisigense*), BR 11175^T (*H. seropedicae*) and ERR 532 (*Bacillus* sp.) could also solubilize aluminum phosphate but showed lower solubilization compared to ERR 587, ERR 589, ERR 733 and ERR 719. These isolates were identified as *Burkholderia* sp. (ERR 587 and ERR 589), *Microbacterium* sp. (ERR 733) and *Serratia* sp. (ERR 719). A reduction in the pH of the media was observed with increasing solubilization for the majority of the bacteria (Table 3).

Production of indole compounds

Fifty-eight bacteria were able to synthetize indole compounds in media supplemented with tryptophan (Figure 2a). The production of isolates ERR 596 (184.47 µg ml^{-1}), ERR 626 (104.61 µg ml^{-1}), and ERR 723 (92.43 µg ml^{-1}), as well as the type strain *H. seropedicae* BR 11175^T (104.67 µg ml^{-1}), was significantly greater than that of the other bacteria and the type strain *A. brasilense* BR 11001^T. These bacteria were identified as *Pantoea* sp. (ERR 596), *Klebsiella* sp. (ERR 626) and *Pseudomonas* (ERR 723). However, in the absence of tryptophan, the production decreased from ERR 744 (*Achromobacter* sp.) with 33.77 µg mL^{-1}, to ERR 779 (*Pseudomonas* sp.) (31.61 µg mL^{-1}), ERR 710 (*Pseudomonas* sp.) (30.96 µg mL^{-1}), ERR 656 (*Lysinibacillus* sp.) (30.81 µg mL^{-1}) and ERR 626 (*Klebsiella* sp.) (30.24 µg mL^{-1}) (Figure 2b).

Selection of bacteria

After all analyses, 22 bacterial isolates (Table 4) were selected for best results in growth promotion, and as potential candidates for inoculation during seed germination and seedling production of Brazil nut trees. They belonged to 12 genera (*Burkholderia*, *Pantoea*, *Enterobacter*, *Klebsiella*, *Stenotrophomonas*, *Lysinibacillus*, *Bacillus*, *Pseudomonas, Serratia*, *Microbacterium*, *Achromobacter* and *Delftia*) and were obtained from the agroforestry system (10), monoculture (6) and *terra firme* forest (4).

