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ENDOPHYTIC RADICULAR AND RHIZOSPHERIC MICROBIOTA ASSOCIATED WITH THE ENDEMIC CERRADO PALM, *BUTIA ARCHERI*

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Abstract

Endophytic and rhizospheric microorganisms form complex communities that play pivotal role in plant growth and development, and contribute to the resilience of the host plant. As *Butia archeri*, a palm species endemic to the Cerrado savanna, is resistant to acidic soils and high concentrations of iron and aluminum, we decided to test the hypothesis that the endophyte and rhizosphere communities associated with this plant comprise microbiota rich in functional traits. To test this hypothesis, we isolated the cultivable radicular (endophytic) and rhizospheric microbiota of this palm and evaluated the capacity of the isolates to solubilize calcium ($CaHPO_4$) and iron ($FePO_4$) phosphates, synthesize indole acetic acid (IAA), and suppress the development of *Aspergillus niger*, the principal fungus known to deteriorate the seeds of this species. In total, 115 symbiotic bacterial and 17 fungal lineages were isolated, together with 40 seed-degrading fungi, primarily *A. niger*. The seed-deteriorating fungi presented a higher diversity index ($H' = 1.82$) than the other groups analyzed. Moreover, we confirmed the hypothesis that the microbiota associated with *B. archeri* has functional traits that contribute to plant growth. The BA81RB isolate of the bacterium *Bacillus cereus* solubilized 570.4 mg L^{-1} $CaHPO_4$ and 750.2 mg L^{-1} $FePO_4$. Furthermore, two isolates also solubilized remarkable amounts of $CaHPO_4$, with the BA367EF and BA99RF lineages of *Stagonosporopsis cucurbitacearum* and *Bionectria ochroleuca* solubilizing 798.90 and 493.20 mg L^{-1} phosphorous, respectively. Additionally, we confirmed the presence of isolates that synthesized high concentrations of IAA, such as the BA147RB lineage of *Enterobacter* sp., which synthesized 97.0 µg mL^{-1}, and others with potential for suppression of *A. niger* (BA68EB of *Bacillus amyloliquefaciens* and BA89RB of *B. cereus*). The present study provides novel insights into the symbiotic microorganisms associated with *B. archeri*, and reveals potentially important perspectives for the application of these isolates as promoters of plant growth, in particular for crops cultivated on the soils of the Cerrado biome.

Key words: Phosphate-solubilizing microorganisms; Auxin; Antibiosis; Arecaceae; Promotion of plant growth.

Introduction

Endophytic and rhizospheric microorganisms promote growth of the plants with which they are associated, considering the provision of various functions that result in adaptive gains for the host species. The functional traits of endophytic microbes include the provision of nutrients, via fixation of nitrogen and solubilization of phosphates (Puri *et al.*, 2018; Richard *et al.*, 2018; Shabanamol *et al.*, 2018; Sahoo & Gupta, 2018; Jeong *et al.*, 2018); synthesis of phytohormones such as auxins, cytokinins, and gibberellins (Shi *et al.*, 2009; Ali *et al.*, 2017); production of siderophores (Loaces *et al.*, 2011; Verma *et al.*, 2011; Rungin *et al.*, 2012; Dolatabad *et al.*, 2017); synthesis of the enzyme ACC-desaminase (e.g. Cedeño-Garcia *et al.*, 2018; Kruasuwan & Thamchaipenet, 2018; Tavares *et al.*, 2018; Win *et al.*, 2018); and antibiosis of phytopathogens (e.g. Devi *et al.*, 2018; Nigris *et al.*, 2018; Vurukonda *et al.*, 2018).

Endophytic microorganisms are deeply connected to the internal tissue of plants, where they provoke physiohormonal alterations, including the regulation of internal hormones (e.g. Shahzad *et al.*, 2016; Bilal *et al.*, 2018). For example, bacteria producing IAA may affect root elongation, development of radicular hairs, and radicular exudate, considering that this phytohormone triggers changes in the permeability of the plasma membrane (Golubev *et al.*, 2011). Similarly, the microorganisms that live in the rhizosphere form a complex community involving numerous connections with the plant. Rhizosphere is the region where interactions between plant and microorganisms occur (Dantas *et al.*, 2011). This close interaction may guarantee resilience of the plant in situations of physiological stress, such as a lack of water or excess of salts and heavy metals (e.g. Sheng *et al.*, 2008; Li *et al.*, 2011; Fan *et al.*, 2018; Khan *et al.*, 2019).

The soils of the Cerrado domain are naturally acidic, and contain high concentrations of aluminum, iron, and manganese, which at low pH, become highly soluble, have toxic effects on plants, and fix phosphorus in the soil by forming insoluble phosphoric compounds (Rodrigues *et al.*, 2016). Phosphorus is poorly mobile in the soil, where it is precipitated in the form of orthophosphate or is absorbed by calcium, iron, or aluminum cations, to form insoluble compounds, such as $AlPO_4$, $FePO_4$, $Ca_3(PO_4)_2$, which remarkably reduce the availability of this nutrient to plants. To overcome these issues, various plants associate with phosphate-solubilizing microorganisms (Khan *et al.*, 2014), which produce organic acids, such as oxalic acid, citric acid, and gluconic acid, as well as phosphatase acids, that are capable of mineralizing phosphorus and increasing its availability to plants (Sharma *et al.*, 2011; Mendes *et al.*, 2014).

The dwarf jelly palm, *Butia archeri* Glassman, known as coqueirinho-do-campo ou butiá in Brazil, is a

short-trunked palm endemic to the Cerrado savanna, and is highly adapted to the soils of this biome. *B. archeri* is found only in the Brazilian states of Goiás, Minas Gerais, and São Paulo, and the Federal District (Lima *et al.*, 2003; Soares, 2015). While *B. archeri* has been classified as extinct in the red list of endangered species of São Paulo (Mamede *et al.*, 2007), the species is locally abundant in the Brazilian Midwest. While this palm has recently gained immense interest from landscape gardeners (e.g. Hazir & Buyukozturk, 2013), it grows slowly and its seeds are difficult to germinate (Lorenzi *et al.*, 2010), which hampers the production of seedlings.

One of the principal difficulties for the germination of *B. archeri* seeds is the high incidence of seed-deteriorating fungi, which grow on the fruit, and reduce the viability of the seeds, considering that the fleshy mesocarp of this palm fruit attracts mold. Numerous studies have reported that several endophytic organisms have an antibiotic effect on seed-deteriorating fungi, and thus, reduce the incidence of disease and increase seed viability (e.g. Mahmood & Kataoka, 2018; Verma *et al.*, 2018). Da Silva *et al.*, (2018) recently found that rhizospheric isolates of the genus *Bacillus* obtained from a congener of *B. archeri*, *Butia purpurascens*, efficiently inhibit the seed-deteriorating fungi *Neodeightonia phoenicum* and *Penicillium purpurogenum* isolated from the seeds, in addition to numerous endophytic bacteria, with an enhanced capacity for synthesizing IAA. As *B. archeri* is a wild species, endemic to the Cerrado, we determined the genetic and functional diversity of its endophytic radicular and rhizospheric microbiota. We verified whether these communities contained organisms with functional traits, including the solubilization of calcium ($CaHPO_4$) and iron ($FePO_4$) phosphates, synthesis of IAA, and suppression of the seed-deteriorating fungi that affect these species.

Material and Methods

Collection of the plant material: Samples were obtained from a *B. archeri* specimen aged approximately 10 years, collected from an area of Cerrado *sensu stricto* in the vicinity of the town of Rio Verde, Goiás, Brazil (17º35'10.51" S, 50º59'13.06" W, altitude 822 m a.s.l.). This palm was apparently healthy, and was extracted from the soil using a hoe, by keeping the block of rhizospheric soil intact to protect the root system. The material was placed immediately in a cooler and transferred to the Agricultural Microbiology Laboratory at the Rio Verde campus of the Goiás Federal Institute, where it was processed.

Isolation of microorganisms: The endophytic microorganisms were isolated by fragmentation; herein, the sample roots were first being rinsed under running water to remove the excess of attached soil, and then agitated in water and neutral detergent at 70 rpm for 10 min, in order to reduce the number of epiphytic microorganisms. Thereafter, the tissue surface was disinfested by successive rinsing, immersion in 70% ethanol for 1 min, sodium chloride (2.5% active chlorine) for 5 min, and 70% ethanol for 30 s, followed by three rinses with autoclaved distilled water. To test the efficiency of disinfestation, 500 µL of water of the final rinse was extracted and inoculated in the nutrient broth (3 g meat extract; 5 g peptone) at 28ºC, for 24 h.

The disinfested fragments were cut into pieces of approximately 1 cm length and were then placed in Petri dishes containing Potato Dextrose Agar (PDA; 200 g potato, 20 g dextrose, and 5 g agar). The endophytic microorganisms were isolated by the 10th day of culture. The relative frequency of colonization was evaluated, based on the number of fragments containing at least one endophytic strain, divided by the total number of isolates.

The rhizospheric microorganisms were randomly selected for isolation, with 10 g of the root fragments being agitated in sterilized peptone water containing Tween 80 (0.1%) for 30 min at 70 rpm, at ambient temperature. The supernatant was then diluted in a series of saline solutions, and 50 µL aliquots were seeded in GELP medium (Sylvester-Bradley *et al.*, 1982) using the pour plate technique. The plates were then incubated at ambient temperature. Phosphate solubilization of $CaHPO_4$ was determined based on the appearance of a transparent halo, considered to be a positive signal (Barroso & Oliveira, 2001; Souchie *et al.*, 2007), after 4 days. Colonies that solubilized $CaHPO_4$ were isolated for purification and storage.

The seed-deteriorating fungi were isolated from *B. archeri* seeds, which were wrapped in Germitest® paper for germination in a Mangelsdorf germinator, adjusted to 30°C for 30 days. Once the mycelia developed, small fragments of the fungi growing around the seeds were removed using an inoculation loop. These fragments were cultivated in PDA medium, and the frequency of colonization was recorded for each isolate. The most common isolate was considered to be the principal deteriorator of the *B. archeri* seeds.

Identification of microorganisms: The genomic DNA of bacteria was extracted using the approach of Cheng & Jiang (2006). The Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) molecular marker, with oligonucleotides ERIC-1 and ERIC-2 (Bose & Sarma, 1975), was used to differentiate the purified strains, and those varying by at least one amplicon were identified as distinct lineages. Species were identified based on a partial sequence of the 16S rDNA gene, using the oligonucleotides 27F and 1492R (Weisburg *et al.*, 1991).

The fungi were grouped in lineages based on their morphological characteristics, including the color of the mycelium, their texture, and the extension of mycelial and microculture growth. The genomic DNA was extracted from a representative of each morphotype using a Miniprep extraction kit (Axygen Biosciences, USA), following the manufacturer's recommendations. The inter-retrotransposon amplified polymorphism (IRAP-PCR) molecular marker with the oligonucleotides CL IRAP1 and CL IRAP4 (Santos *et al.*, 2012), was used to differentiate the lineages through their banding patterns. Species were identified based on a partial sequence of the ITS (Internal Transcribed Spacer) rDNA region, using the oligonucleotides ITS 4 and ITS 5 (White *et al.*, 1990).

