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ORIGINAL PAPER

Multifunctional potential of endophytic and rhizospheric microbial isolates associated with *Butia purpurascens* roots for promoting plant growth

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Abstract The functional diversity of endophytic and rhizospheric microorganisms associated with the promotion of plant growth includes increased availability of plant nutrients, phytohormone synthesis and phytopathogen suppression. We used the hypothesis that the unknown root and rhizospheric community associated with the *Butia purpurascens* palm, an endemic species of the Cerrado, could be composed of microbiota with great functional diversity. Thus, the potential of the isolates of this community for four functional traits was evaluated: solubilization of calcium phosphate ($CaHPO_4$) and iron phosphate ($FePO_4$), synthesis of indoleacetic acid (IAA) and suppression of seed- and fruit-spoilage fungi of *B. purpurascens*. A total of 166 bacterial isolates, most belonging to the phylum Proteobacteria (94%), and 46 fungal isolates (Ascomycota) were tested. None of the isolates showed the four functional traits tested, but 72% presented two traits ($CaHPO_4$ solubilization and IAA synthesis). Fifteen fungi (27% of the isolates) presented only the trace for IAA, whereas the capacity for antibiosis was observed in only eight bacteria. $CaHPO_4$-solubilization capacity was evidenced by all bacterial isolates and by some fungal isolates. The functional trait for IAA production was present in all isolates, and production levels were significantly above 100 μg mL^{-1} for some bacteria. Isolates of the genus *Bacillus* efficiently suppressed the growth of spoilage fungi tested, with relative inhibition rates reaching levels higher than 60% when using *Bacillus subtilis*. These results attest to the multifunctionality of the endophytic and rhizospheric isolates of *B. purpurascens* for the promotion of plant growth. This is the first study that sought to identify the root endophytic and rhizospheric microbiota associated with the *B. purpurascens* palm for the bioprospection of species with functional traits related to the promotion of plant growth, thus opening the way for in vivo tests in plants of commercial or ecological interest.

Keywords Bacteria · Fungi · Phosphate solubilization · Auxin · Antibiosis

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Introduction

The interactions between plants and microorganisms have been widely used to promote the growth of species of agronomic or environmental interest. This promotion usually occurs taking advantage of the potential of endophytic and rhizospheric fungi and bacteria as microbial inoculants (Gaggia et al. 2013; Rangel de Souza et al. 2016; Karthik et al. 2017; Berthelot et al. 2017; Murphy et al. 2018), as plant growth and development depend directly on access to minerals and the actions of phytohormones (Vitorino et al. 2012). Furthermore, rhizospheric and endophytic microorganisms can maximize the supply of nutrients, or even regulate the concentrations of the plant hormone such as indoleacetic acid (IAA), gibberellins, abscisic acid and jasmonic acid accessible to plant cells (Behie and Bidochka 2014; Waqas et al. 2015; Bacon and White 2016; Bilal et al. 2018).

Several authors have confirmed that microbial species associated with the rhizosphere, or even other plant organs, such as leaves, flowers and fruits, perform a range of functions that can positively affect the growth and yield of plant species. Because they have at least one property that stimulates the development of plants, these microorganisms are known as growth promoters and can belong to several genera, including *Rhizobium* (Abbaszadeh-Dahaji et al. 2012; Laranjo et al. 2014; Marks et al. 2015), *Bacillus* and *Pseudomonas* (Esitken et al. 2010), *Aerococcus, Alteromonas, Enterobacter, Xanthomonas, Aspergillus, Penicillium* (Srinivasan et al. 2012; Bilal et al. 2018), and *Azospirillum* (Pedraza et al. 2010; Cassán et al. 2014).

The selection of strains with multifunctional potential is strategic for agricultural areas or reforestation programs that prioritize healthy and highly productive seedlings (Vassilev et al. 2012). The benefits of plant-microorganism interactions can be direct: uptake of essential nutrients through biological nitrogen fixation, phosphate solubilization (Marra et al. 2012; Ludueña et al. 2018) and siderophore production; and modulation of hormone levels through the synthesis of indoleacetic acid (IAA), gibberellins, cytokinins, nitric oxide and polyamines (Cassán et al. 2014).

The promotion of plant growth can also be induced indirectly through production of stress-related phytohormones, such as abscisic acid, jasmonic acid and cadaverine, or ethylene catabolism, related to the production of the enzyme ACC deaminase (Khan et al. 2016; Santoyo et al. 2016), even when phytopathogen suppression occurs (Ghyselinck et al. 2013).

Plant growth promoting microorganisms can also act by increasing the available phosphorus (P) levels for the plant. P is a macronutrient required by plants for activities such as cell division, development, photosynthesis, sugar metabolism, nutrient transport, transmission of genetic traits between generations and regulation of metabolic pathways (Behera et al. 2014). Therefore, P limitation directly affects soil fertility and consequently plant growth and crop yield (Vassilev et al. 2012; Kvakić et al. 2018).

Tropical soils have low P availability due to the reactivity of soluble forms of this mineral with calcium, iron, magnesium and aluminium, forming low-solubility compounds (Barroso and Nahas 2008; Chagas Junior et al. 2010; Behera et al. 2014). Soil P can be divided into inorganic (P_i) and organically-bound P (P_o) forms, which are commonly termed "organic P". Inorganic P occurs in P containing minerals such as apatite and in dissolved state as phosphate ions or polyphosphates (Turner et al. 2007; Vestergren et al. 2013; Missong et al. 2016). Reactions such as fixation and immobilization convert the fractions of P into forms unavailable to the plant. As a consequence, about 70–90% of the phosphate fertilizers applied to the soil becomes fixed, that is, unavailable for root absorption (Behera et al. 2014). In vitro studies have shown that some endophytic and rhizospheric microorganisms are able to release phosphate ions from inorganic compounds, making P available to the plant (Vassilev et al. 2012; Santoyo et al. 2016). These microorganisms contribute to a decrease in the pH near the root through the production and release of organic acids, which is the main mechanism involved in phosphate solubilization (Vassilev et al. 2006; Marra et al. 2012; Khan et al. 2014). Thus, phosphate-solubilizing microorganisms (PSMs) function as efficient bio-fertilizers, especially in areas with P deficiency, increasing the resistance and general growth of plants (e.g., Liu et al. 2018; Delfim et al. 2018).

Another important functional feature of some microorganisms is antibiosis against phytopathogens (Rocha et al. 2009). This activity has been frequently employed in agricultural and agroforestry systems for pest control, where endophytic species, such as the fungi *Trichoderma harzianum* (Zhang et al. 2016) and

Phialocephala fortinii (Surono and Narisawa 2018), bacteria of the genera *Bacillus* (El-Bendary et al. 2016; Zouari et al. 2016), *Paenibacillus* and *Pseudomonas* (Lin et al. 2014) and yeasts such as *Pichia ohmeri* and *Candida guilliermondii* (Coelho et al. 2011), have shown great antibiosis potential.

The antibiosis activity is particularly important for some plants native to the Cerrado, a Brazilian biome considered a biodiversity *hotspot*, characterized by the presence of different phytophysiognomies such as savannas, semideciduous seasonal forests and fields. This importance is due to the fact that many plants of this biome, such as the Jataí-palm (*Butia purpurascens* Glassman), that have fruits with succulent and aromatic mesocarp, which are highly appreciated by the local fauna as food (Lorenzi et al. 2010). This is because these mesocarp act as an attractant for spoilage fungi, which compromise the quality and germination of the seeds. This Areacaceae species is endemic to the Cerrado areas of Southwest Goiania, where local populations use their leaves and pulp in the therapeutic treatment of skin diseases and as an antivenom (Hoffmann et al. 2014; Martins et al. 2014). The leaves are also used in the manufacture of brooms, providing an alternative income source for many low-income families (Guilherme et al. 2015). As the distribution of this species is very restricted, it is threatened by the loss of natural habitat. This study is the first aiming to attribute functional traits of growth promotion to the symbiotic microbiota of this species.