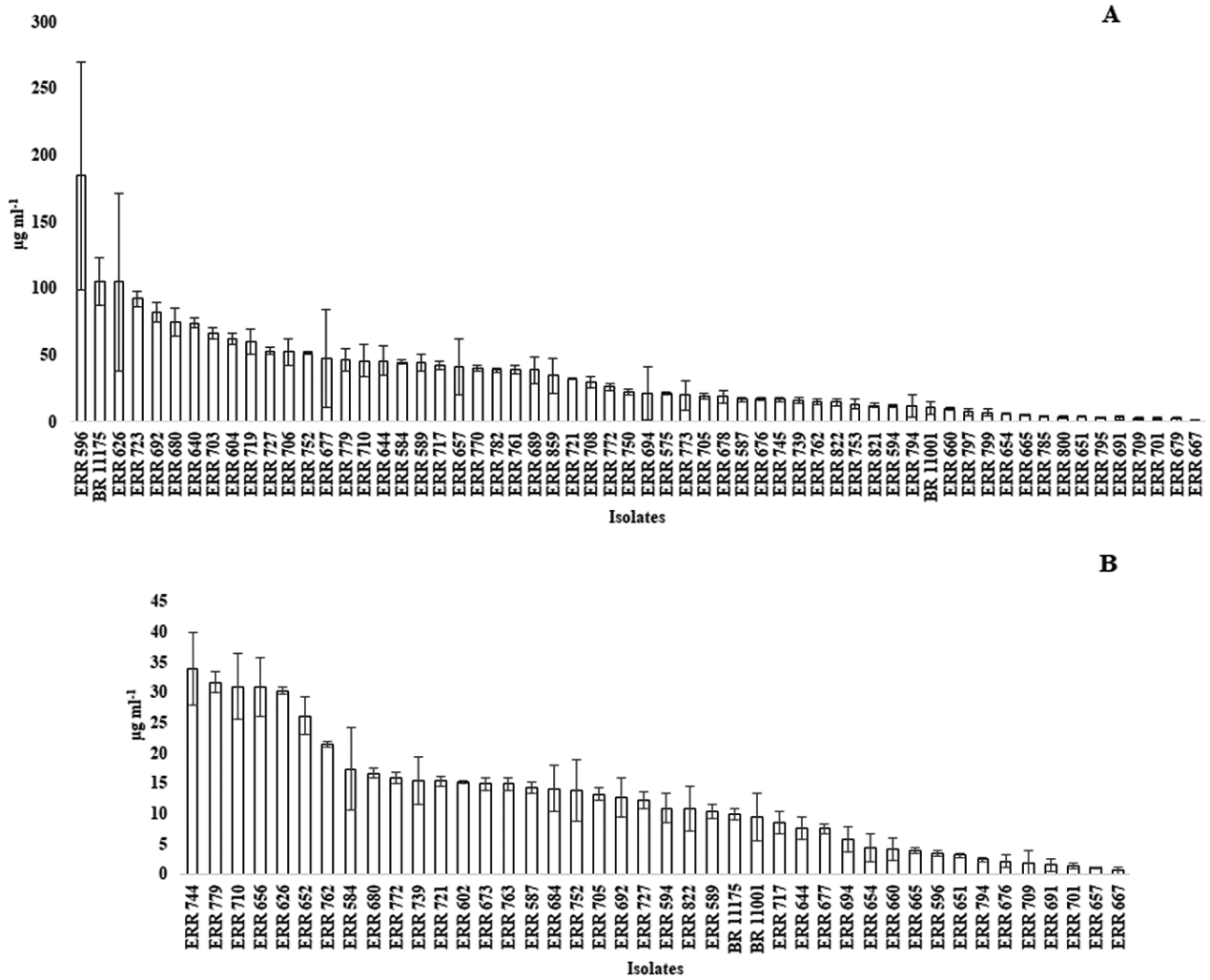

Figure 2. Production of indole compounds by endophytic bacterial isolates obtained from the roots of Brazil nut (*Bertholletia excelsa*) trees in native and cultivated habitats in media supplemented with (A) and without (B) tryptophan.

Table 4. Genus, origin and growth promotion characteristics of selected isolates among 90 bacterial isolates sampled from roots of Brazil–nut (*Bertholletia excelsa*) trees in native and cultivated habitats in Roraima (northern Brazil). *PS = phosphate solubilization; IC^+ = indole compounds production with tryptophan; IC = indole compounds production without tryptophan; NF = nitrogen fixation.

Isolate	Genus	Origin	Growth promotion*
ERR 584	*Burkholderia*	Agroforestry	PS
ERR 587	*Burkholderia*	Native Forest	PS
ERR 589	*Burkholderia*	Monoculture	PS
ERR 596	*Pantoea*	Monoculture	IC^+
ERR 604	*Enterobacter*	Agroforestry	IC^+/ NF
ERR 626	*Klebsiella*	Agroforestry	IC
ERR 640	*Stenotrophomonas*	Agroforestry	IC^+/NF
ERR 656	*Lysinibacillus*	Monoculture	IC^-
ERR 680	*Bacillus*	Monoculture	IC^+
ERR 692	*Enterobacter*	Monoculture	IC
ERR 703	*Lysinibacillus*	Monoculture	IC
ERR 710	*Pseudomonas*	Agroforestry	IC
ERR 719	*Serratia*	Agroforestry	PS/IC^+
ERR 723	*Pseudomonas*	Agroforestry	PS/IC^+
ERR 733	*Microbacterium*	Agroforestry	PS
ERR 744	*Achromobacter*	Agroforestry	IC^-
ERR 753	*Microbacterium*	Agroforestry	PS
ERR 773	*Microbacterium*	Agroforestry	OS/NF
ERR 779	*Pseudomonas*	Native Forest	IC^-
ERR 833	*Enterobacter*	Native Forest	PS
ERR 762	*Delftia*	Agroforestry	IC/NF
ERR 779	*Pseudomonas*	Native Forest	IC