The amplification products of the 16S and ITS were purified (Dunn & Blattner, 1987) and sequenced by the Sanger method, using a Big Dye kit in an ABI3100 automatic sequencer (Applied Biosystems) in the UNESP Centralized multiuser Large-Scale DNA Sequencing and Gene Expression Analysis Laboratory in Jaboticabal, São Paulo, Brazil. The sequences were compared with those available in GenBank (http://www.ncbi.nlm.nih.gov) using BLASTN (Altschul *et al.*, 1990), considering a minimum homology of 97%.

Diversity and phylogenetic relationships of the isolates: The phylogenetic inference was carried out separately for the bacterial and fungal isolates, as well as the seed-deteriorating fungal isolates. The inferences were based on the alignment of the 16S and ITS sequences with the type sequences extracted from the Ribosomal Database Project, run in CLUSTAL OMEGA (Sievers *et al.*, 2011).

The evolutionary model of the sequences was selected based on the Bayesian Information Criterion (BIC) obtained in JMODELTEST 2 (Darriba *et al.*, 2012). The TPM3uf+I+G model was selected for the bacteria and the K80+G model for the fungi (both symbiotic and seed-deteriorating). Moreover, the phylogenetic trees were produced by Bayesian inference in Mr. Bayes v.3.2.6 (Ronquist *et al.*, 2012). Four independent runs were employed for each tree, with chains of 10 × 106 generations, and the *a posteriori* probabilities being evaluated every 500 generations. The first 2500 trees were discarded prior to the calculation of the consensus tree, to guarantee the convergence of the chains. The phylogenies recuperated by this approach were subsequently tested by the bootstrap method, with 5000 replications, run in MEGA 7 (Kumar *et al.*, 2016). Furthermore, the phylogenetic trees were visualized and edited in FigTree v 1.4.2 (Rambaut, 2014). *Chrysiogenes arsenatis* was the outgroup for the bacterial tree, whereas *Cryptosphaeria* sp. was the outgroup for the endophytic/ rhizospheric fungi, and *Spirosphaera beverwijkiana* for the seed-deteriorating fungi.

The Shannon–Wiener index (*H'*) was used to determine the diversity of the endophytic, rhizospheric, and deteriorator isolates, as reported by Kumar & Hyde (2004).

Functional traits

Quantification of the solubilization of $CaHPO_4$ and $FePO_4$: The endophytic and rhizospheric bacteria (previously selected in the qualitative test for the solubilization of $CaHPO_4$) were inoculated into liquid GL medium (10 g glucose, 2 g yeast extract), under constant agitation, in an orbital Shaker (Nova Técnica NT 712) agitator at 90 rpm, for 24 h, at 30°C. The optical density (OD_{600}) of the samples was standardized with 0.85% saline solution at 0.1. Thereafter, the fungal samples were grown in PDA medium for 4 days, at 30°C. All tests were run in triplicate.

The solubilization test comprised inoculation of 1 mL of each standardized bacterial culture or 5-mm mycelial discs in liquid GL medium with 1.26 g L^{-1} of each phosphate sources ($CaHPO_4$ and $FePO_4$) separately. These cultures were incubated under constant agitation (90 rpm) at 30°C, for 72 h. Following growth, the pH was measured and the quantity of inorganic phosphorus was determined by the vitamin C colorimetric method, as mentioned by Gadagi & Sa (2002). The solubilization was determined via comparison with an established standard curve, and values were presented as mg L^{-1} of Pi.

Production of IAA: The production of IAA was quantified based on the approach of Gordon & Weber (1951). For this, 50 µL of the bacterial culture, with the OD_{600} adjusted to 0.1, or 5-mm diameter mycelial discs from each sample were inoculated in nutrient broth, supplemented with 100 µg mL^{-1} of tryptophan. Thereafter, the cultures were incubated for 72 h at 30°C, under constant agitation (90 rpm), in an orbital agitator, and 1 mL of the supernatant of each culture was transferred to a test tube, followed by addition of 2 mL of Salkowski reagent. The tubes were kept in the dark for 20 min, prior to the spectrophotometric reading (530 nm). The synthesis of IAA was determined by the standard curve, with values given in $\mu g.mL^{-1}$ of IAA.

Biocontrol of the principal seed-deteriorating fungus: The antagonistic activity of the endophytic and rhizospheric bacteria toward the most common phytopathogen found in the *B. archeri* seeds, was evaluated. The double culture (Mew & Rosales, 1986) tests were run in PDA medium. Initially, the bacterial isolates were selected using a 5-mm disc of the phytopathogen mycelium, which was placed at the center of the Petri dish, with four different bacteria being placed equidistant around it. The control was a dish with only the fungus in its center. The dishes were incubated at ambient temperature for 8 days. The formation of a halo between the lineages under analysis was considered to indicate antibiosis, and the selected lineages were then transferred to the quantitative test, in triplicate, under the same conditions.

The diameter of the mycelium was measured to determine the percentage growth of the fungus in the presence of the bacterium, calculated by the relative inhibition (RI) index:

$$RI\ (\%) = \frac{(RC - RX)}{RC} \times 100$$

RC = Radius of the phytopathogen colony in the control treatment

RX = Radius of the phytopathogen colony exposed to the endophytic/rhizospheric isolate

Statistical analysis

All tests were completely randomized and conducted in triplicate. The data were evaluated using an analysis of variance, and the mean values recorded for functional traits analyzed in the present study were compared using the Scott–Knott test (5%).

Results

Diversity and phylogenetic relationships of the isolates: All (100%) root fragments were colonized by endophytic microorganisms, resulting in the isolation of 89 bacteria and 15 fungi. In total, 26 bacteria and two fungi capable of solubilizing $CaHPO_4$ were isolated from the rhizosphere.

The 115 endophytic and rhizospheric bacterial lineages isolated here (Table S1) represented seven genera with 13 taxa belonging to the phyla Proteobacteria, classes α and γ (90.43% of the isolates), Firmicutes, class Bacilli (8.70%), and Actinobacteria, class Actinobacteria (0.87%). In the Proteobacteria, the genus *Enterobacter* contributed 67% of the isolates, most of which (85%) were obtained from the endophytic environment, whereas the other 15% were isolated from the rhizospheric environment. *Rhizobium*, which corresponded to 24% of the isolates, was represented by two species found exclusively in the endophytic environment, as was *Acinetobacter*, which corresponded to 3% of the isolates. Two genera were found exclusively in the rhizospheric environment, that is, *Kosakonia* and *Klebsiella*, with 4% and 2% isolates, respectively (Fig. 1A).

In case of Firmicutes and Actinobacteria, only *Bacillus amyloliquefaciens* was isolated in both the environments analyzed, although it was more frequent in the rhizospheric isolates, with 27% of the total isolates (Fig. 1B). All other *Bacillus* species were observed only in the rhizospheric environment. The most prominent of these species was *Bacillus cereus*, the most common bacterium in this environment (37%). *Streptacidiphilus luteoalbus* was the only actinobacterium isolated from the endophytic environment (9% of isolates).

The phylogenetic inference revealed the existence of three monophyletic clades, two of which were more similar, containing bacteria of the genus *Rhizobium*, together with *Enterobacter*, *Klebsiella*, *Kosakonia*, and *Acinetobacter*, with the other clade being made up exclusively of the isolates of genus *Bacillus* (Fig. 2).

All fungi isolated in the present study were members of the phylum Ascomycota, distributed in three classes. The class Sordariomycetes was the most common (70.59% of the isolates), followed by Euromycetes (23.53%) and Dothimycetes (5.88%). In total, seven genera were recorded, including *Fusarium* (47.06% of the isolates), *Talaromyces*, *Penicillium*, and *Phomopsis*, each with 11.76% of the total, and *Stagonosporopsis*, *Hypocrea*, and *Bionectria*, each with 5.88%. None of the species occurred in both the rhizospheric and endophytic environments.

The analysis of the endophytic and rhizospheric fungal isolates revealed the existence of three clades (Table S2, Fig. 3). The most divergent clade was formed exclusively by *Penicillium*, whereas the most ample clade encompassed *Fusarium*, *Talaromyces*, *Stagonosporopsis*, *Phomopsis*, and *Hypocrea*, which shares similarities with *Bionectria*.

In total, 40 taxa of seed-deteriorating fungi were recorded here (Table S3), most of which belong to the class Euromycetes (82.50% of the total), followed by Sordariomycetes (12.5%) and Dothimycetes (5%). Five genera were recorded, in particular *Aspergillus* (67.50% of the isolates), followed by *Talaromyces* (15%), *Fusarium* (7.5%), *Gibberella* (5%), and *Neodeightonia* (5%) (Fig. 4).

The phylogenetic inference revealed three distinct clades, although the arrangement of the species was not well defined. *Talaromyces ruber* formed one clade, which was completely distinct from the other two, including that of *Talaromyces amestolkiae*. Moreover, the *Aspergillus* species were also allocated to two different clades.

The diversity indices (Shannon–Wiener *H'*) recorded for the cultivable microbial populations of *B. archeri* were 0.95 and 1.64 for the endophytic and rhizospheric bacteria, respectively. In fungal isolates, the indices were 1.52, 0.69, and 1.82 for the endophytic, rhizospheric, and seed-deteriorating fungi, respectively.