To identify and determine the importance of microbial diversity and to quantify phosphate solubilization, indole-3-acetic acid (IAA) production and activity against phytopathogens, the culturable endophytic and rhizospheric microbiota of purple yatay palm roots (*Butia purpurascens* Glassman) were isolated and evaluated.

Materials and methods

Phylogenetic diversity and relationships among isolates

A total of 166 bacteria and 46 fungi were isolated from the internal root tissues and rhizosphere of *B. purpurascens*, as described in Da Silva et al. (2015) (Tables S1 and S2). These isolates, currently belonging to the microorganism bank of the Agricultural Microbiology Laboratory of the Goiano Federal Institute—Rio Verde Campus, were initially subjected to preliminary tests for functions related to growth promotion. Only the isolates with potential to promote plant growth were molecularly identified, namely, 33 bacteria (15 endophytic and 18 rhizospheric) and 21 fungi (14 endophytic and seven rhizospheric).

Bacterial genomic DNA was extracted according to the method of Cheng and Jiang (2006), and identification was performed by sequencing the 16S rDNA region. Genomic DNA from fungi was obtained using a kit (Axygen Biosciences, USA) according the manufacturer's recommendations; identification was achieved by partial sequencing of the internal transcribed spacer (ITS) of the rDNA region. The sequences obtained were compared to sequences available in GenBank using BLASTn accessions (http://www.ncbi.nlm.nih.gov).

The phylogenetic relationships were obtained separately for bacterial and fungal isolates. For this purpose, the sequences obtained for the 16S and ITS regions, along with sequences from that region available in GenBank for other isolates, were aligned using CLUSTAL OMEGA software (Sievers et al. 2011). The references for the determination of the phylogenetic relationship were obtained by selection of the evolution model of the 16S sequences for bacteria and ITS sequences for fungi using the Bayesian Information Criterion (BIC) available in the JMODELTEST 2 software (Darriba et al. 2012). The model TIM3 + G (− lnL = 9280.2761, wBIC = 0.9483, partition = 012032, K = 143, freqA = 0.2489, freqC = 0.2316, freqG = 0.3118, freqT = 0.2077, R(a) [AC] = 0.6703, R(b) [AG] = 1.4990, R(c) [AT] = 1.0000, R(d) [CG] = 0.6703, R(e) [CT] = 2.4282, R(f) [GT] = 1.0000, gamma shape = 0.5320) was selected for bacteria, and the model TrNef + G (-lnL = 7453.0714, wBIC = 0.7546), partition = 010020, K = 109, R(a) [AC] = 1.0000, R(b) [AG] = 1.6881, R(c) [AT] = 1.0000, R(d) [CG] = 1.0000, R(e) [CT] = 2.9724, R(f) [GT] = 1.0000, gamma shape = 0.7320) was selected for fungi. Phylogenetic analyses were performed using Bayesian statistics in MRBAYES v.3.2.6 (Ronquist et al. 2012). Four independent runs were performed, with 10×10^6 generations, sampling the posterior probability distribution every 500 generations. Before calculating the consensus tree and to ensure the convergence of the chains, the first 2500 trees sampled were discarded. Subsequently, the generated phylogeny was tested by

the bootstrap method, with 5000 replications, using MEGA 7 software (Kumar et al. 2016). The species *Methylobacterium* sp. and *Rhizopus oryzae* were used as outgroups in the bacterial and fungal trees, respectively.

Multifunctionality of isolates

Preliminary tests for the selection of microorganisms with potential for plant growth promotion

A total of 166 bacteria and 46 fungi isolated from the roots and rhizosphere of *B. purpurascens* were tested, and qualitative tests of $CaHPO_4$ solubilization and IAA synthesis were performed. Only isolates that stood out for the two traits in question were evaluated in subsequent quantification tests of $CaHPO_4$ and $FePO_4$ solubilization and IAA synthesis.

Qualitative evaluation of $CaHPO_4$ solubilization capacity

Bacterial and fungal isolates were inoculated onto Petri dishes containing GELP growth medium (10 g of glucose; 5 g of peptone, 0.05 g of yeast extract and 15 g of agar), supplemented with 25 mL of $CaCl_2$ (10%) and 12.5 mL of K_2HPO_4 (10%), forming a precipitate of inorganic phosphate $CaHPO_4$ (10%), as described by Sylvester-Bradley et al. (1982). The capacity of the microorganism to solubilize $CaHPO_4$ was confirmed by visualization of a clear halo around the bacterial or fungal colony, in contrast to the opaque medium (Souchie et al. 2007). Plates containing GELP culture medium with $CaHPO_4$ absent from inoculum were used as negative control of solubilization.

Qualitative evaluation of IAA synthesis

Only the bacteria were submitted to the qualitative test for IAA production, the fungal isolates were assigned directly to the quantitative test. The bacteria were inoculated into 10 mL of nutrient broth, supplemented with 100 µL of tryptophan. After 72 h of incubation at 30 °C in the dark, the cultures were centrifuged (16,000×*g*) for 5 min at 4 °C. Then, 1 mL of the supernatant from each isolate was transferred to a test tube, and 1 mL of Salkowski's reagent (1.875 g of $FeCl_3.6H_2O$, 100 mL of H_2O and 150 mL of H_2SO_4) was added. The tubes were kept in the dark for 20 min and then read in a spectrophotometer (530 nm). The microorganism's capacity to synthesize IAA was demonstrated by the red-pink coloration of the mixture in the test tubes. It was used as negative control, nutrient broth without inoculum.

Quantification tests of $CaHPO_4$ and $FePO_4$ solubilization in liquid medium and of IAA synthesis

For these tests, the bacterial samples were grown under constant agitation with the aid of an orbital shaker (Nova Técnica NT 712), at 90 rpm, for 24 h at 30 °C in 7 mL of liquid GL culture medium (10 g of glucose, 2 g of yeast extract). Subsequently, 3 mL of each culture was aseptically removed to determine the optical density (OD) at 600 nm. All bacterial samples had their OD adjusted to 0.1 by dilution with saline solution sterile (0.85%). The fungal samples were grown in PDA medium (infusion of 200 g of potato, 20 g of dextrose and 15 g of agar) for 4 days at 30 °C. The tests were performed in triplicate. For all quantification tests, was used as negative control, culture medium absent from inoculum.

For quantification of $CaHPO_4$ and $FePO_4$ solubilization in liquid medium, 1 mL of previously standardized bacterial culture was inoculated into 10 mL of liquid GL medium supplemented with 1.26 g L^{-1} of each phosphate source ($CaHPO_4$ and $FePO_4$). For the evaluation of the fungi, 5-mm-diameter discs with mycelial growth were removed and inoculated into penicillin flasks containing GL medium (a disc per glass). The cultures were shaken at 90 rpm at 30 °C for 72 h. After growth, the pH was measured, and the amount of inorganic P was determined by the colorimetric method for ascorbic acid determination at 725 nm, as described by Gadagi and Sa (2002).

The IAA production was determined by the colorimetric method described by Gordon and Weber (1951). For this purpose, 1 mL of each standard bacterial culture was inoculated into 9 mL of nutrient broth supplemented with 100 µL of tryptophan, while for fungi, 5-mm-diameter discs with mycelial growth were inoculated. The same incubation and growth standards applied in the qualitative test of IAA synthesis described above were used here. The IAA concentrations were obtained using the standard calibration curve equation (Pereira et al. 2012).