DISCUSSION

We report the first isolation and characterization of endophytic bacteria from the roots of Brazil nut trees. The bacterial classes identified are generally dominated by endophytes in diversity analyses (Santoyo *et al.* 2016). However, most studies using plant growth-promoting bacteria have been performed with grasses (Moreira *et al.* 2010), whereas few such works have involved trees. In Brazil, only one study involved endophytic plant growth-promoting bacteria isolated from a native tree species [*Araucaria angustifolia* (Bertol.) Kuntze.] in southern Brazil (Neroni and Cardoso 2007), which identified diazotrophic bacteria in soil and root samples as belonging to the genus *Burkholderia*, while the PGPB belonged mainly to *Bacillus* and *Enterobacter.* In our Brazil–nut tree root samples, *Bacillus* was the predominant genus, followed by *Stenotrophomonas* and *Pseudomonas.*

The highest diversity of bacterial isolates in our samples was found in the agroforestry system (15 genera), relative to Brazil nut trees in native forest and monoculture (eight and seven genera, respectively). The cultivation of agroforestry systems represents a sustainable practice for carbon sequestration in soil (Abbas *et al.* 2017). Agroforestry can enrich soil organic carbon, improve soil nutrient availability and fertility due to the presence of trees in the system, litter decomposition and mineralization, and the increase of soil microbial dynamics (Dollinger and Jose 2018). Our agroforestry area also received limestone and fertilizer application, which could be related to an increase in the quantity and quality of litter inputs and, consequently, more soil organic matter accumulation in the soil. In Amazon soils, bacterial community structure and composition are related to land use, likely through the effects of soil attributes, particularly those related to soil acidity (Jesus *et al.* 2009). These characteristics may explain the higher bacterial diversity associated with Brazil nut roots agroforestry system in our samples, as the soil in this area had lower acidity and higher nutrient content (see Table 1).

The observed diversity was also influenced by the medium utilized for isolation, as the highest diversity was found in DYG'S medium (12 genera), that is rich and nonselective (Rodrigues Neto *et al.* 1986). For example, the JMV medium is selective for *Burkholderia* (Estrada De Los Santos *et al.* 2001), and most isolates obtained from this medium belonged to *Burkholderia.* We did not detect the presence of *Azospirillum* spp. using NFb or LGI media, which are known to favor these species (Silva *et al.* 2011a).

Endophytic bacteria can increase host fitness via many different functions, such as N_2 fixation, P solubilization, and phytohormone production (Compant *et al.* 2010). Nitrogen fixation has been the focus of many studies seeking alternatives to reduce the use of nitrogen fertilizers (Moreira *et al*. 2010; Glick 2012). Fourteen of the 90 isolates showed positive amplification of the *nif*H gene, which qualifies them as potential N_2-fixing bacteria that could presumably be considered diazotrophs. The majority were obtained using LGI, NFb and DYG's media and belonged to *Klebsiella*, *Enterobacter*, *Burkholderia*, *Stenotrophomonas*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Delftia* and *Microbacterium.*