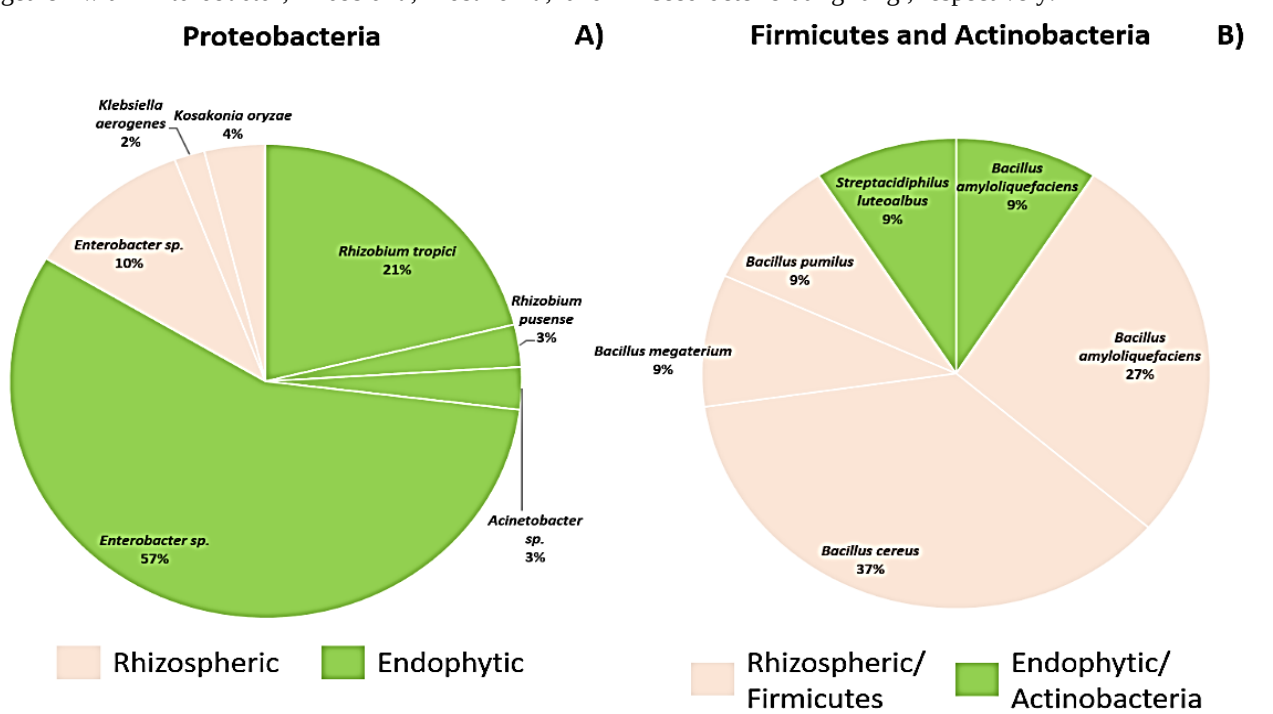

Fig. 1. Distribution (percentage) of the endophytic and rhizospheric bacterial isolates (phyla: Proteobacteria, Firmicutes, and Actinobacteria) recorded in *Butia archeri*, a palm (Arecaceae) endemic to the Cerrado biome.

Table S1. Molecular identification based on 16S region sequencing of isolates of the endophytic radicular and rhizospheric bacteria of *B. archeri*, a palm (Arecaceae) endemic to the Cerrado biome.

Isolate		Environment / Quantity	Identification	Filo, Class, Order, Family	GenBank access
BA71EB	BA227EB	E(22)	*Rhizobium tropici*	Proteobacteria; Alphaproteobacteria, Rhizobiales, Rhizobiaceae	KP687380
BA72EB	BA230EB				
BA76EB	BA234EB				
BA81AEB	BA235EB				
BA84EB	BA241EB				
BA185EB	BA246EB				
BA186EB	BA257EB				
BA195EB	BA259EB				
BA198EB	BA346EB				
BA209EB	BA368EB				
BA220EB	BA372EB				
BA212EB	BA253EB	E(3)	*Rhizobium pusense*	Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae	KF297587
BA215EB					
BA57EB	BA219EB	E(59) / R(11)	*Enterobacter* sp.	Proteobacteria, Gammaproteobacteria, Enterobacterales, Enterobacteriaceae	KF420155
BA62EB	BA222EB				
BA63EB	BA223EB				
BA65EB	BA224EB				
BA67EB	BA225EB				
BA69EB	BA228EB				
BA73EB	BA232EB				
BA74EB	BA233EB				
BA75EB	BA240EB				
BA76BEB	BA243EB				
BA77EB	BA248EB				
BA78EB	BA250EB				
BA78BEB	BA252EB				
BA80EB	BA254EB				
BA82EB	BA255EB				
BA83EB	BA256EB				
BA85EB	BA258EB				
BA98EB	BA279EB				
BA116EB	BA283EB				
BA182EBB	BA286EB				
A183EB	BA288EB				
BA184EB	BA290EB				
BA189EB	BA295EB				
BA190EB	BA298EB				
BA191EB	BA96RB				
BA192EB	BA103RB				
BA197EB	BA105RB				
BA199EB	BA109RB				
BA200EB	BA110RB				
BA201EB	BA116RB				
BA202EB	BA117RB				
BA203EB	BA120RB				
BA206EB	BA121RB				
BA207EB	BA123RB				
BA216EB	BA147RB				
BA73RB		R(2)	*Klebsiella aerogenes*	Proteobacteria; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae	MG546216
BA80RB					
BA106RB	BA115RB	R(4)	*Kosakonia oryzae*	Proteobacteria; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae	LT799040
BA112RB	BA118RB				
BA229EB	BA247EB	E(3)	*Acinetobacter* sp.	Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae	GQ478266
BA239EB					
BA68EB	BA98RB	E(1) / R(3)	*Bacillus amyloliquefaciens*	Firmicutes, Bacilli, Bacillales, Bacillaceae	KF418240
BA84RB	BA122RB				
BA78RB	BA88RB	R(4)	*Bacillus cereus*	Firmicutes, Bacilli, Bacillales, Bacillaceae	HQ694315
BA81RB	BA89RB				
BA98RB		R(1)	*Bacillus megaterium*	Firmicutes, Bacilli, Bacillales, Bacillaceae	MG774438
BA77RB		R(1)	*Bacillus pumilus*	Firmicutes, Bacilli, Bacillales, Bacillaceae	KX242456
BA188EB		E(1)	*Streptacidiphilus luteoalbus*	Actinobacteria, Actinobacteria, Streptomycetales, Streptomycetaceae	AY530190

E = Endophytic; R = Rhizospheric

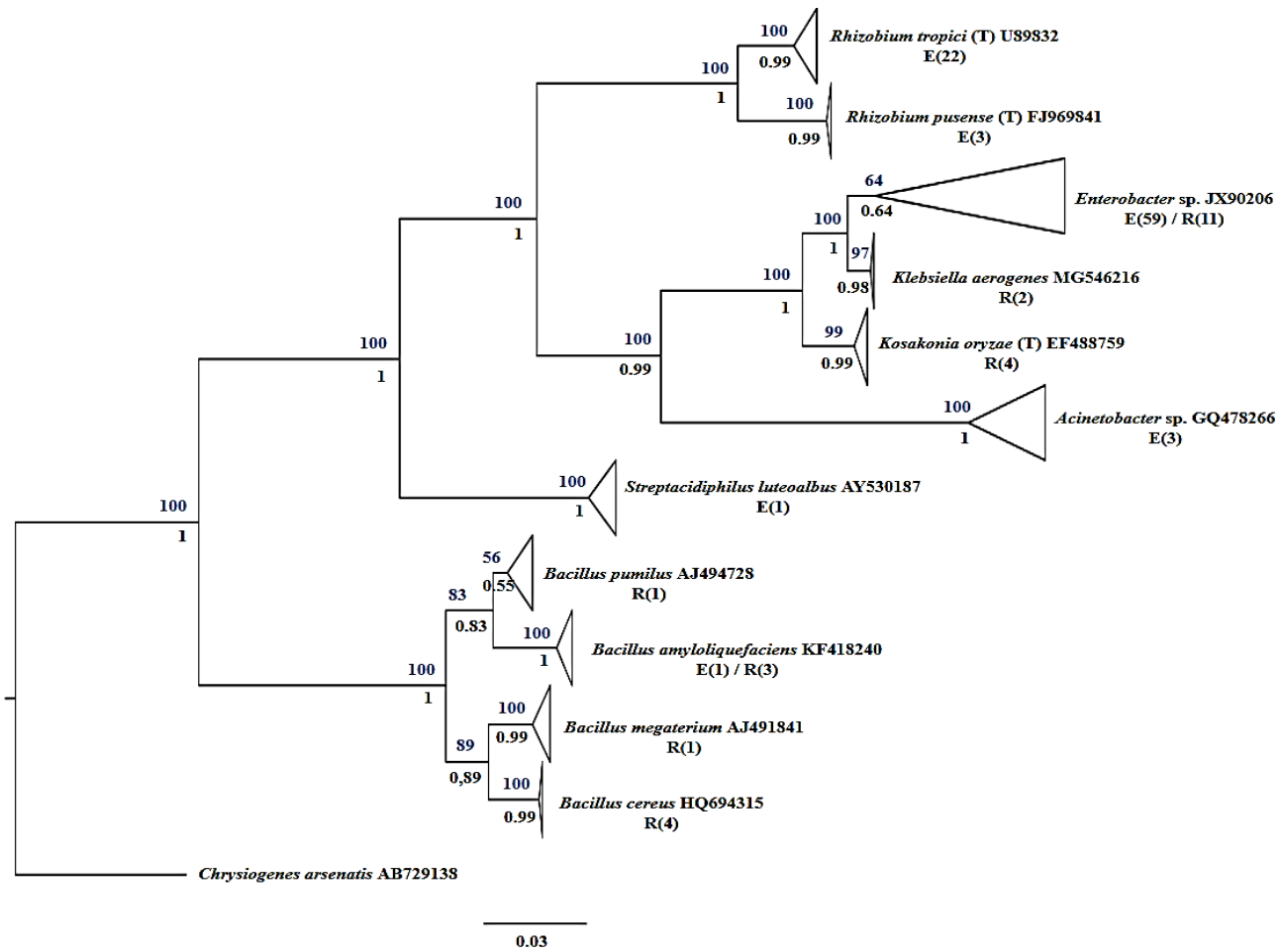

Fig. 2. Phylogenetic relationships and classification of the isolates of the endophytic radicular and rhizospheric bacteria of *Butia archeri*, a palm (Arecaceae) endemic to the Cerrado biome. The values in black are the *a posteriori* probabilities of the nodes and those in blue are the bootstrap values. E = endophytic; R = rhizospheric; ( ) the quantity of isolates recorded in each environment.