Antibiosis in vitro

Bacteria were tested for antagonism against the spoilage fungi *Neodeightonia phoenicum* BP91DF and *Penicillium purpurogenum* BP110DF, according to the dual culture method (Mew and Rosales 1986). These fungi were previously isolated from *B. purpurascens* seeds, where they naturally deteriorate the tissues. An initial selection was conducted in PDA medium, inoculating four bacteria per dish, 3 cm apart from the centre of the dish, where 5-mm-diameter mycelial discs of the tested pathogens were deposited. In the test with *P. purpurogenum* BP110DF, inoculation occurred 48 h earlier, and the distance between mycelium and bacteria was reduced to 2 cm due to the slow growth of this phytopathogen. A dish containing only the phytopathogen's mycelium in the central region was used as a control. The potential for antibiosis was evaluated when the mycelia of the phytopathogens reached 6 cm in diameter in the control.

The test resulted in the selection of eight bacterial isolates (seven rhizospheric and one endophytic) that showed some degree of suppression of the two spoilage fungi evaluated. Further dual culture tests were then conducted by culturing the phytopathogens and each of the selected bacteria separately. The dishes were incubated at room temperature, between 4 and 8 days, according to the growth rate of the fungus. The diameter of the fungus was measured with a calliper, and the percentage of suppression of each bacterium was calculated according to the relative inhibition (RI) rate:

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

where RC radius of the pathogen colony in the control treatment, RX radius of the pathogen colony paired with the endophytic isolate.

Experimental design and statistical analysis

The tests were conducted in a completely randomized design, always in triplicate. The data were subjected to analysis of variance, and the means of the phosphate solubilization capacity and IAA synthesis were compared by the Scott-Knott test (5%) using SISVAR software (Ferreira 2011). The functional variables analysed, namely, isolation environment (endophytic or rhizospheric), $CaHPO_4$ solubilization, pH in the $CaHPO_4$ solubilization, pH in the $FePO_4$ solubilization and IAA synthesis, were evaluated separately for bacterial and fungal isolates using a matrix of correlation and combined in a principal component analysis (PCA). The number of components was chosen according to the eigenvalues (> 1.0) and the explained variance (above 80%). The analyses were conducted using Statistica 13.3 software (StatSoft, Tulsa).

Results

Phylogenetic diversity and relationship between isolates

Most bacterial isolates identified (94%) belonged to the phylum Proteobacteria, whereas the remaining 6% are members of phylum Firmicutes (Fig. 1). Those belonging to the phylum Firmicutes were characterized as belonging to class Bacilli, order Bacillales. The proteobacteria were more diverse, with species of classes α, β and γ-Proteobacteria identified. The γ-Proteobacteria had the highest percentage of isolates (39%), with the order Enterobacteriales being the most observed in this class.

All fungal isolates obtained belong to the phylum Ascomycota, most of which belong to the class Sordariomycetes (70%), while the rest belong to the classes Eurotiomycetes (20%) and Dothideomycetes (10%) (Fig. 2). Among the Sordariomycetes, the order Hypocreales was the most numerous, accounting for 64% of the isolates, followed by Diaporthales and Microascales, each with 14%, and Chaetosphaeriales, with only 7% of the isolates. All Eurotiomycetes belong to the class Eurotiales, and the Dothideomycetes were divided between Pleosporales and Botryosphaeriales.

Multifunctionality of isolates

Analyzing the multifunctionality of the isolates, none presented the four functional traits tested, but 33 bacteria, 15 endophytes and 18 rhizospheric, had 2 functional traits ($CaHPO_4$ solubilization and IAA synthesis), while only six fungi, three endophytic and three rhizospheric presented these two traits (Fig. 3a). With respect to the functional trait synthesis of IAA, only fungi expressed this trait alone, 11 endophytic

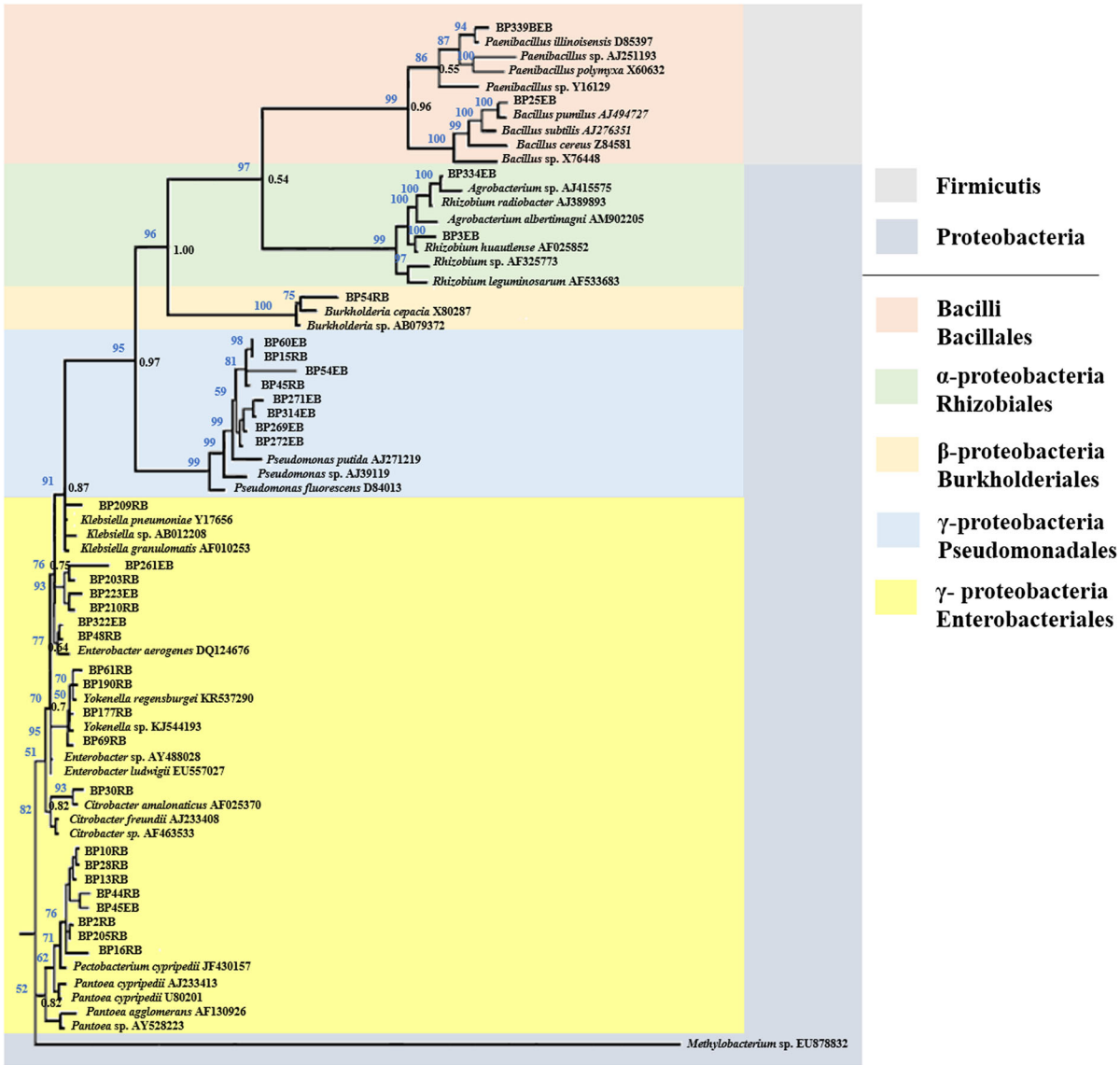

Fig. 1 Relationship and classification of root endophytic and rhizospheric bacterial isolates obtained from the *B. purpurascens* palm, endemic to the state of Goiás — Brazil. Bacterial isolates whose codes end in EB are endophytic, whereas those whose codes end in RB are rhizospheric. The outer colors of the tree represent the classification of the isolates for phyla and the internal colors represent together the class and the order of the bacterial isolates. Black values indicate the posterior probability of the nodes, and the blue values represent the bootstraps

and 4 rhizospheric, whereas the antibiosis trait was expressed only by bacteria, and these did not present any other traits tested (Fig. 3b).