In acidic soils in tropical regions, most P is precipitated with iron and aluminum, as is the case in soils from Roraima, which have low natural fertility and contain aluminum (Vale Junior and Leitão Sousa 2005). There are fewer studies involving the solubilization of aluminum phosphate than that of calcium phosphate, but some have reported a halo of solubilization on solid media (Marra *et al.* 2011, 2012; Oliveira-Longatti *et al.* 2014), while others have indicated the absence of a halo of solubilization on media with aluminum phosphate (Pérez *et al.* 2007; Silva *et al.* 2011a). For our isolates, as well as the type or reference strains tested, no halo of solubilization was observed on the utilized media. Phosphate solubilization tests (Ca, Al and Fe) have been widely used for the selection of plant growth-promoting bacteria (Hara and Oliveira 2004, 2005; Marra *et al.* 2011, 2012; Nautiyal, 1999; Oliveira-Longatti *et al.* 2014; Silva Filho and Vidor 2000; Silva Filho *et al.* 2002; Silva *et al.* 2011b; Silva *et al.* 2012). However, solubilization tests in solid

media show relatively poor effectiveness for universal selection to isolate solubilizing bacteria *in vitro* (Bashan *et al.* 2012). Bacteria that have performed solubilization many times could grow on solid media without a visible halo and may present other mechanisms of solubilization that do not result in a visible halo (Bashan *et al.* 2012; Fankem *et al.* 2008; Illmer *et al.* 1995). In liquid media, most of our bacterial isolates could solubilize aluminum phosphate, therefore, a visible halo on solid media should not be used as a unique test for solubilizing bacteria, and quantitative tests should also be used (Bashan *et al.* 2012). As happened in our samples, Illmer *et al.* (1995) also reported a pH reduction in cultures of bacteria and fungi that are able to solubilize aluminum phosphate and attributed the decrease to the production of organic acids and/or H^+ excretion accompanying NH_4 assimilation. Among the five best phosphorus-solubilizing bacteria in our work, three belong to the *Burkholderia* genus.

Indole compound production was variable among the isolates, but most of them could produce indoles with and without tryptophan addition. Tryptophan has been identified as a main precursor for IAA biosynthesis pathways in bacteria (Spaepen *et al.* 2007), which could explain our results regarding greater numbers of bacterial isolates able to produce indolic compounds with tryptophan. The IAA produced by bacteria acts in seed germination and in the radicular system, increasing the size and number of adventitious roots and deep ramifications, allowing a greater volume of soil to be explored by the roots (Glick 2012). This mechanism is an important tool for use in seed germination and seedlings production of Brazil nut.

CONCLUSIONS

Our results indicated that there is a great diversity of endophytic bacteria in the roots of Brazil nut trees in Roraima state, in the northern Brazilian Amazon. The diversity of genera was higher in isolates obtained from an agroforestry area than in those from a Brazil nut monoculture and a native *terra firme* forest area. The obtained endophytic bacteria presented plant growth–promotion characteristics, such as nitrogen fixation, phosphate solubilization and indole compound production. Among the 90 isolates obtained, at least 22 that presented the best results for plant-growth promotion traits are promising candidates for use in the seed germination and seedling production of Brazil nut.

REFERENCES

Abbas, F.; Hammad, H.M.; Fahad, S.; Cerdà, A.; Rizwan, M.; Farhad, W.; Ehsan, S.; Bakhat, H.F. 2017. Agroforestry: a sustainable environmental practice for carbon sequestration under the climate change scenarios – a review. *Environmental Science and Pollution Research*, 24: 11177-11191.

Afzal, I.; Shinwari, Z.K.; Sikandar, S.; Shahzad, S. 2019. Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. *Microbiological Research*, 221: 36–49.

Baldani, J.I.; Baldani, V.; Seldin, L.; Döbereiner; J. 1986. Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. *International Journal of Systematic Bacteriology*, 36: 86-93.

Bashan, Y.; Kamnev, A.A.; De-Bashan, L.E. 2012 Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. *Biology and Fertility of Soils*, 49: 465–479.

Camargo, F.F.; Costa, R.B.; Resende, M.D.V.; Roa, R.A.R.; Rodrigues, N.B.; Santos, L.V.; Freitas, C.A. 2010. Genetic variability for morphometric characteristics in Brazilian nut parent trees from northern Mato Grosso, Amazon rain forest. *Acta Amazonica*, 40: 705–710.