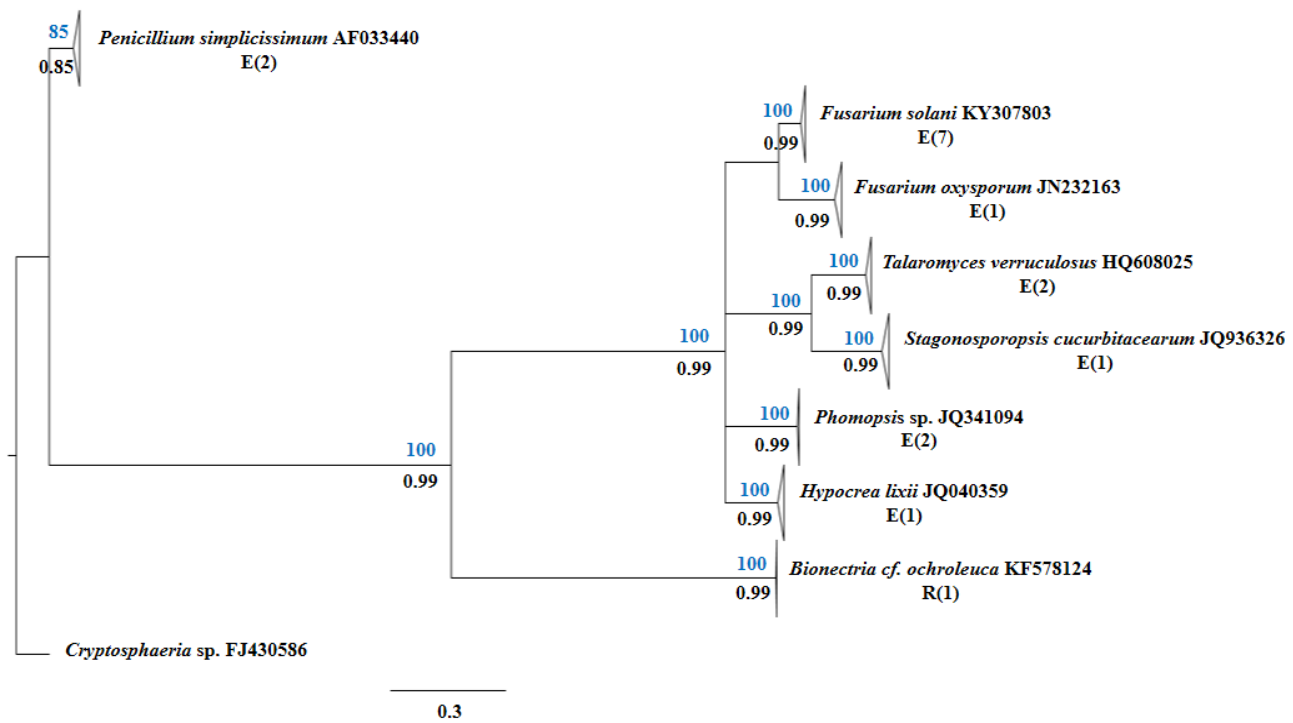

Fig. 3. Phylogenetic relationships and classification of the isolates of the endophytic radicular and rhizospheric fungi of *Butia archeri*, a palm (Arecaceae) endemic to the Cerrado biome. The values in black are the *a posteriori* probabilities of the nodes and those in blue are the bootstrap values. E = endophytic; R = rhizospheric; ( ) the quantity of isolates recorded in each environment.

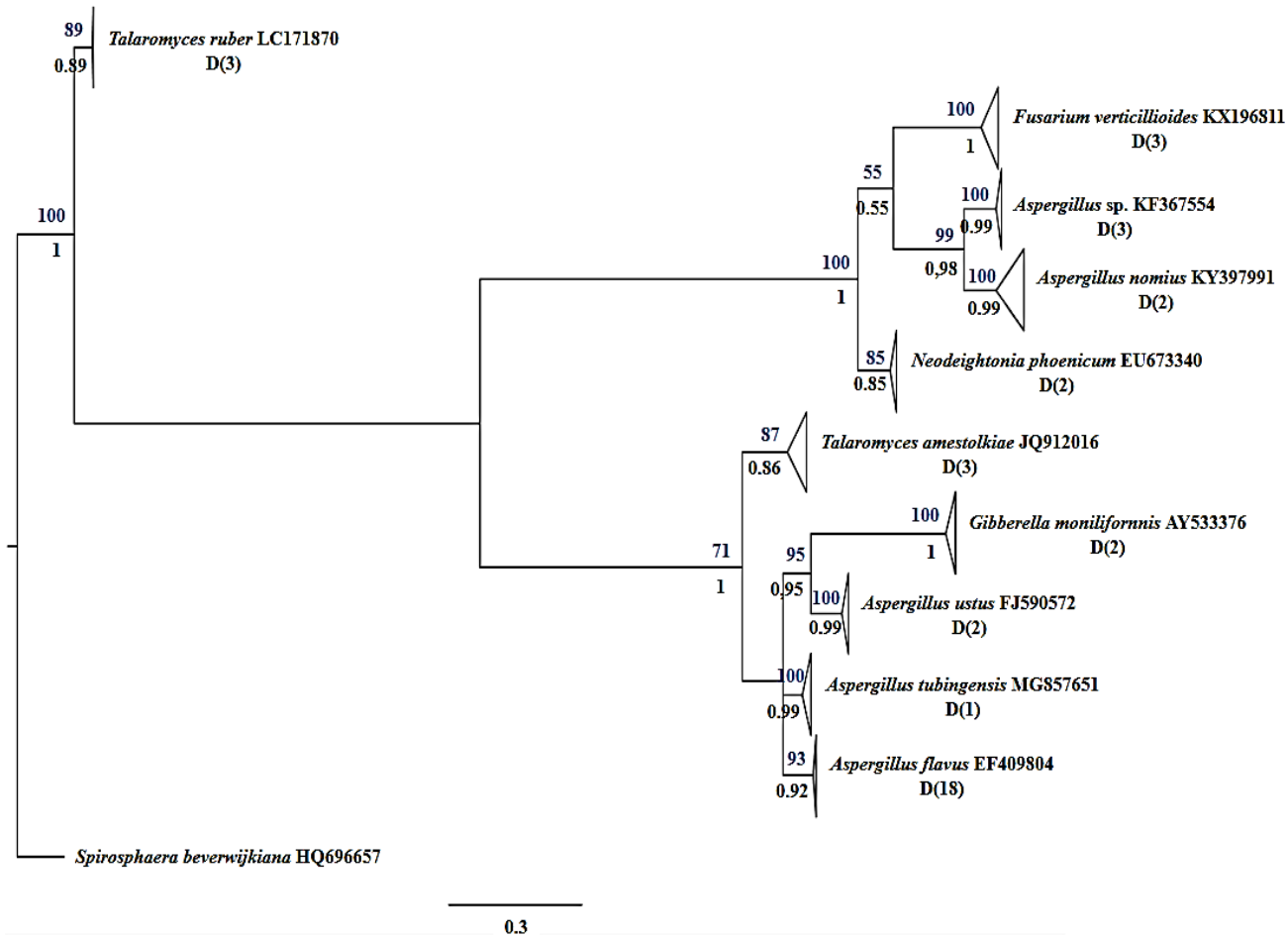

Fig. 4. Phylogenetic relationships and classification of the isolates of the seed-deteriorating fungi of *Butia archeri*, a palm (Arecaceae) endemic to the Cerrado biome. The values in black are the *a posteriori* probabilities of the nodes and those in blue are the bootstrap values. D = seed-deteriorating fungi; ( ) the quantity of isolates recorded in each environment.

Table S2. Molecular identification based on ITS region sequencing of isolates of the endophytic radicular and rhizospheric fungi of *B. archeri*, a palm (Arecaceae) endemic to the Cerrado biome.

Isolate	Environment / Quantity	Identification	Filo, Class, Order, Family	GenBank access
BA63AEF BA104EF BA71BEF BA287EF BA81BEF BA301EF BA101EF	E (7)	*Fusarium solani*	Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae	KY307803
BA296EF	E(1)	*Fusarium oxysporum*	Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae	JN232163
BA72EF BA90EF	E(2)	*Talaromyces verruculosus*	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae	HQ608025
BA292EF BA380EF	E(2)	*Penicillium simplicissimum*	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	HM469430
BA367EF	E(1)	*Stagonosporopsis cucurbitacearum*	Ascomycota, Dothideomycetes, Pleosporales, Didymellaceae	JQ936326
BA108RF	R(1)	*Hypocrea lixii*	Ascomycota, Sordariomycetes, Hypocreales, Hypocreaceae	JQ040359
BA99RF	R(1)	*Bionectria ochroleuca*	Ascomycota, Sordariomycetes, Hypocreales, Bionectriaceae	KF578124
BA214EF BA284EF	E(2)	*Phomopsis sp.*	Ascomycota, Sordariomycetes, Diaporthales, Valsaceae	JQ341094

E = Endophytic; R = Rhizospheric

Tabela S3. Molecular identification based on ITS region sequencing of seed deteriorating fungi of *B. archeri*, a palm (Arecaceae) endemic to the Cerrado biome. D = Deteriorator.

Isolate		Environment / Quantity	Identification	Filo, Class, Order, Family	GenBank access
BA139DF BA141DF	BA151DF	D(3)	*Fusarium verticillioides*	Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae	KX196811
BA152DF BA156DF	BA172DF	D(3)	*Talaromyces ruber*	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae	LC171870
BA148DF BA150DF	BA178DF	D(3)	*Talaromyces amestolkiae*	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae	JQ912016
BA146DF BA147DF BA153DF BA154DF BA155DF BA157DF BA158DF BA159DF BA160DF BA163DF	BA168DF BA167DF BA173DF BA174DF BA175DF BA177DF BA179DF BA180DF BA183DF	D(19)	*Aspergillus flavus*	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	EF409804
BA140DF BA142DF	BA161DF	D(3)	*Aspergillus* sp.	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	JF312217
BA176DF BA181DF		D(2)	*Aspergillus nomius*	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	KY397991
BA149DF BA182DF		D(2)	*Aspergillus ustus*	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	AY213637
BA162DF		D(1)	*Aspergillus tubingensis*	Ascomycota, Eurotiomycetes, Eurotiales, Aspergillaceae	MG857651
BA143DF BA145DF		D(2)	*Gibberella moniliformis*	Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae	AB374142
BA144DF BA170DF		D(2)	*Neodeightonia phoenicum*	Ascomycota, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae	EU673340

Functional traits: In general, 10 of the isolated bacteria were capable of solubilizing significant concentrations of $CaHPO_4$, in particular, the BA81RB strain of *B. cereus* (570.4 mg L^{-1}) and the BA123RB strain of *Enterobacter* sp. (591.1 mg L^{-1}). In the case of $FePO_4$, the BA81RB *B. cereus* strain was highly effective, and solubilized 750.2 mg L^{-1}, similar to the BA105RB strain of *Enterobacter* sp., which solubilized 732.9 mg L^{-1} (Fig. 5a); however, the high indices of solubilization observed here were not associated with reduced pH of the cultures, considering that the pH remained close to neutrality in both $CaHPO_4$ and $FePO_4$ (Fig. 5b).

Only two bacterial isolates did not synthesize IAA, and the highest rates were recorded for the rhizospheric isolates BA147RB (*Enterobacter* sp.), which synthesized 97.0 µg mL^{-1} of IAA, and BA118RB (*Kosakonia oryzae*), which synthesized 85.9 µg mL^{-1} (Fig. 2), although the isolates BA68EB (*B. amyloliquefaciens*) and BA105RB (*Enterobacter* sp.) also synthesized more than 80.0 µg mL^{-1} of IAA. None of the fungal isolates synthesized IAA.

Only two bacterial isolates, the endophytic BA68EB strain of *B. amyloliquefaciens* and the rhizospheric BA89RB strain of *B. cereus* presented antagonistic effects on the seed-deteriorating fungus *Aspergillus niger*, with the highest percentage of inhibition being recorded for *B. cereus* (RI = 14.29%). As for IAA, no fungal isolates inhibited the growth of *A. niger*.