Phosphate solubilization

The qualitative test for $CaHPO_4$ solubilization revealed solubilization capacity for 33 bacterial isolates. In turn, the $CaHPO_4$ solubilization quantification test showed that all bacterial isolates efficiently solubilized phosphate (Table 1), with the highest solubilization rates obtained by isolates *Pantoea* sp. BP205RB (0.37 mg L^{-1}), *Yokenella regensburgei* BP190RB (0.35 mg L^{-1}), *Pantoea cypripedii* BP28RB (0.35 mg L^{-1}), *P. cypripedii* BP44 EB (0.35 mg L^{-1}), *Y. regensburgei* BP69RB

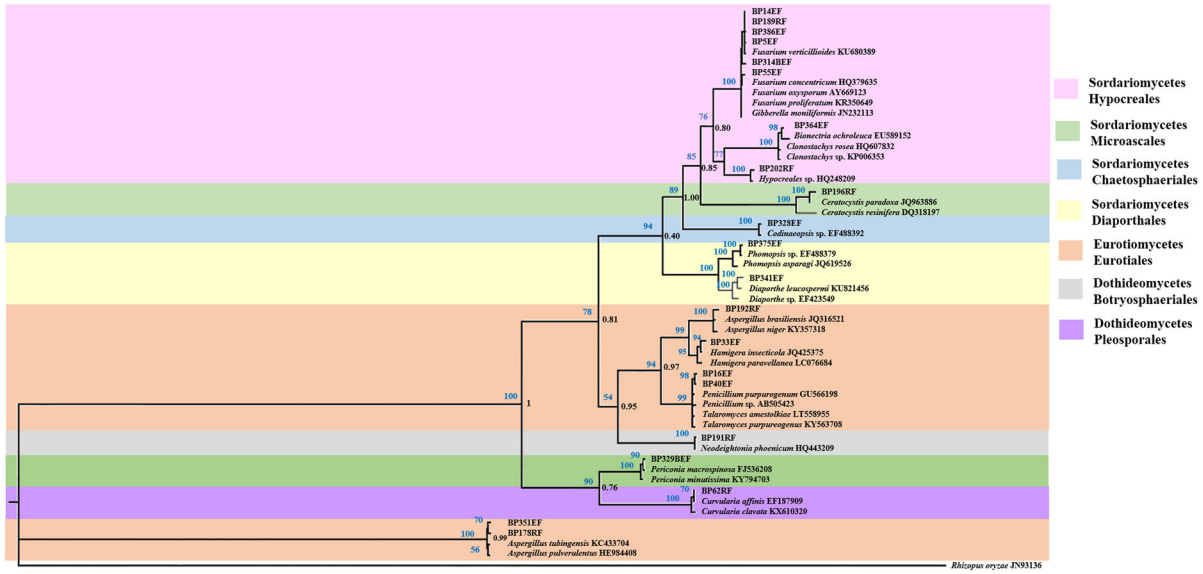

Fig. 2 Relationship and classification of root endophytic and rhizospheric fungal isolates obtained from the *B. purpurascens* palm, endemic to the state of Goiás — Brazil. Isolates whose codes end in EF are endophytic, while those whose codes end in RF are rhizospheric. The colors represent the classification of the fungal isolates together for class and order. Black values indicate the posterior probability of the nodes, and the blue values represent the bootstraps

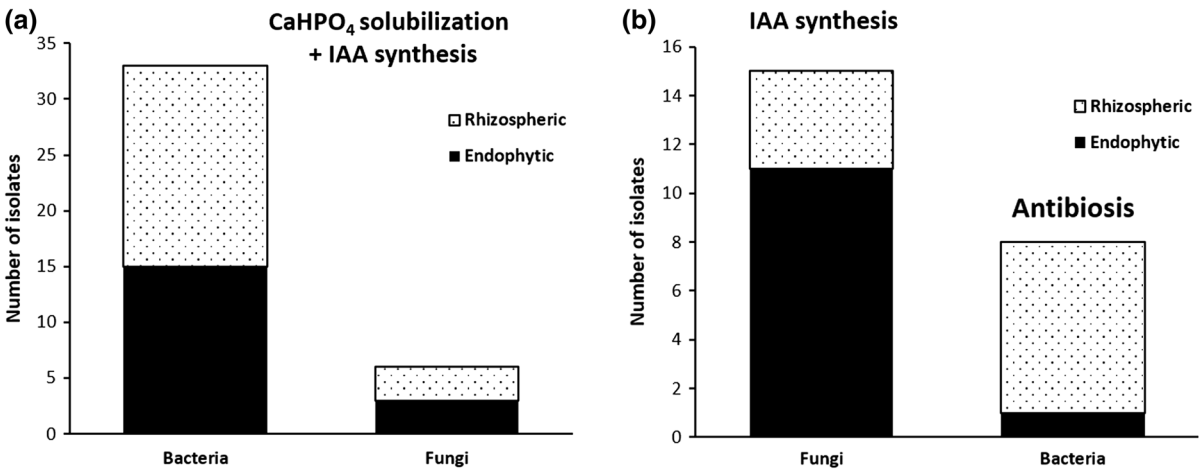

Fig. 3 Multifunctional potential of root endophytic and rhizospheric bacteria and fungi, isolates from the *B. purpurascens* palm, endemic to the state of Goiás — Brazil. **a** Number of endophytic and rhizospheric bacterial and fungal isolates that presented two of the functional traits tested. **b** Number of fungal isolates that presented only the functional trait synthesis of IAA and number of bacterial isolates that presented only the antibiosis functional trait

(0.33 mg L^{-1}), *Citrobacter amalonaticus* BP30RB (0.33 mg L^{-1}), *P. cypripedii* BP45 EB (0.33 mg L^{-1}), *P. cypripedii* BP10RB (0.33 mg L^{-1}), *Y. regensburgei* BP177RB (0.33 mg L^{-1}), *Pseudomonas* sp. BP54 EB (0.32 mg L^{-1}), *Pseudomonas putida* BP45RB (0.31 mg L^{-1}) and *Agrobacterium tumefaciens* BP324BEB (0.31 mg L^{-1}). The pH of the culture medium was acidified by 87.9% of the evaluated bacteria, while 12.1% made the culture medium more alkaline than the control; therefore, the latter showed lower $CaHPO_4$ solubilization capacity.