Carvalho, T.S.; Jesus, E.C.; Barlow, J.; Gardner, T.A.; Soares, I.C.; Tiedje, J.M.; Moreira, F.M.S. 2016. Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria. *Ecology*, 97: 2760-2771.

Chanway, C.P. 1997. Inoculation of tree roots with plant growth promoting soil bacteria: an emerging technology for reforestation. *Forest Science*, 43: 99-112.

Compant, S.; Clément, C.; Sessitsch, A. 2010. Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, 42: 669-678.

Döbereiner, J.; Baldani, V.L.D.; Baldani, J.I. 1995. *Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas*. Embrapa-SPI, Brasília, 60p.

EMBRAPA. 1997. *Manual de métodos de análise de solos*. 2nd. ed. Centro Nacional de Pesquisa de Solos, Rio de Janeiro, 212p.

Dollinger, J.; Jose, S. 2018. Agroforestry for soil health. *Agroforestry Systems,* 92: 213-219.

Estrada-De Los Santos, P.; Bustillos-Cristales, R.; Caballero-Mellado, J. 2001. *Burkholderia* a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. *Applied and Environmental Microbiology*, 67: 2790-2798.

Fankem, H.; Ngo Nkot, L.; Quinn, J.; Merbach, W.; Deubel, A.; Etoa, F.X.; Nwaga, D. 2008. Solubilization of inorganic phosphates and plant growth promotion by strains of *Pseudomonas fluorescens* isolated from acidic soils of Cameroon. *African Journal of Microbiology Research*, 2: 171-178.

FEMACT. 1993. *O Brasil do hemisfério norte: diagnóstico científico e tecnológico para o desenvolvimento*. Ed. Ambtec, Boa Vista, 512p.

Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, 35: 1039–1042.

Glick, B.R. 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012: 1-15.

Guariguata, M.R.; Cronkleton, P.; Duchelle, A.E.; Zuidema, P.A. 2017. Revisiting the 'cornerstone of Amazonian conservation': a socioecological assessment of Brazil nut exploitation. *Biodiversity and Conservation*, 26: 2007–2027.

Hallmann, J.; Quadt-Hallmann, A.; Mahaffee, W.F.; Kloepper, J.W. 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology*, 43: 895-914.

Hara, F.A.S.; Oliveira, L.A. 2004. Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos e álicos de Presidente Figueiredo, Amazonas. *Acta Amazonica*, 34: 343-357.

Hara, F.A.S.; Oliveira, L.A. 2005. Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos de Iranduba, Amazonas. *Pesquisa Agropecuária Brasileira*, 40: 667-672.

Illmer, P.; Barbato, A.; Schinner, F. 1995. Solubilization of hardly soluble $AlPO_4$ with P-solubilizing microorganisms. *Soil Biology and Biochemistry*, 27: 265–270.

Jesus, E.C.; Marsh, T.L.; Tiedje, J.M.; Moreira, F.M.S. 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. *The ISME Journal* 3: 1004–1011.

Kimura, M.A. 1980. Simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16: 111-120.

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

Long, H.H.; Schmidt, D.D.; Baldwin, I.T. 2008. Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. *PLoS ONE*, 3: e2702.

Lorenzi, A. 2002. *Árvores brasileiras: manual de identificação e cultivos de plantas arbóreas do Brasil*, *v.1*. Instituto Plantarum, Nova Odessa, 368p.

Marasco, R.; Rolli, E.; Ettoumi, B.; Vigani, G.; Mapelli, F.; Borin, S.; *et al*. 2012. A drought resistance-promoting microbiome is selected by root system under desert farming. *PLoS ONE,* 7: e48479.

Marra, L.; Oliveira, S.M.D.; Soares, C.R.F.S.; Moreira, F.M.D.S. 2011 Solubilization of inorganic phosphates by inoculant strains from tropical legumes. *Scientia Agricola*, 68: 603-609.