Fewer fungal isolates solubilized phosphates, in general. The highest rates of solubilization of $CaHPO_4$ were recorded for the BA367EF strain of *Stagonosporopsis cucurbitacearum* (798.90 mg L^{-1} of P) and the BA99RF strain of *Bionectria ochroleuca*, which solubilized 493.20 mg L^{-1} of P (Fig. 6a). Moreover, solubilization was observed in other isolates, such as the BA108RF strain of *Hypocrea lixii* and the BA72RF strain of *Talaromyces verruculosus*, although the process was less intense. In the specific case of the BA108RF strain of *H. lixii*, the solubilization was associated with an abrupt reduction in the pH of the medium, which indicates the liberation of organic acids by the microorganism. Furthermore, the BA367EF isolate of *S. cucurbitacearum* was capable of solubilizing $FePO_4$, reaching 383.83 mg L^{-1} of P (Fig. 6b).

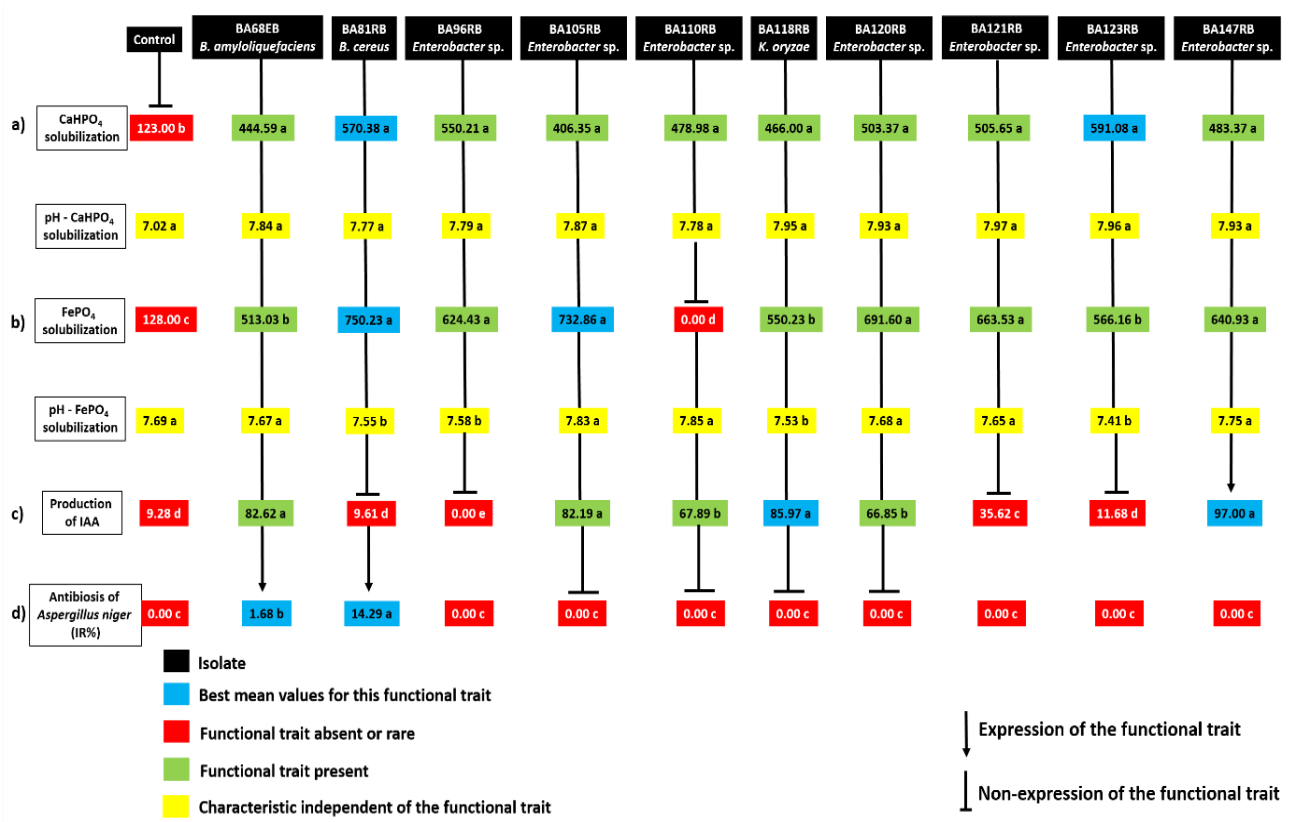

Fig. 5. Functional traits presented by the different endophytic radicular and rhizospheric bacterial isolates of *Butia archeri*, a palm (Arecaceae) endemic to the Cerrado biome. Mean values followed by different letters are significantly different (5%) from one another, based on the Scott-Knott test.

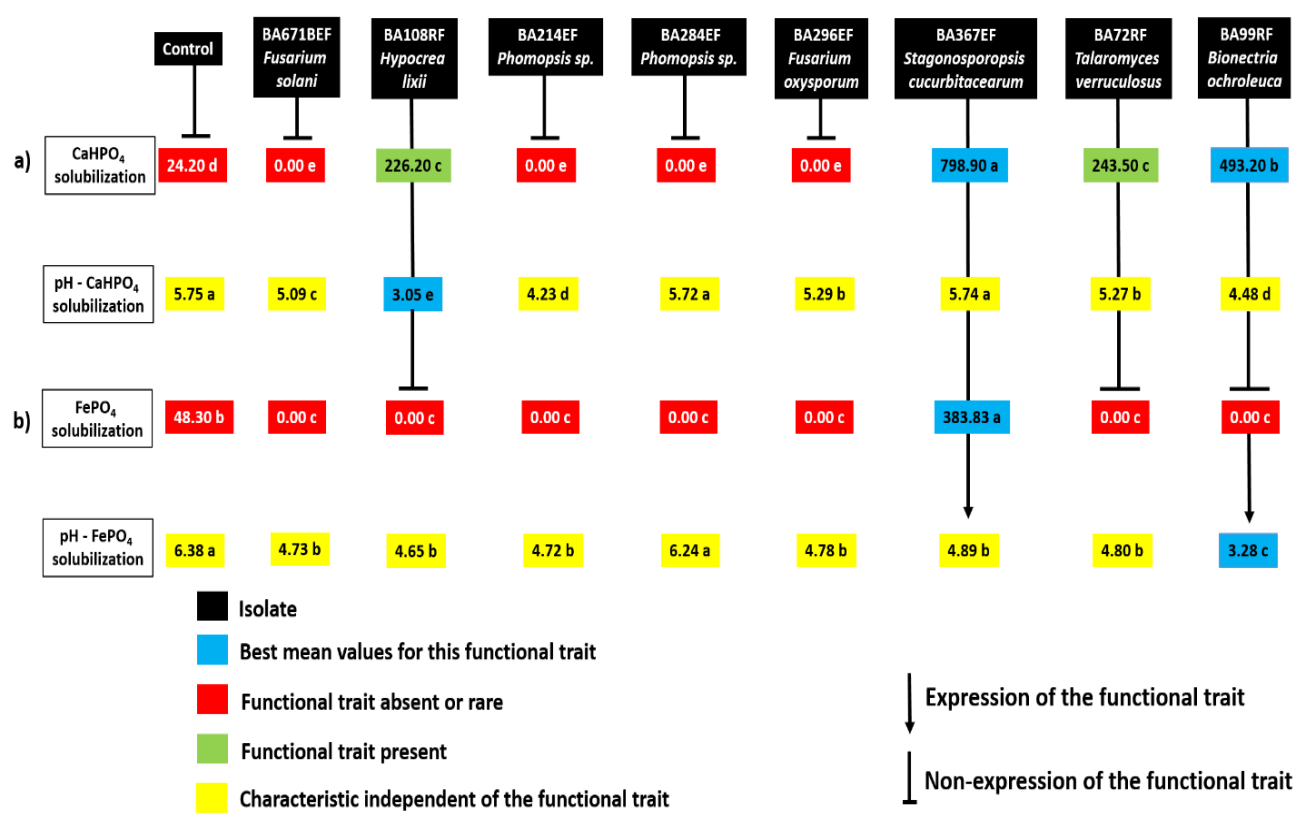

Fig. 6. Functional traits presented by the different endophytic radicular and rhizospheric fungal isolates of *Butia archeri*, a palm (Arecaceae) endemic to the Cerrado biome. Mean values followed by different letters are significantly different (5%) from one another, based on the Scott–Knott test.

Discussion

Species of the genera *Bacillus*, *Rhizobium*, and *Enterobacter*, as observed in the present study, are commonly known to share endophytic radicular relationships with both native and introduced plants of the Cerrado biome (e.g. Inácio *et al.*, 2017; de Abreu *et al.*, 2017; Braga *et al.*, 2018). Da Silva *et al.*, (2015; 2018) isolated species of these genera from the roots of *B. purpurascens*, a second *Butia* palm endemic to the Cerrado. The endophytic fungi of the *B. archeri* roots included one species of *Penicillium* and two of *Fusarium* genera, which are known to have endophytic relationships with several Cerrado plants (e.g. Noriler *et al.*, 2018). Faria *et al.*, (2016) isolated species of these genera from the roots of the native tree, *Anacardium othonianum* Rizzini; whereas, Almeida *et al.*, (2005) obtained endophytic isolates of *Fusarium oxysporum* and *Fusarium* sp. from *In vitro* samples of the *Bactris gasipaes* palm. The occurrence of pathogenic lineages of *Penicillium* and *Fusarium* is known to be influenced by climatic factors, such as temperature and humidity (e.g. Ono *et al.*, 1999; Doohan *et al.*, 2003; Backhouse, 2014), which indicates that the highly seasonal tropical climate of the Cerrado may also be favorable to endophytic lineages of these genera.

The highest indices of diversity (Shannon–Wiener *H'*) were recorded for the seed-deteriorating fungi, which support the hypothesis that the fleshy mesocarp of palm (Arecaceae) fruit may attract these fungi due to their water content. Moreover, excess water may reduce the germination rate by impeding the penetration of oxygen and affecting seed metabolism and viability (Figliola *et al.*, 1993). Kobori *et al.*, (2009) observed that seed germination of *Livistona chinensis* (Arecaceae) was impacted by the presence of seed-deteriorating fungi, primarily *Fusarium*. In another study, Pereira *et al.*, (2014) found that the presence of decomposing fungi, such as *Fusarium*, may avoid predation of the palm fruit by beetles (Coleoptera) in *Acrocomia aculeata*. Furthermore, this indicates that fungal incidence in these fruits may be related to seed protection provided by them through elimination of volatile compounds.

Rhizospheric strains of *B. cereus* (BA81RB) and *Enterobacter* sp. (BA123RB and BA105RB) with potential for solubilizing calcium and iron phosphates were identified in the present study. Numerous previous studies have also confirmed the solubilizing capacity of rhizospheric strains of *B. cereus*, including that of Ku *et al.*, (2018), who tested their potential for the promotion of growth in soybean, wheat, and kale. The bacteria of this genus are known to promote plant growth under low phosphorus availability (*Bacillus subtilis*, *Bacillus pumilus*, *Bacillus sphaericus*, *Bacillus polymyxa*, *Bacillus brevis*, *B. amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus atrophaeus*, and *Bacillus velezensis*) (de Freitas *et al.*, 1997; Vazquez *et al.*, 2000; Mohamed *et al.*, 2018; Ribeiro *et al.*, 2018; Chen *et al.*, 2019; Dipta *et al.*, 2019), alkaline (*Bacillus marisflavi* and *B. subtilis*) (Ahmad *et al.*, 2018; Prabhu *et al.*, 2018) and volcanic (*Bacillus thuringiensis*) (Delfim *et al.*, 2018) soils, or even on soils with high metal concentrations (*B. cereus*) (Yang *et al.*, 2018).