For $FePO_4$ solubilization, the values were zero for all of the isolates, demonstrating that the endophytic and rhizosphere bacteria of *B. purpurascens* were not

Table 1 pH values and soluble P contents in liquid GL medium, supplemented with calcium phosphate ($CaHPO_4$) or iron phosphate ($FePO_4$) and incubated with endophytic and rhizospheric bacteria of *B. purpurascens*

Isolate		Environment	$CaHPO_4$				$FePO_4$			
			Soluble P (mg L^{-1})		pH		Soluble P (mg L^{-1})		pH	
Pantoea sp.	BP2EB	Endophytic	0.22	b	5.31	a	–		3.44	a
Rhizobium huautlense	BP3EB	Endophytic	0.30	b	4.53	a	–		7.32	e
Pantoea cypripedii	BP44EB	Endophytic	0.35	a	4.38	a	–		3.82	a
Pantoea cypripedii	BP45EB	Endophytic	0.33	a	4.36	a	–		3.45	a
Pseudomonas sp.	BP54EB	Endophytic	0.32	a	4.52	a	–		7.38	e
Pseudomonas putida	BP60EB	Endophytic	0.28	b	4.56	a	–		7.26	e
Enterobacter aerogenes	BP261EB	Endophytic	0.02	d	6.84	c	–		7.42	e
Pseudomonas plecoglossicida	BP269EB	Endophytic	0.29	b	4.50	a	–		7.17	e
Pseudomonas putida	BP271EB	Endophytic	0.29	b	4.64	a	–		7.44	e
Pseudomonas putida	BP272EB	Endophytic	0.28	b	4.21	a	–		7.62	e
Pseudomonas putida	BP314EB	Endophytic	0.29	b	4.24	a	–		7.20	e
Enterobacter sp.	BP322EB	Endophytic	0.03	d	6.68	c	–		7.74	e
Enterobacter ludwigi	BP323EB	Endophytic	0.01	d	6.92	c	–		7.73	e
Agrobacterium tumefaciens	BP324EB	Endophytic	0.31	a	4.39	a	–		4.52	b
Paenibacillus illinoisensis	BP339EB	Endophytic	0.05	d	6.57	c	–		4.59	b
Pantoea cypripedii	BP10RB	Rhizospheric	0.33	a	4.40	a	–		3.48	a
Pectobacterium cypripedii	BP13RB	Rhizospheric	0.30	b	4.25	a	–		3.53	a
Pseudomonas putida	BP15RB	Rhizospheric	0.30	b	4.50	a	–		6.35	d
Pantoea sp.	BP16RB	Rhizospheric	0.30	b	4.30	a	–		3.53	a
Bacillus pumilus	BP25RB	Rhizospheric	0.26	b	5.02	a	–		4.87	b
Pantoea cypripedii	BP28RB	Rhizospheric	0.35	a	4.40	a	–		3.54	a
Citrobacter amalonaticus	BP30RB	Rhizospheric	0.33	a	4.40	a	–		4.51	b
Pseudomonas putida	BP45RB	Rhizospheric	0.31	a	4.51	a	–		7.46	e
Enterobacter sp.	BP48RB	Rhizospheric	0.03	d	6.68	c	–		7.52	e
Burkholderia sp.	BP54RB	Rhizospheric	0.02	d	6.56	c	–		3.22	a
Yokenella regensburgei	BP61RB	Rhizospheric	0.25	b	4.56	a	–		4.56	b
Yokenella regensburgei	BP69RB	Rhizospheric	0.33	a	4.56	a	–		4.54	b
Yokenella regensburgei	BP177RB	Rhizospheric	0.33	a	4.56	a	–		4.92	b
Yokenella regensburgei	BP190RB	Rhizospheric	0.35	a	4.58	a	–		4.63	b
Enterobacter asburiae	BP203RB	Rhizospheric	0.02	d	7.08	c	–		7.27	e
Pantoea sp.	BP205RB	Rhizospheric	0.37	a	4.48	a	–		3.41	a
Klebsiella pneumoniae	BP209RB	Rhizospheric	0.15	c	6.03	b	–		5.68	c
Enterobacter ludwigii	BP210RB	Rhizospheric	0.01	d	7.04	c	–		7.32	e
Negative control			–		6.77	c	–		5.83	c

Means followed by the same letter, in the column, do not differ among themselves by the Scott-Knott test (5%). – not detected

able to solubilize this phosphate source under in vitro conditions. However, 54.6% of the isolates acidified the culture medium, while 45.5% made the culture medium more alkaline (Table 1).

Regarding the fungal isolates evaluated, only 33.3% were able to solubilize $CaHPO_4$, with greater efficiency for the isolates *Neodeightonia phoenicum* BP191RF, *Hypocreales* sp. BP202RF and *P. purpurogenum* BP16EF (2.47, 2.47 and 1.70 mg L^{-1},

respectively). For $FePO_4$ solubilization, no solubilization effect was observed under in vitro conditions; however, all isolates reduced the pH of the media relative to the control without inoculation (Table 2).

IAA production

All 33 bacterial strains tested were able to synthesize IAA in the presence of tryptophan as the precursor. The mean value of synthesis was 44.46 μg mL^{-1}, and these values were significantly higher for the isolates *Enterobacter* sp. BP322 EB, *Enterobacter ludwigi* BP323 EB, *Enterobacter asburiae* BP203RB and *Enterobacter* sp. BP48RB (108.8, 106.2, 102.1 and 96.7 μg mL^{-1}, respectively) (Table 3).

All of the fungal isolates evaluated also showed potential for IAA synthesis, particularly *Fusarium concentricum* BP55EF (12.5 μg mL^{-1}) and *Fusarium proliferatum* BP314BEF (3.9 μg mL^{-1}), since the other isolates produced IAA levels below 3.1 μg mL^{-1} (Table 4).

Antibiosis in vitro

In the antibiosis test, seven rhizospheric and one endophytic bacteria demonstrated inhibitory potential against the tested phytopathogens. For the fungus *N. phoenicum* BP91DF, higher relative inhibition rates were observed for the rhizospheric bacteria *Bacillus subtilis* BP186RB (63%) and *Bacillus amyloliquefaciens* BP1RB (57%), while for *P. purpurogenum* BP110DF, the isolates that led to higher relative inhibition were *B. amyloliquefaciens* BP60RB (44%), *B. subtilis* BP186RB (42%), *B. amyloliquefaciens* BP70RB (40%), *B. amyloliquefaciens* BP66RB (38%), *B. amyloliquefaciens* BP1RB (38%) and *B.*

Table 2 pH values and soluble P contents in liquid GL medium, supplemented with calcium phosphate ($CaHPO_4$) or iron phosphate ($FePO_4$) and incubated with endophytic and rhizospheric fungi of *B. purpurascens*

Isolate		Environment	$CaHPO_4$				$FePO_4$		
			Soluble P (mg L^{-1})		pH		Soluble P (mg L^{-1})	pH	
Gibberella moniliformis	BP5EF	Endophytic	–		5.25	c	–	5.10	e
Fusarium oxysporum	BP14EF	Endophytic	–		5.23	c	–	5.15	e
Penicillium purpurogenum	BP16EF	Endophytic	1.70	a	5.52	c	–	4.78	d
Hamigera insecticol	BP33EF	Endophytic	–		6.11	d	–	3.87	c
Talaromyces amestolkiae	BP40EF	Endophytic	–		5.14	c	–	5.21	e
Fusarium concentricum	BP55EF	Endophytic	–		4.80	b	–	4.43	d
Fusarium proliferatum	BP314EF	Endophytic	–		5.31	c	–	4.93	e
Periconia macrospinosa	BP329EF	Endophytic	–		6.20	d	–	5.82	e
Codinaeopsis sp.	BP328EF	Endophytic	–		4.38	b	–	4.64	d
Diaporthe sp.	BP341EF	Endophytic	0.25	b	4.95	c	–	4.07	c
Aspergillus tubingensis	BP351EF	Endophytic	–		4.57	b	–	4.53	d
Bionectria ochroleuca	BP364EF	Endophytic	0.82	b	3.85	b	–	3.19	b
Phomopsis sp.	BP375EF	Endophytic	–		5.72	c	–	5.15	e
Gibberella moniliformis	BP386EF	Endophytic	–		5.44	c	–	5.09	e
Curvularia affinis	BP62RF	Rhizospheric	–		5.58	c	–	4.79	d
Aspergillus tubingensis	BP178RF	Rhizospheric	–		5.22	c	–	4.62	d
Fusarium concentricum	BP189RF	Rhizospheric	0.96	b	5.62	c	–	5.00	e
Neodeightonia phoenicum	BP191RF	Rhizospheric	2.47	a	4.30	b	–	4.34	d
Aspergillus brasiliensis	BP192RF	Rhizospheric	–		2.93	a	–	2.29	a
Ceratocystis paradoxa	BP196RF	Rhizospheric	–		4.64	b	–	4.38	d
Hypocreales sp.	BP202RF	Rhizospheric	2.47	a	4.23	b	–	4.00	c
Negative control			–		6.56	d	–	5.85	e