Marra, L.; Soares, C.R.F.S.; Oliveira, S.M.; Ferreira, P.A.A.; Soares, B.L.; Carvalho, R.F.; Lima, J.M.; Moreira, F.M.S. 2012. Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. *Plant and Soil*, 357: 289-307.

Moreira, F.M.S.; Gillis, M.; Pot, B.; Kersters, K.; Franco, A.A. 1993. Characterization of rhizobia isolated from different divergence groups of tropical Leguminosae by comparative gel electrophoresis of their total proteins. *Systematic and Applied Microbiolgy*, 16: 135–146.

Moreira, F.M.S.; Silva, K.; Nóbrega, R.S.A.; Carvalho, F. 2010. Bactérias diazotróficas associativas: diversidade, ecologia e potencial de aplicações. *Comunicata Scientiae*, 1: 74-99.

Mori, S.A. 1992. The Brazil nut industry: past, present, and future. In: Plotkin, M.J.; Famolare, L.M. (Ed.). *Sustainable Harvest and Marketing of Rainforest Products*. Island Press, Washington, p.241-251.

Muller, C.H.; Fiqueiredo, F.J.C.; Kato, A.K.; Carvalho, J.E.U.; Stein, R.L.B.; Silva, A.B. 1995. *Castanha-do-Brasil*. Embrapa SPI, Brasília, 65p.

Murphy, J.; Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27: 31-36.

Nautiyal, C.S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, 170: 265-270.

Neroni, R.F.; Cardoso, E.J.B. 2007. Occurrence of diazotrophic bacteria in *Araucaria Angustifolia*. *Scientia Agricola*, 64: 303-304.

Oliveira Junior, M.C.M.; Xaud, M.R.; Xaud, H.A.M.; Moura Neto, M.A.; Arco-Verde, M.F.; Pereira, P.R.V.S.; Tonini, H. 2003. *Precipitação pluviométrica em áreas de transição savana-mata de Roraima: campos experimentais Serra da Prata e Confiança*. Comunicado Técnico nº 17. Embrapa Roraima, Boa Vista, 7p.

Oliveira-Longatti, S.M.; Marra, L.M.; Soares, B.L.; Bomfeti, C.A.; Silva, K.; Ferreira, P.A.A.; Moreira, F.M.S. 2014. Bacteria isolated from soils of the western Amazon and from rehabilitated bauxite-mining areas have potential as plant growth promoters. *World Journal of Microbiology and Biotechnology*, 30: 1239-1250.

Ortiz, E. 2002. Brazil nut (Bertholletia excelsa). In: Shanley, P.; Pierce, A.R.; Laird, S.A.; Guillen, A. (Ed.). *Tapping the green market: certification and management of non-timber forest products*. Earthscan, London, p.61–74.

Pérez, E.; Sulbarán, M.; Ball, M.M.; Yarzábal, L.A. 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biology and Biochemistry*, 39: 2905-2914.

Pot, B.; Vandamme, P.; Kersters, K. 1994. Analysis of electrophoretic whole organism protein fingerprints. In: Goodfellow, M.; O'Donnell, A.G. (Ed.). *Chemical methods in prokaryotic systematics*. Wiley, New York, p.493–521.

Radwan, T.; Mohamed, Z.K.; Reis, V.M. 2002. Production of indole-3-acetic acid by different strains of *Azospirillum* and *Herbaspirillum* spp. *Symbiosis*, 32: 39–54.

Rodrigues Neto, J.; Malavolta Júnior, V.A.; Victor, O. 1986. Meio simples para o isolamento e cultivo de *Xanthomonas campestris* pv. *citri* tipo B. *Summa Phytopathologica*, 12: 16.

Santoyo, G.; Moreno-Hagelsieb, G.; Orozco-Mosqueda, M.C.; Glick, B.R. 2016. Plant growth-promoting bacterial endophytes. *Microbiological Research*, 183: 92–99.