Similarly, the genus *Enterobacter*, in particular the species *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Enterobacter ludwiigi*, are known to promote plant growth by solubilizing phosphates (see e.g. Shrivastava *et al.*, 2018; Singh, 2018) on salinized soils (e.g. Hafeez *et al.*, 2018), and those with high concentrations of heavy metals, by helping to reduce their toxicity (e.g. Adhikari, *et al.*, 2018; Pramanik *et al.*, 2018a).

The mechanisms through which these bacteria solubilize inorganic phosphates remain unclear, although they may involve the release of organic acids, processes of chelation and reduction (Altomare *et al.*, 1999), or even stimulation of plant metabolism, which may promote the efflux of protons and release of organic acids by the roots (Carrillo *et al.*, 2002; Shrivastava *et al.*, 2018). We observed high levels of phosphate solubilization by the BA81RB strain of *B. cereus*, and the BA123RB and BA105RB strains of *Enterobacter* sp., although the expression of this functional trait did not result reduced pH of the medium, which contradicts the hypothesis that these strains may have liberated acids to access the Pi. In contrast, numerous studies have confirmed the capacity of *B. cereus* and *Enterobacter* to hydrolyze organic phosphate compounds and produce soluble phosphorus, via synthesis of phosphatase enzymes (e.g. Danial & Alkhalf, 2018; Pramanik *et al.*, 2018b; Muslim *et al.*, 2018). Sato *et al.*, (2016) described a new ectophosphatase acid that is produced by *Enterobacter* and concluded that it may constitute a mechanism for solubilizing mineral phosphates by the microorganisms capable of solubilizing insoluble minerals, which increases the availability of nutrients for the plant, in particular in soils with less phosphorus content.

The fungal BA367EF isolate of *S. cucurbitacearum* solubilized extremely high levels of $CaHPO_4$, in some cases, higher that those recorded for the bacterial isolates analyzed in this study. Moreover, this isolate was also effective for the solubilization of $FePO_4$. Biz *et al*., (2017) isolated an endophytic radicular strain of this genus from *Vochysia divergens* in the Pantanal biome, but did not evaluate the functional traits of this strain. In contrast with the results of the present study, Nandhini *et al.*, (2018) isolated an endophytic strain of *S. cucurbitacearum* from millet, although it did not appear to promote plant growth in any perceivable way. One other isolate that was effective in solubilizing $CaHPO_4$ was the BA99RF strain of *B. ochroleuca*. This endophytic microorganism was tested biotechnologically for the production of peptides (Abdalla & Matasyoh, 2014) and exopolysaccharides with antitumoral activity (Li *et al.*, 2016), and also for its potential influence on plant growth, as described by Faria *et al.*, (2016), who confirmed the capacity of *B. ochroleuca* for solubilizing various phosphates.

The BA108RF isolate of *H. lixii* solubilized relatively low concentrations of $CaHPO_4$, although its activity markedly reduced the pH of the medium. *H. lixii* is the sexual stage of *Trichoderma harzianum* (Chaverri *et al.*, 2003), a species that is commonly found in soil samples worldwide. Hong *et al.*, (2010) recorded reduced pH values in a test experiment in Petri dishes, revealing that *H. lixii* can solubilize insoluble metallic phosphates, and accumulate Cu and Zn in the biomass. Altomare *et al.*,

(1999) also demonstrated that *T. harzianum* is able to solubilize insoluble minerals (MnO_2, metallic zinc, and $CaHPO_4$). From a slightly different perspective, *H. lixii* is also known to synthesize phytase-type phosphatases, in particular, via solid (Thyagarajan *et al*., 2014a) or liquid fermentation, under agitation (Thyagarajan *et al*., 2014b). Thus, *H. lixii* may also be considered as a potential promoter of plant growth.

Only one of the symbiotic bacteria analyzed in the present study did not produce IAA, and the highest concentrations of this phytohormone were synthesized by the BA147RB strain of *Enterobacter* sp. These findings are in accordance to those of Da Silva *et al.*, (2018), in their study of the palm *B. purpurascens* (Arecaceae), in which the highest concentrations of IAA were also produced by a strain of *Enterobacter*. Shoebitz *et al.*, (2009) isolated a strain of *Enterobacter* that may promote plant growth, considering its capacity for producing IAA, solubilization of phosphates, production of positive nitrogenase, and its potential as an antagonist of *Fusarium solani*.

In contrast, only two of the bacteria analyzed presented antibiotic activity against *A. niger*. This indicates that this functional trait is not developed primarily by the symbiotic bacteria of *B. archeri*. The BA68EB strain of *B. amyloliquefaciens* was effective in all the functional traits analyzed, as was the BA81RB strain of *B. cereus*, although it did synthesize low concentrations of IAA.

This is the first study to analyze the endophytic and rhizospheric microbial diversity of the dwarf jelly palm, *B. archeri* Glassman, a species that is widely used as an ornamental plant for landscaping and urban afforestation, but is poorly-known in biological terms. Furthermore, the functional capacities of the microorganisms associated with this palm have important implications in developing effective agricultural practices, in particular for the soils of the Cerrado biome. Phosphate-solubilizing strains, for example, may be used as biofertilizers, with the potential to minimize or eliminate the current dependence on chemical fertilizers (Coutinho *et al.*, 2012). We hope that the present study will also expand the current perspective on the potential use of endemic species for the prospection of biotechnologically viable strains of microorganisms.

Conclusions

We confirmed the hypothesis that the microbiota associated with *B. archeri* presents functional traits for the solubilization of phosphates, such as the bacterial isolates BA81RB (*B. cereus*) and BA105RB (*Enterobacter* sp.), and the fungal isolates BA367EF, of *S. cucurbitacearum*, and BA99RF, of *B. ochroleuca*. We also confirmed the presence of isolates that synthesize IAA (the BA147RB strain of *Enterobacter* sp. and BA118RB of *K. oryzae*) and have potential for suppressing *A. niger*, the principal seed-deteriorating fungus that affects *B. archeri* (BA68EB of *B. amyloliquefaciens* and BA89RB of *B. cereus*). Collectively, the results of the present study provide important insights into the symbiotic microorganisms associated with a native palm (Arecaceae) endemic to the Brazilian Cerrado savanna.

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References

Abdalla, M.A. and J.C. Matasyoh. 2014. Endophytes as producers of peptides: an overview about the recently discovered peptides from endophytic microbes. *Nat. Prod. Bioprospect*, 4: 257-270.

Adhikari, A., K.E. Lee, M.A. Khan, S.M. Kang and I.J. Lee. 2018. Rhizobacterium *Enterobacter ludwigii* GAK2 mitigates cadmium toxicity in rice (*Oryza sativa*) through silicate and phosphate solubilization. *Conference of the Korean Society of Crop Science*, 10: 119-119.

Ahmad, M., I. Ahmad, T.H. Hilger, S.M. Nadeem, M.F. Akhtar, M. Jamil, A. Hussain and Z.A. Zahir. 2018. Preliminary study on phosphate solubilizing *Bacillus subtilis* strain Q3 and *Paenibacillus* sp. strain Q6 for improving cotton growth under alkaline conditions. *Peer J.*, 4: 6: e5122.

Ali, S., T.C. Charles and B.R. Glick. 2017. Endophytic phytohormones and their role in plant growth promotion. In: (Ed.): Doty, S. *Functional Importance of the Plant Microbiome Springer*, 89-105.

Almeida, C.D., R. Yara. and M.D. Almeida, 2005. Fungos endofíticos isolados de ápices caulinares de pupunheira cultivada *In vivo* e *In vitro*. *Pesq. Agropec. Bras.*, 40: 467-470.

Altomare, C., W.A. Norvell, T. Björkman and G.E. Herman 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Appl. Environ. Microbiol.*, 65: 2926-2933.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman 1990. Basic local alignment search tool. *J. Mol. Biol.*, 215: 403-410.

Backhouse, D. 2014. Global distribution of *Fusarium graminearum*, *F. asiaticum* and *F. boothii* from wheat in relation to climate. *Eur. J. Plant Pathol.*, 139: 161-173.

Barroso, C.B. and L.A. Oliveira. 2001. Ocorrência de bactérias solubilizadoras de fosfato de cálcio nas raízes de plantas na Amazônia Brasileira. *Rev. Bras. Ciênc. Solo*, 25: 575-581.

Bilal, S., R. Shahzad, A.L. Khan, S.M. Kang, Q.M. Imran, A. Al-Harrasi, B.W. Yun and I.J. Lee. 2018. Endophytic microbial consortia of phytohormones-producing fungus *Paecilomyces formosus* LHL10 and bacteria *Sphingomonas* sp. LK11 to *Glycine max* L. regulates physio-hormonal changes to attenuate aluminum and zinc stresses. *Front. Plant Sci.*, 9: 1273.

Biz, A.R., E.A.F. Mendonça, E.G. Almeida and M.A. Soares. 2017. Endophytic fungal diversity associated with the roots of cohabiting plants in the Pantanal wetland. In: *Natural resources in wetlands: from Pantanal to Amazonia*, pp. 37-70.

Bose, K.S. and R.H. Sarma. 1975. Delineation of the intimate details of the backbone conformation of pyridine nucleotide coenzymes in aqueous solution. *Biochem. Biophys. Res. Commun.*, 66: 1173-1179.

Braga, L.F., F.A. Oliveira, E.A.P. Couto, K.F.d'E.N. Santos, E.P.B. Ferreira and C.C.G. Martin-Didonet. 2018. Polyphasic characterization of bacteria obtained from upland rice cultivated in Cerrado soil. *Braz. J. Microbiol.*, 49: 20-28.

Carrillo, A.E., C.Y. Li and Y. Bashan. 2002. Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense*. *Naturwissenschaften*, 89: 428-432.

Cedeño-García, G.A., M. Gerding, G. Moraga, L. Inostroza, S. Fischer, M. Sepúlveda-Caamaño and P. Oyarzúa. 2018. Plant growth promoting rhizobacteria with ACC deaminase activity isolated from Mediterranean dryland areas in Chile: Effects on early nodulation in alfalfa. *Chil. J. Agric. Res.*, 78: 360-369.

Chaverri, P., L.A. Castlebury, G.J. Samuels and D.M. Geiser 2003. Multilocus phylogenetic structure within the *Trichoderma harzianum*/*Hypocrea lixii* complex. *Mol. Phylogenet. Evol.*, 27: 302-313.

diptaChen, L., H. Shi, J. Heng, D. Wang and K. Bian. 2019. Antimicrobial, plant growth-promoting and genomic properties of the peanut endophyte *Bacillus velezensis* LDO2. *Microbiol. Res.*, 218: 41-48.