Means followed by the same letter in the column do not differ by Scott - Knott test (5%). – not detected

Table 3 *In vitro* production of indoleacetic acid (IAA) by endophytic and rhizospheric bacteria of *B. purpurascens*

Bacterial isolates		Environment	IAA (μg mL^{-1})	
Pantoea sp.	BP2EB	Endophytic	31.92	c
Rhizobium huautlense	BP3EB	Endophytic	47.68	b
Pantoea cypripedii	BP44EB	Endophytic	42.98	b
Pantoea cypripedii	BP45EB	Endophytic	53.58	b
Pseudomonas sp.	BP54EB	Endophytic	28.55	c
Pseudomonas putida	BP60EB	Endophytic	36.30	c
Enterobacter aerogenes	BP261EB	Endophytic	22.47	d
Pseudomonas plecoglossicida	BP269EB	Endophytic	32.39	c
Pseudomonas putida	BP271EB	Endophytic	17.98	d
Pseudomonas putida	BP272EB	Endophytic	21.21	d
Pseudomonas putida	BP314EB	Endophytic	23.71	d
Enterobacter sp.	BP322EB	Endophytic	108.80	a
Enterobacter ludwigi	BP323EB	Endophytic	106.20	a
Agrobacterium tumefaciens	BP324EB	Endophytic	55.53	b
Paenibacillus illinoisensis	BP339EB	Endophytic	66.12	b
Pantoea cypripedii	BP10RB	Rhizospheric	34.79	c
Pectobacterium cypripedii	BP13RB	Rhizospheric	24.11	d
Pseudomonas putida	BP15RB	Rhizospheric	21.51	d
Pantoea sp.	BP16RB	Rhizospheric	31.92	c
Bacillus pumilus	BP25RB	Rhizospheric	37.70	c
Pantoea cypripedii	BP28RB	Rhizospheric	30.28	c
Citrobacter amalonaticus	BP30RB	Rhizospheric	56.72	b
Pseudomonas putida	BP45RB	Rhizospheric	33.63	c
Enterobacter sp.	BP48RB	Rhizospheric	96,70	a
Burkholderia sp.	BP54RB	Rhizospheric	17.21	d
Yokenella regensburgei	BP61RB	Rhizospheric	46.80	b
Yokenella regensburgei	BP69RB	Rhizospheric	25.42	d
Yokenella regensburgei	BP177RB	Rhizospheric	39.29	c
Yokenella regensburgei	BP190RB	Rhizospheric	25.61	d
Enterobacter asburiae	BP203RB	Rhizospheric	102.13	a
Pantoea sp.	BP205RB	Rhizospheric	38.31	c
Klebsiella pneumoniae	BP209RB	Rhizospheric	51.74	b
Enterobacter ludwigii	BP210RB	Rhizospheric	58.06	b
Negative control			ND	

Means followed by the same letter in the column do not differ by Scott-Knott test (5%)

ND = not detected

amyloliquefaciens BP201RB (36%) (Fig. 4). The rhizospheric isolates *B. amyloliquefaciens* BP1RB and *B. subtilis* BP186RB demonstrated greater capacity to suppress the growth of the two spoilage fungi tested.

PCA revealed a cluster of bacterial isolates 1, 3 and 4 based on the functional traits evaluated (Fig. 5a). These isolates were identified as belonging to the genus *Pantoea*. Other isolates of this genus (16, 19, 21 and 31) were also grouped together. Likewise, *Enterobacter* (7, 12 and 13), *Pseudomonas* (5, 6, 8, 9, 10 and 11) and *Yokenella* (26, 27, 28 and 29) isolates tended to cluster together. The soluble P content, released during $CaHPO_4$ solubilization, was the most significant variable for the scores; that is, it contributed the most to explain the variance between the data, followed by the variable pH-$CaHPO_4$ for PC1 and the variable Environment for PC2. The variable P-$CaHPO_4$ was plotted in the opposite direction to the other variables; therefore, it was weakly correlated to

Table 4 *In vitro* production of indoleacetic acid (IAA) by endophytic and rhizospheric fungi of *B. purpurascens*

Fungus isolates		Environment	IAA (μg mL^{-1})	
Gibberella moniliformis	BP5EF	Endophytic	2.52	d
Fusarium oxysporum	BP14EF	Endophytic	2.21	d
Penicillium purpurogenum	BP16EF	Endophytic	3.09	c
Hamigera insecticol	BP33EF	Endophytic	1.02	e
Talaromyces amestolkiae	BP40EF	Endophytic	1.05	e
Fusarium concentricum	BP55EF	Endophytic	12.49	a
Fusarium proliferatum	BP314EF	Endophytic	3.90	b
Codinaeopsis sp.	BP328EF	Endophytic	1.12	e
Periconia macrospinosa	BP329EF	Endophytic	1.32	e
Diaporthe sp.	BP341EF	Endophytic	1.27	e
Aspergillus tubingensis	BP351EF	Endophytic	0.73	f
Bionectria ochroleuca	BP364EF	Endophytic	0.89	e
Phomopsis sp.	BP375EF	Endophytic	1.71	e
Gibberella moniliformis	BP386EF	Endophytic	2.50	d
Curvularia affinis	BP62RF	Rhizospheric	1.00	e
Aspergillus tubingensis	BP178RF	Rhizospheric	1.00	e
Fusarium concentricum	BP189RF	Rhizospheric	1.86	d
Neodeightonia phoenicum	BP191RF	Rhizospheric	1.34	e
Aspergillus brasiliensis	BP192RF	Rhizospheric	0.58	f
Ceratocystis paradoxa	BP196RF	Rhizospheric	0.46	f
Hypocreales sp.	BP202RF	Rhizospheric	1.87	d
Negative control			ND	

Means followed by the same letter in the column do not differ by Scott-Knott test (5%)

ND not detected

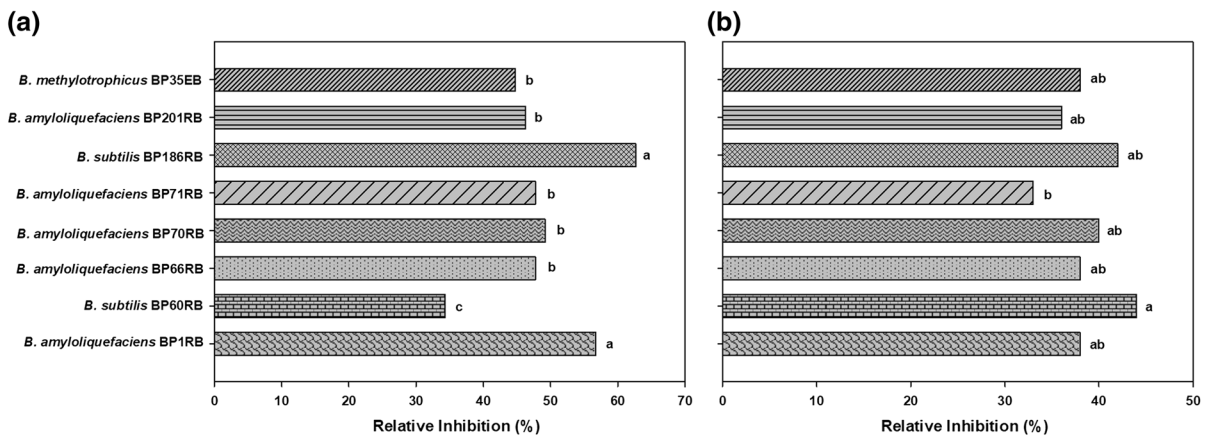

Fig. 4 Relative inhibition of mycelial growth (RI%) of the phytopathogens *Neodeightonia phoenicum* BP91DF (**a**) and *Penicillium purpurogenum* BP110DF (**b**) by endophytic and rhizospheric bacteria of *B. purpurascens* in Rio Verde, GO

them, particularly in relation to the pH-$CaHPO_4$ variable, which corresponds to the pH values during $CaHPO_4$ solubilization. This finding indicates a negative correlation between $CaHPO_4$ and pH-$CaHPO_4$; that is, the higher the soluble P content, the lower the pH values identified.