Sarwar, M.; Kremer, R.J. 1995. Determination of bacterially derived auxins using a microplate method. *Letters in Applied Microbiology*, 20: 282-285.

Scott, A.J.; Knott, M.A. 1974. Cluster analysis method for grouping means in the analysis of variance. *Biometrics*, 30: 507–512.

SEPLAN. 2010. *Informações Socioeconômicas do Município de São João da Baliza– RR*. CGEES/SEPLAN, Boa Vista, 68p.

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

Silva, A.C.S.; Junior, A.F.C.; Oliveira, L.A.; Chagas, L.F.B. 2011a. Ocorrência de bactérias solubilizadoras de fosfato nas raízes de plantas de importância econômica em Manaus e Rio Preto da Eva, Amazonas. *Journal of Biotechnology and Biodiversity*, 2: 37-42.

Silva Filho, G.N.; Narloch, C.; Scarf, R. 2002. Solubilização de fosfatos naturais por microrganismos isolados de cultivos de *Pinus* e *Eucalyptus* de Santa Catarina. *Pesquisa Agropecuária Brasileira*, 37: 847-854.

Silva Filho, G.N.; Vidor, C. 2000. Solubilização de fosfatos por microrganismos na presença de fontes de carbono. *Revista Brasileira de Ciência do Solo*, 24: 311-319.

Silva, K.; Cassetari, A.S.; Lima, A.S.; Brandt, E.; Pinnock, E.; Vandamme, P.; Moreira, F.M.S. 2012. Diazotrophic *Burkholderia* species isolated from the Amazon region exhibit phenotypical, functional and genetic diversity. *Systematic and Applied Microbiology*, 35: 253-262.

Silva, K.; Nóbrega, R.S.A.; Lima, A.S.; Barberi, A.; Moreira, F.M.S. 2011b. Density and diversity of diazotrophic bacteria isolated from Amazonian soils using N-free semi-solid media. *Scientia Agricola*, 68: 518-525.

Souza, R.; Ambrosini, A.; Passaglia, L.M.P. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology*, 38: 401-419.

Spaepen, S.; Vanderleyden, J.; Okon, Y. 2009. Plant growth-promoting actions of rhizobacteria. In: Van Loon, L.C. (Ed.). *Advances in Botanical Research*. Academic Press, Burlington, p.283–320.

Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28: 2731–2739.

Thompson, J.D.; Higgins, D.G.; Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22: 4673-4680.

Ueda, T.; Suga, Y.; Yahiro, N.; Matsuguchi, T. 1995. Genetic diversity of N2-fixing bacteria associated with rice roots by molecular evolutionary analysis of a *nif*D library. *Canadian Journal of Microbiology*, 41: 235-240.

Vale Júnior, J.F.; Leitão Sousa, M.I. 2005. Caracterização e distribuição dos solos das Savanas de Roraima. In: Barbosa, R.I.; Xaud, H.A.M.; Souza, J.M.C. (Ed.). *Savanas de Roraima: Etnoecologia, biodiversidade e potencialidades agrosilvopastoris*. FEMACT, Boa Vista, p.79-91.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255: 571-586.

Vieira, S.; Trumbore, S.; Camargo, P.B.; Selhorst, D.; Chambers, J.Q.; Higuchi, N.; Martinelli, L.A. 2005. Slow growth rates of Amazonian trees: consequences for carbon cycling. *Proceedings of the National Academy of Sciences of the United States of America*, 102: 18502-18507.

Wadt, L.H.O.; Kainer, K.A.; Staudhammer, C.L.; Serrano, R.O.P. 2008. Sustainable forest use in Brazilian extractive reserves: natural regeneration of Brazil nut in exploited populations. *Biological Conservation*, 141: 332–346.

RECEIVED: 26/12/2018
ACCEPTED: 21/08/2019
ASSOCIATE EDITOR: Oscar Valverde