Cheng, H.R. and N. Jiang. 2006. Extremely rapid extraction of DNA from bacteria and yeasts. *Biotechnol. Lett.*, 28: 55-59.

Coutinho, F.P., W.P. Felix and A.M.Y. Melo. 2012. Solubilization of phosphates *In vitro* by *Aspergillus* spp. and *Penicillium* spp. *Ecol. Eng.*, 42: 85-89.

Da Silva, C.F., J.A. Senabio, L.C. Pinheiro, M.A. Soares and E.L. Souchie. 2015. Isolation and genetic characterization of endophytic and rhizospheric microorganisms from *Butia purpurascens* Glassman. *Afr. J. Microbiol. Res.*, 9: 1907-1916.

Da Silva, C.F., L.C. Vitorino, M.A. Soares and E.L Souchie. 2018. Multifunctional potential of endophytic and rhizospheric microbial isolates associated with *Butia purpurascens* roots for promoting plant growth. *Antonie van Leeuwenhoek*, 111: 2157-2174.

Danial, E.N. and M.I. Alkhalf. 2018. Effect of physical and chemical parameters on the activity of purified phosphatase enzyme produced by *Bacillus cereus*. *Int. J. Biotechnol. Wellness Ind.*, 6: 64-71

Dantas, J.S., A.P.D. Souza, M.F.D. Farias and V.D.F.B. Nogueira. 2011. Interações entre grupos de microorganismos com a rizosfera. *Pesqui. Apl. Agrotec.*, 2: 213-224.

Darriba, D., G.L. Taboada, R. Doallo and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods*, 9: 772.

De Abreu, C.S., J.E. Figueiredo, C.A. Oliveira, V.L. Dos Santos, E.A. Gomes, V.P. Ribeiro, B.A. Barros, U.G. Lana and I.E. Marriel. 2017. Maize endophytic bacteria as mineral phosphate solubilizers. *Genet. Mol. Res.*, 16(1): gmr16019294.

De Freitas, J.R., M.R. Banerjee and J.J. Germida 1997. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). *Biol. Fertil. Soils*, 24: 358-364.

Delfim, J., M. Schoebitz, L. Paulino, J. Hirzel and E. Zagal. 2018. Phosphorus availability in wheat, in volcanic soils inoculated with phosphate-solubilizing *Bacillus thuringiensis*. *Sustainability*, 10: 144.

Devi, A., P.K. Rajappan and V. Ravi. 2018. Evoluation of endophytic biocontrol agentes against sheath rot of rice. *Agrica*, 7: 24-36.

Dipta, B., S. Bhardwaj, M. Kaushal, S. Kirti and R. Sharma. 2019. Obliteration of phosphorus deficiency in plants by microbial interceded approach. *Symbiosis*, 1-14.

Dolatabad, H.K., M. Javan-Nikkhah and W.T. Shier. 2017. Evaluation of antifungal, phosphate solubilisation, and siderophore and chitinase release activities of endophytic fungi from *Pistacia vera*. *Mycol. Prog.*, 16: 777-790.

Doohan, F.M., J. Brennan and B.M. Cooke. 2003. Influence of climatic factors on *Fusarium* species pathogenic to cereals. In: (Eds.): Xu, X., J.A. Bailey and B.M. Cooke. *Epidemiology of Mycotoxin Producing Fungi.* Springer, Dordrecht.

Dunn, I.S. and F.R. Blattner. 1987. Charons 36 to 40: multienzyme, high capacity, recombination deficient replacement vectors with polylinkers and polystuffers. *Nucl. Acids Res.*, 15: 2677-2698.

Fan, M., Z. Liu, L. Nan, E. Wang, W. Chen, Y. Lin and G. Wei. 2018. Isolation, characterization, and selection of heavy metal-resistant and plant growth-promoting endophytic bacteria from root nodules of *Robinia pseudoacacia* in a Pb/Zn mining área. *Microbiol. Res.*, 217: 51-59.

Faria, P.S.A., J.A. Senábio, M.A. Soares, F.G. Silva, A.P.A. Cunha and E.L. Souchie. 2016. Assessment of functional traits in the assemblage of endophytic fungi of *Anacardium Othonianum* Rizzini. *Pak. J. Bot.*, 48: 1241-1252.

Figliola, M.B., E.C. Oliveira and F.C.M. Piña-Rodrigues. 1993. Análise de sementes. *In:* Aguiar, I.B., Piña-Rodrigues, F.C.M. and Figliolia, M.B. (Coords.). *Sementes florestais tropicais.* Brasília, DF: ABRATES, pp. 137-174.

Gadagi, R.S. and T. Sa. 2002. New isolation method for microorganisms solubilizing iron and aluminum phosphates using dyes. *J. Soil Sci. Plant Nutr*., 48: 615-618.

Golubev, S.N., A.Y. Muratova, L. Wittenmayer, A.D. Bondarenkova, F. Hirche, L.Y. Matora, W. Merbach and O.V. Turkovskaya. 2011. Rhizosphere indole-3-acetic acid as a mediator in the *Sorghum bicolor*-phenanthrene-*Sinorhizobium meliloti* interactions. *Plant Physiol. Biochem.*, 49: 600-608.

Gordon, S.A. and R.P. Weber. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiol.*, 26: 192-195.

Hafeez, M., P.W. Ramteke, R. Lawrence, Rambharose, B.G. Suresh, S. Kumari, A.K. Singh, A. Singla, A. Paul, S. Masih and H. Masih. 2018. Bio-formulation of halotolerant phosphate solubilizing *Enterobacter cloacae* hfz-h4 strain to screen different carrier materials and their shelf life study. *Int. J. Curr. Microbiol. App. Sci.*, 7: 2373-2380.

Hazir, A. and H.D. Buyukozturk. 2013. *Phoenix* spp. and other ornamental palms in Turkey: The threat from red palm weevil and red palm scale insects. *Emir. J. Food Agric.*, 25: 843-853.

Hong, J.W., J.Y. Park and G.M. Gadd. 2010. Pyrene degradation and copper and zinc uptake by *Fusarium solani* and *Hypocrea lixii* isolated from petrol station soil. *J. Appl. Microbiol. Biochem*., 108: 2030-2040.

Inácio, M.C., T.A. Paz, A.M.S. Pereira and M. Furlan. 2017. Endophytic *Bacillus megaterium* and exogenous stimuli affect the quinonemethide triterpenes production in adventitious roots of *Peritassa campestris* (Celastraceae). *Plant Cell Tiss. Organ. Cult.*, 131: 15-26.

Jeong, J.J., M.K. Sang, D.W. Lee, I.G. Choi and K.D. Kim. 2018. *Chryseobacterium phosphatilyticum* sp. nov., a phosphate-solubilizing endophyte isolated from cucumber (*Cucumis sativus* L.) root. *Int. J. Syst. Evol. Microbiol.*, 69: 610-615.

Khan, M.A., I. Ullah, M. Waqas, M. Hamayun, A.L. Khan, S. Asaf, S.M. Kang, K.M. Kim, R. Jan and I.J. Lee. 2019. Halo-tolerant rhizospheric *Arthrobacter woluwensis* AK1 mitigates salt stress and induces physio-hormonal changes and expression of *GmST1* and *GmLAX3* in soybean. *Symbiosis*, 77: 9-21.

Khan, M.S., A. Zaidi and J. Musarrat. (Eds). 2014. Phosphate Solubilizing Microorganisms. In: *Principles and Application of Microphos Technology*.

Kobori, N.N., K.F.L. Pivetta, M.E.S.P. Dematte, B.M.S. Silva, P.B. Luz and R.S. Pimenta. 2009. Efeito da temperatura e do regime de luz na germinação de sementes de Palmeira-leque-da-China (*Livistona chinensis* (Jack.) R. Br. ex. Mart.). *Ornam. Hort.*, 15: 29-36.

Kruasuwan, W. and A. Thamchaipenet 2018. 1-Aminocyclopropane-1-carboxylate (ACC) deaminase-producing endophytic diazotrophic *Enterobacter* sp. EN-21 modulates salt–stress response in sugarcane. *J. Plant Growth Regul.*, 37: 849-858.

Ku, Y., G. Xu, X. Tian, H. Xie, X. Yang, C. Cao and Y. Chen. 2018. Correction: Root colonization and growth promotion of soybean, wheat and Chinese cabbage by *Bacillus cereus* YL6. *PLoS One*, 13(12): e0210035.

Kumar, D.S.S. and K.D. Hyde. 2004. Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. *Fungal Divers.*, 17: 69-90.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.*, 33: 1870-1874.

Li, T., M.J. Liu, X.T. Zhang, H.B. Zhang, T. Sha and Z.W. Zhao. 2011. Improved tolerance of maize (*Zea mays* L.) to heavy metals by colonization of a dark septate endophyte (DSE) *Exophiala pisciphila*. *Sci. Total Environ.*, 409: 1069-1074.

Li, Y., S. Guo and H. Zhu. 2016. Statistical optimization of culture medium for production of exopolysaccharide from endophytic fungus *Bionectria ochroleuca* and its antitumor effect *In vitro*. *EXCLI J*, 11: 211-220.

Lima, E.S., J.M. Felfili, B.S. Marimon and A. Scariot. 2003. Diversidade, estrutura e distribuição espacial de palmeiras em um cerrado sensu stricto no Brasil Central-DF. *Rev. Bras. Bot.*, 26: 361-370.

Loaces, I., L. Ferrando and A.F. Scavino. 2011. Dynamics, Diversity and function of endophytic siderophore-producing bacteria in rice. *Microb. Ecol.*, 61: 606-618.

Lorenzi, H., F. Kahn, L.R. Noblick and E. Ferreira. 2010. *Flora Brasileira: Arecaceae (Palmeiras)*. Nova Odessa, SP: Instituto Plantarum. pp. 159-160.

Mahmood, A. and R. Kataoka. 2018. Potential of biopriming in enhancing crop productivity and stress tolerance. In: (Eds.): Rakshit, A. and H. Singh. *Advances in Seed Priming*, 127-145.

Mamede, M.C.H., V. Souza, J. Prado, F. Barros, W. MG and J.G. Rando. 2007. *Livro vermelho das espécies vegetais ameaçadas do Estado de São Paulo*. Edição 1, Instituto de Botânica.

Mendes, G.O., A.L.M. Freitas, O.L. Pereira, I.R. Silva, N.B. Vassilev and M.D. Costa. 2014. Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. *Ann. Microbiol.*, 64: 239-249.

Mew, T.W. and A.M. Rosales. 1986. Bacterization of rice plants for control of sheath blight caused by *Rhizoctonia solani*. *Phytopathology*, 76: 1260-1264.