Fungal clusters were not as representative of genera as bacterial clusters, and the *Gibberella* isolates (1 and 14) were the only ones presenting similar behaviours (Fig. 6a). *Aspergillus brasiliensis* isolate 19 presented the most disparate behaviour among the isolates, because the fungus greatly reduced the pH of the

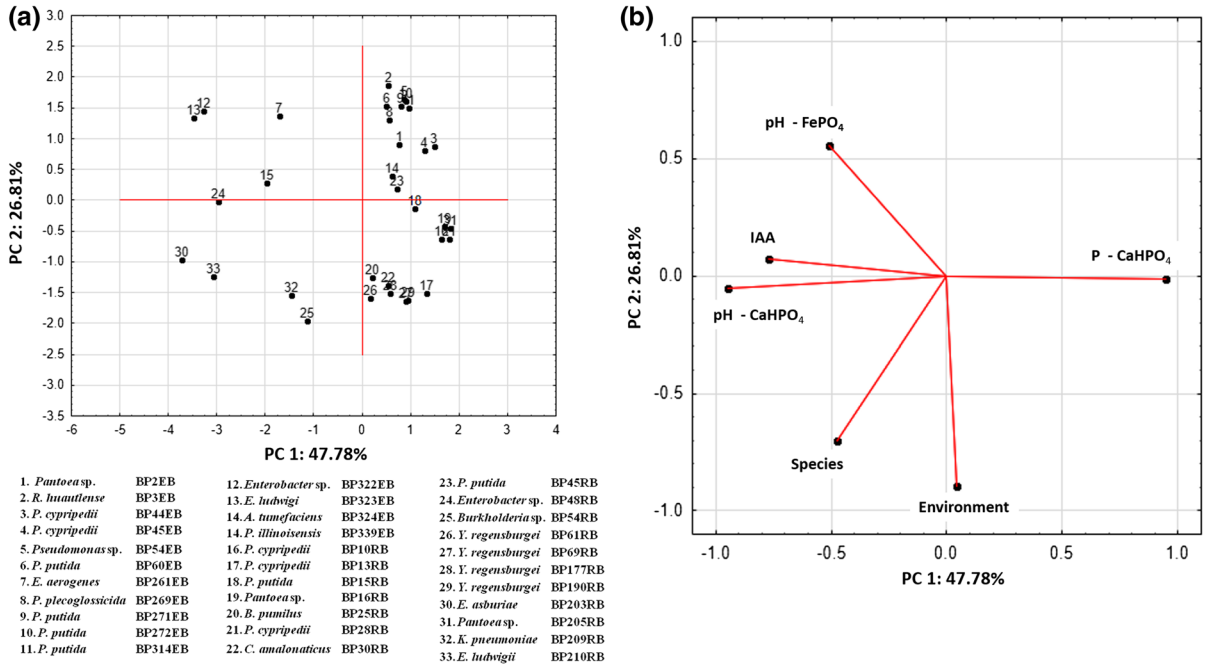

Fig. 5 Projection plane of principal component analysis of root endophytic and rhizospheric bacterial isolates of *B. purpurascens* (**a**) and different growth promotion variables (**b**) as a function of the two principal components (PC1 and PC2) in Rio Verde, GO

culture medium but could not solubilize $CaHPO_4$ or solubilized $FePO_4$ at a very low percentage. The pH rates were highly correlated (r = 0.78), indicating similar behaviours of the analysed fungi during $CaHPO_4$ and $FePO_4$ solubilization (Fig. 6b).

Discussion

The phylum Proteobacteria was the best represented among the isolates. In fact, many proteobacteria have been described as plant-growth-promoting rhizobacteria (PGPR). Bruto et al. (2014) evaluated the co-occurrence of growth-promoting genes in 25 strains of this group and concluded that plant-bacteria symbiosis may have been established separately in several taxa of this phylum, producing PGPR strains using different gene assortments. The authors also concluded that the accumulation of genes and possibly of different beneficial characteristics for plants may be an intrinsic characteristic of PGPR. The α and β-Proteobacteria isolated are classically related to symbiotic nitrogen fixation (Udvardi and Poole 2013; Meyer et al. 2016; Estrada de los Santos et al. 2016), indicating that they may play an important role in the N uptake pathway of *B. purpurascens*.

Fungi of the class sordariomycetes, order Hypocreales, were the most represented among the tested isolates. This order includes many known entomopathogenic species, described as endophytic in various plant species (Guesmi-Jouini et al. 2014; Russo et al. 2015; Ghobad-Nejhad et al. 2018). The presence of these fungi associated with plant tissues has been potentially studied as an alternative for the biological control of pests (Kepler et al. 2017). It is possible that the presence of these fungi in the roots of *B. purpurascens* is an important strategy for the control of root phytopathogens in this species. Among the endophytic isolates of this order, a large number were identified as belonging to the genus *Fusarium*, but *Clonostachys* was also found. These results corroborate those of Mahmoud et al. (2017), who analysed the diversity of endophytic fungi associated with the roots of the *Phoenix dactylifera* palm from three coastal sites in Southeast Spain and identified these two genera as the most frequent in both the roots and the sampling areas.

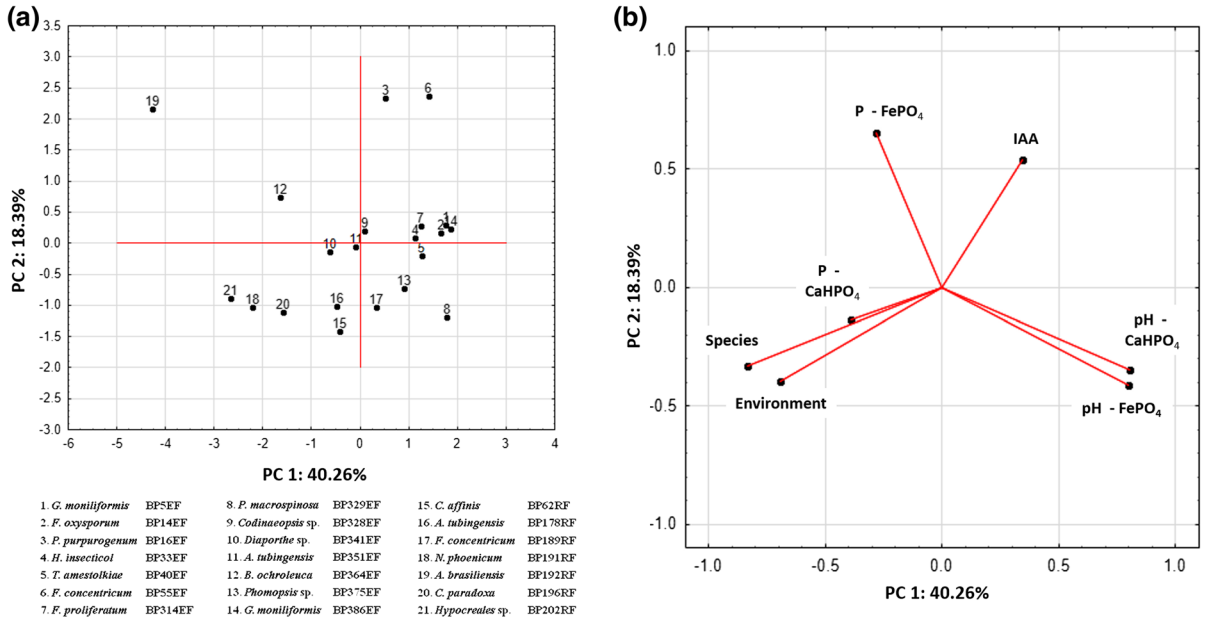

Fig. 6 Projection plane of principal component analysis of root endophytic and rhizospheric fungal isolates of *B. purpurascens* (**a**) and different growth promotion variables (**b**) as a function of the two principal components (PC1 and PC2) in Rio Verde, GO

In the present work, there was a relationship between rhizospheric and endophytic isolates of the same species, both for bacteria and for fungi (Figs. 5, 6). Therefore, it is assumed that many root endophytes of *B. purpurascens* may have originated from the rhizosphere, i.e., rhizospheric bacteria that colonized root tissues of this species, establishing an endophytic relationship.