Mohamed, E.A.H., A.G. Farag and S.A. Youssef. 2018. Phosphate solubilization by *Bacillus subtilis* and *Serratia marcescens* isolated from tomato plant rhizosphere. *J. Environ. Prot.*, 9: 266-277.

Muslim, S.N., Mohammed, A.N. Ali, I.M.S. Al-Kadmy, S.S. Khazaal, S.A. Ibrahim, N.A. Al-Saryi, L.G. Al-Saadi, S.N. Muslim, B.K. Salman and S.N. Aziz. 2018. Screening, nutritional optimization and purification for phytase produced by *Enterobacter aerogenes* and its role in enhancement of hydrocarbons degradation and biofilm inhibition. *Microb. Pathog.*, 115: 159-167.

Nandhini, M., S.B. Rajini, A.C. Udayashankar, S.R. Niranjana, O.S. Lund, H. Shetty and H.S. Prakash. 2018. Diversity, plant growth promoting and downy mildew disease suppression potential of cultivable endophytic fungal communities associated with pearl millet. *Biol. Control*, 127: 127-138.

Nigris, S., E. Baldan, A. Tondello, F. Zanella, N. Vitulo, G. Favaro, V. Guidolin, N. Bordin, A. Telatin, E. Barizza, S. Marcato, M. Zottini, A. Squartini, G. Valle and B. Baldan. 2018. Biocontrol traits of *Bacillus licheniformis* GL174, a culturable endophyte of *Vitis vinifera* cv. Glera. *BMC Microbiol.*, 18: 133.

Noriler, S.A., D.C. Savi, R. Aluizio, A.M. Palácio-Cortes, Y.M. Possiede and C. Glienke. 2018. Bioprospecting and Structure of Fungal Endophyte Communities Found in the Brazilian Biomes, Pantanal, and Cerrado. *Front Microbiol.*, 9: 1526.

Ono, E.Y.S., Y. Sugiura, M. Homechin, M. Kamogae, E. Vizzoni, Y. Ueno and E.Y. Hirooka. 1999. Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvested corn of the State of Paraná, Brazil. *Mycopathologia*, 147: 139-148.

Pereira, A.C.F., F.S.A. Fonseca, G.R. Mota, A.K.C. Fernandes, M. Fagundes, R. Reis-Júnior and M.L. Faria. 2014. Ecological Interactions Shape the Dynamics of Seed Predation in *Acrocomia aculeata* (Arecaceae). *PLoS One*, 9(5): e98026.

Prabhu, N., S. Borkar and S. Garg. 2018. Phosphate solubilization mechanisms in alkaliphilic bacterium *Bacillus marisflavi* FA7. *Curr. Sci*., 114: 845-853.

Pramanik, K., S. Kundu, S. Banerjee, P.K. Ghosh and T.K. Maiti. 2018a. Computational-based structural, functional and phylogenetic analysis of *Enterobacter* phytases. *3 Biotech.*, 8: 262.

Pramanik, K., S. Mitra, A. Sarkar and T.K. Maiti. 2018b. Alleviation of phytotoxic effects of cadmium on rice seedlings by cadmium resistant PGPR strain *Enterobacter aerogenes* MCC 3092. *J. Hazard Mater.*, 351: 317-329.

Puri, A., K.P. Padda and C.P. Chanway. 2018. Nitrogen-Fixation by endophytic bacteria in agricultural crops: Recent Advances. *Nitrogen in Agriculture – Updates*, 73-94.

Rambaut, A. 2014. FigTree version 1.4.2 [computer program] http://tree.bio.ed.ac.uk/software/figtree/

Ribeiro, V.P., I.E. Marriel, S.M. Sousa, U.G.P. Lana, B.B. Mattos, C.A. Oliveira and E.A. Gomes. 2018. Endophytic *Bacillus* strains enhance pearl millet growth and nutrient uptake under low-P. *Braz. J. Microbiol.*, 49: 40-46.

Richard, P.O., A.O. Adekanmbi and A.A. Ogunjobi. 2018. Screening of bacteria isolated from the rhizosphere of maize plant (*Zea mays* L.) for ammonia production and nitrogen fixation. *Afr. J. Microbiol. Res.*, 12: 829-834.

Rodrigues, A.L., L.F. Watzlawick, A.M. Genú, A.F. Hess and A.A. Ebling. 2016. Atributos de um solo florestal em uma topossequência e relações com a comunidade arbórea. *Floresta*, 46: 145-154.

Ronquist, F., M. Teslenko, P.V.D. Mark, A. Darling, S. Hohna, B. Larget, L. Liu, M.A. Suchard and J.P. Huelsenbeck. 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.*, 61: 539-542.

Rungin, S., C. Indananda, P. Suttiviriya, W. Kruasuwan, R. Jaemsaeng and A. Thamchaipenet. 2012. Plant growth enhancing effects by a siderophore-producing endophytic streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105) *Antonie Van Leeuwenhoek*, 102: 463-472.

Sahoo, H.R. and N. Gupta. 2018. Diversity of endophytic phosphate solubilising fungi associated with *Pomatocalpa decipiens* (Lindl.) JJ Smith – an endangered orchid in Barbara forest of Odisha, India. *Studies in Fungi*, 3: 84-99.

Santos, L.V. Dos, M.V. De Queiroz, M.F. Santana, M.A. Soares, E.G. De Barros, E.F. De Araújo and T. Langin. 2012. Development of new molecular markers for the *Colletotrichum* genus using *RetroCl1* sequences. *World J. Microbiol. Biotechnol.*, 28: 1087-1095.

Sato, V.S., R.F. Galdiano Júnior, G.R. Rodrigues, E.G. Lemos and J.M. Pizauro Junior. 2016. Kinetic characterization of a novel acid ectophosphatase from *Enterobacter asburiae*. *J. Microbiol.*, 54: 106-113.

Shabanamol, S., T.K. George, K.S. Rishad, T.S. Sreekumar and M.S. Jisha. 2018. Characterization and in planta nitrogen fixation of plant growth promoting endophytic diazotrophic *Lysinibacillus sphaericus* isolated from rice (*Oryza sativa*). *Physiol. Mol. Plant Pathol.*, 102: 46-54.

Shahzad, R., M. Waqas, A. LatifKhan, S. Asaf, M.A. Khan, S.M. Kang, B.W. Yun and I.J. Lee. 2016. Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. *Plant Physiol. Biochem.*, 106: 236-243.

Sharma, S., V. Kumar and R.B. Tripathi. 2011. Isolation of phosphate solubilizing microorganism (PSMs) from soil. *J. Microbiol. Biotech. Res.*, 1: 90-95.

Sheng, X.F., J.J. Xia, C.Y. Jiang, L.Y. He and M. Qian. 2008. Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environ. Pollut.*, 156: 1164-1170.

Shi, Y., K. Lou and C. Li. 2009. Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. *Biol. Fertil. Soils*, 45: 645-653.

Shoebitz, M., C.M. Ribaudo, M.A. Pardo, M.L. Cantore, L. Ciampi and J.A. Curá. 2009. Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perene* rhizosphere. *Soil Biol. Biochem.*, 41: 1768-1774.

Shrivastava, M., P.C. Srivastava and S.F. D'Souza. 2018. Phosphate-Solubilizing Microbes: Diversity and Phosphates Solubilization Mechanism. In: (Ed.): Meena, V. *Role of Rhizospheric Microbes in Soil*, 137-165.

Sievers, F., A. Wilm, D. Dineen, T.J. Gibson, K. Karplus, W. Li, R. Lopez, H. Mcwilliam, M. Remmert, J. Soding, J.D. Thompson and D.G. Higgins. 2011. Fast, scalable generation of highquality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.*, 7: 1-6.

Singh, M. 2018. Isolation and characterization of insoluble inorganic phosphate solubilizer rice rhizosphere strain *Enterobacter cloacae* BAU3. *IJANS*, 10(4): 1204-1209.

Soares, K.P. 2015. "Le genre *Butia*". Principes 1: 12-57.

Souchie, E.L., A.C.S. Abboud, A.L. Caproni. 2007. Solubilização de fosfato *İn vitro* por micro-organismos rizosféricos de guandu. *Biosci. J.*, 23: 53-60.

Sylvester-Bradley, R., N. Asakawa, S. Latorraca, F.M.M. Magalhães, L.A. Oliveira and R.M. Pereira. 1982. Levantamento quantitativo de micro- organismos solubilizadores de fosfatos na rizosfera de gramíneas e leguminosas forrageiras na Amazônia. *Acta Amaz.*, 12: 15-22.

Tavares, M.J., F.X. Nascimento, B.R. Glick and M.J. Rossi. 2018. The expression of an exogenous ACC deaminase by the endophyte Serratia grimesii BXF1 promotes the early nodulation and growth of common bean. *Lett. Appl. Microbiol.*, 66: 252-259.

Thyagarajan, R., S.K.R. Namasivayam and G. Narendrakumar. 2014a. Evaluation of phytase production by *Hypocrea lixii* SURT01 in submerged and solid-state fermentation. *Int. J. Pharm. Pharm. Sci.*, 6: 352-355.

Thyagarajan, R., S.K.R. Namasivayam and G.N. Kumar. 2014b. Optimization of medium components for phytase production by *Hypocrea lixii* SURT01 using response surface methodology. *J. Pure Appl. Microbio.*, 8: 2485-2490.

Vazquez, P., G. Holguin, M.E. Puente, A. Lopez-Cortes and Y. Bashan. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol. Fertil. Soils*, 30: 460-468.

Verma, S.K., K.L, Kingsley, M.S. Bergen, K.P. Kowalski and J.F. White. 2018. Fungal disease prevention in seedlings of rice (*Oryza sativa*) and other grasses by growth-promoting seed-associated endophytic bacteria from invasive *Phragmites australis*. *Microorganisms*, 8: 1.

Verma, V.C., S.K. Singh and S. Prakash. 2011. Bio-control and plant growth promotion potential of siderophore producing endophytic Streptomyces from *Azadirachta indica* A. Juss. *J. Basic Microbiol.*, 51: 550-556.

Vurukonda, S.S.K.P., D. Giovanardi and E. Stefani. 2018. Plant growth promoting and biocontrol activity of *Streptomyces* spp. as Endophytes. *Int. J. Mol. Sci.*, 19: 952.

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane. 1991. 16S Ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.*, 173: 697-703.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *Academic Press*, 315-322.

Win, K.T., T. Fukuyo, O. Keiki and Y. Ohwaki. 2018. The ACC deaminase expressing endophyte Pseudomonas spp. Enhances NaCl stress tolerance by reducing stress-related ethylene production, resulting in improved growth, photosynthetic performance, and ionic balance in tomato plants. *Plant Physiol. Biochem.*, 127: 599-607.

Yang, P., X-F. Zhou, L-L. Wang, Q-S. Li, T. Zhou, Y-K. Chen, Z-Y. Z-Y. Zhao and B-Y. He. 2018. Effect of phosphate-solubilizing bacteria on the mobility of insoluble cadmium and metabolic analysis. *Int. J. Environ. Res. Public Health*, 15: 1330.

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