A total of 87.9% of the evaluated bacteria acidified the culture medium used in the $CaHPO_4$ solubilization test. These results indicate that the production of organic acids can be one of the mechanisms used by these bacteria to solubilize $CaHPO_4$; this hypothesis is also suggested by the PCA, which shows opposite behaviours of the variables $CaHPO_4$ solubilization and pH during $CaHPO_4$ solubilization. The organic acids involved in the solubilization of inorganic phosphates include glutamic, glycolic, gluconic, citric, oxalic and succinic acids, which reduce the pH and release protons to the soil solution (Osorio Vega 2007; Barroso and Nahas 2008). Factors such as the concentration and type of acid synthesized by the strain may potentiate the solubilization of phosphates (Marra et al. 2012).

The fungal isolates reached higher values of phosphate solubilization than the bacterial isolates; however, a greater number of bacteria expressed this functional trait. In plant growth promotion tests, P-solubilizing fungi have been preferred by researchers in general (Osorio Vega 2007; Verma et al. 2010), as many bacterial strains may lose their capacity after several cycles of culture in vitro.

The highest values of $CaHPO_4$ solubilization by bacteria were of the order of 0.37 mg L^{-1}, attributed to the activity of *Pantoea* sp. BP205RB. However, these values are lower than those reported in other studies, such as Zhao et al. (2014), which found solubilization rates ranging from 58.2 to 452.2 µg mL^{-1} of soluble P, using bacteria (*Agrobacterium, Burkholderia, Mesorhizobium, Pseudomonas, Rhizobium, Streptomyces* and *Bacillus*) isolated from maize rhizosphere. Some of these genera have been described in the present work, such as *Agrobacterium*, *Burkholderia*, *Pseudomonas* and *Bacillus*, but these genera have not demonstrated potential for exploration of the phosphate solubilization functional trait, indicating that this function may be secondarily expressed by the microorganisms associated with *B. purpurascens.*

In the present work, some bacterial isolates stood out regarding IAA synthesis, obtaining rates of 108.8, 106.2, 102.1 and 96.7 µg $mL^{-}1$. These isolates

belong to the genus *Enterobacter* sp., and the rates are similar or even higher than those found in other studies evaluating root endophytic and/or rhizospheric bacteria. For example, Kavamura et al. (2013) worked with cacti rhizobacteria from the Brazilian Caatinga and found *Pantoea* sp. and *Arthrobacter* sp. strains producing IAA contents similar to those found in this study (113.6 and 135.2 μg mL^{-1}, respectively). In turn, Goswami et al. (2014) observed IAA production levels of about 25 μg mL−1, below the levels observed in this study, by bacteria isolated from the rhizosphere of *Suaeda fruticosa.* The same was observed by Palaniyandi et al. (2013), who evaluated the yam rhizosphere actinomycetes and found that 96% of isolates had very low IAA production levels of up to 6.7 μg $mL^{-1.}$ These results indicate that IAA synthesis is an important functional role performed by the symbiotic microbiota on the *B. purpurascens* roots.

The production of IAA by microorganisms has often been evaluated because this phytohormone allows the maximization of root development and, consequently, the absorption of more significant amounts of nutrients (Goswami et al. 2014). The genes responsible for auxin biosynthesis are located on the chromosome or a plasmid. When located in the chromosomal DNA, they result in lower IAA production, but if present in the plasmid, in multiple copies, they promote greater biosynthesis (Spaepen and Vanderleyden 2011).

Plant exudates such as L-tryptophan stimulate IAA synthesis by microbial strains that colonize plants (Idris et al. 2007; Karnwal and Dohroo 2018). For the production of IAA, growth-promoting microorganisms use tryptophan-independent and tryptophan-dependent pathways. There are four main dependent pathways, including indole-3-pyruvate acid (IPyA), tryptamine (TAM), indole-3-acetonitrile (IAN) and indole-3-acetamide (IAM), along with a tryptophan-independent pathway (Kochar et al. 2011).

Symbiotic microorganisms can also contribute to plant health through the induction of systemic resistance (Pieterse et al. 2014; Misha et al. 2018) and the production of substances with antimicrobial activity, such as lipopeptides of the iturin, surfacin and fengicin families (Chen et al. 2016; Soares et al. 2016). These substances represent important mechanisms by which selected bacteria and fungi can stimulate increased plant defences against a wide range of pathogens.

The genus *Bacillus* has been successfully used to control various plant pathogens (Hinarejos et al. 2016; Lozano et al. 2016; Chen et al. 2016; Gotor-Vila et al. 2017). In this study, strains of *B. subtilis*, *B. amyloliquefaciens* and *B. methylotrophicus*, along with endophytic and rhizospheric strains of *B. purpurascens,* were tested and demonstrated potential for inhibition of the fungus *N. phoenicum*, which causes severe palm rot (Ligoxigakis et al. 2013), and *P. purpurogenum*, a recognized fruit- and seed-spoiling fungus (De Silva 2016; Esua et al. 2017; Elshahawy et al. 2017). *B. subtilis* also inhibited *P. purpurogenum* in vitro. According to Alsohiby et al. (2016), the use of bacteria of this genus may potentially suppress the proliferation of fruit- and seed-spoiling fungi or phytopathogens of *B. purpurascens*. This function expressed by the endophytic bacteria in question may contribute to increased fitness of *B. purpurascens* in the environment in which it occurs.

Some of the clusters established for the functional traits in PCA, such as the bacterial genera *Pantoea*, *Enterobacter*, *Pseudomonas* and *Yokenella*, and the fungal genus *Gibberella*, directly reflect the phylogenetic relationship between the isolates. This can be explained by the natural tendency of characteristics to be shared by species that have a recent common ancestry (Kraft et al. 2007). The genetic conservatism of some traits between microbial groups has been reported in several papers (e.g., Martiny et al. 2013, 2015), although this topic needs to be better discussed within microbiology.

This work is the first bioprospecting study of endophytic and rhizospheric microorganisms of *B. purpurascens* with functional traits related to plant growth promotion, and by the multifunctionality observed, we indicate *B. purpurascens* as a good source for these microorganisms. However, the functional characteristics observed in vitro not necessarily are expressed in the field, therefore, the effectiveness of the microbial species described here must be tested. The goal is to promote the growth and development of species of commercial and ecological interest and the protection of *B. purpurascens* seeds, allowing the healthy propagation of this species, which presents reduced populations and restricted distribution.

Conclusions

Endophytic and rhizospheric microorganisms of *B. purpurascens* present multifunctions related to plant growth promotion, making this species be characterized as a source for microorganisms with traits for solubilization of $CaHPO_4$, IAA synthesis and antibiosis to phytopathogens.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This article does not contain any studies with human participants and/or animals performed by any of the authors. The formal consent is not required in this study.

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