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Isolation and genetic characterization of endophytic and rhizospheric microorganisms from *Butia purpurascens* Glassman

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Interactions between plants and microorganism are complex and can affect the establishment of plant communities and change ecosystem properties. These interactions are of interest for researchers and have many ecological and biotechnological applications. The present study is the first report on the microbial diversity associated with *Butia purpurascens* Glassman, and the goal was to isolate and identify the genetic diversity of endophytic and rhizospheric growable microorganisms from *B. purpurascens* using molecular biology techniques. Endophytic and rhizospheric microorganisms were isolated from the roots and rhizospheric soil of the purple yatay palm. DNA was extracted, and the 16S region for bacteria and internal transcribed spacer (ITS) region for fungi were amplified and sequenced. The resulting sequences were compared with known sequences from GenBank by similarity search using BLASTn. The rhizosphere and roots of *B. purpurascens* harbor a diverse set of microorganism groups. Fourteen (14) genera of endophytic and 12 genera of rhizospheric bacteria were identified that belonged to three phyla (Proteobacteria, Firmicutes and Actinobacteria), and 11 endophytic and six rhizospheric genera of fungi were identified that belonged to the phylum Ascomycota. The most frequent isolated genera were *Enterobacter* and *Pseudomonas* for endophytic bacteria, *Gibberella* and *Codinaeopsis* for endophytic fungi, *Bacillus* and *Enterobacter* for rhizospheric bacteria, and *Ceratocystis* for rhizospheric fungi. Differences were observed between the endophytic and rhizospheric microbial communities of *B. purpurascens,* with some microorganisms only detected in one environment. Further studies with a higher number of individuals are required to confirm these results.

Key words: Purple yatay palm, genetic diversity, molecular biology, microbial ecology.

INTRODUCTION

The purple yatay palm (*Butia purpurascens* Glassman) is a native species to the Brazilian Cerrado. It is restricted to southwest regions of the state of Goiás (especially in the region of Jataí), Minas Gerais Triangle, state of Mato Grosso do Sul and in neighboring regions of the state of Goiás. This species presents pronouncedly arched leaves,

is used as an ornamental plant, and has a great potential for use in landscaping and urban afforestation in tropical regions (Lorenzi et al., 2010; Guilherme and Oliveira, 2011).

The absence of sustainable management practices has contributed to the extinction of this species because of the use of its leaves in the manufacturing of broom (Oliveira, 2010). In addition, its seeds have slow and heterogeneous germination and low germination percentages because of the physical dormancy imposed by the seed-coat, which is considerably lignified (Lorenzi et al., 2010; Rubio Neto, 2010; Rubio Neto et al., 2012).

Novel uses of microorganisms may benefit integrations between plants and microorganisms in natural and agricultural ecosystems (Philippot et al., 2013). Plant growth promotion by microorganisms is commonly used to improve crop productivity, and microorganisms have a great potential for remediation of environmental problems, such as in phytoremediation for decontamination of soils and water (Bashan et al., 2012).

Microorganisms are essential components of ecosystems that participate in 80 to 90% of soil processes, such as the carbon and nitrogen biogeochemical cycles, and are efficient and dynamic indicators of soil quality (Bresolin et al., 2010). Different types of soils and plants possess specific microbial communities, and each plant species is colonized by a different microbial population that is mostly controlled by edaphic variables, especially pH (Berg and Smalla, 2009).

The rhizosphere is the narrow region of soil surrounding the roots. This microenvironment is inhabited by countless microorganisms and invertebrates and is considered one of the most dynamic interfaces on the earth (Philippot et al., 2013). The rhizosphere can vary according to factors related to soil, age, plant species, etc. (Campbell and Greaves, 1990). Complex biological and ecological processes occur through the release of exudates by the plant in which the microbiota can have a direct influence on the plant's growth, development, nutrition and health (Bais et al., 2006; Philippot et al., 2013).

The composition of root exudates varies according to the plant species and affects microbial diversity because it can supply nutrients to the microorganisms (in certain plant species) and may contain unique antimicrobial metabolites (Berg and Smalla, 2009). The released compounds are organic acids, amino acids, proteins, sugars, phenols, and other secondary metabolites that are readily used by microorganisms (McNear Jr., 2013).

Plant growth promoting rhizobacteria (PGPR) are commonly found in the rhizosphere and can also be isolated from plant tissues (endophytic bacteria). PGOR can be used as inoculants for plant growth promotion through the production of plant hormones or their precursors, and they can affect both the production and degradation of more than one group of hormones, such as abscisic acid, auxins, cytokinins, ethylene, gibberellin, jasmonic acid, and salicylic acid, and minimize agricultural environmental impacts (Dodd et al., 2010).

Endophytic microorganisms colonize plant tissues without damaging the host (Reinhold-Hurek and Hurek, 2011). Their symbiotic life strategy can vary between facultative saprophyte, parasitism, and mutualism (Schulz and Boyle, 2005). The benefits depend on the balance between the microorganism and plant response. If the interaction becomes unbalanced, symptoms of disease can appear or the microorganism may be excluded through induced defense reactions by the host plant (Kogel et al., 2006).

Plant colonization by endophytes may occur through natural openings and wounds, such as fissures (especially at the root region) that are caused by lateral root emergence or abrasions resulting from root growth and soil penetration, or through stomata, hydathodes, openings caused by insects, and pathogenic fungi structures (Santos and Varavalho, 2011).

Independent of the microorganisms' location on the plant, they can promote plant growth through several mechanisms that are important to the host plant (Dodd et al., 2010). In endophytic interactions, such mechanisms include the supply of nutrients to the host plant, a decrease in abiotic stress, competition between microbes (Schulz and Boyle, 2005), changes to plant physiology, and production of phytohormones. The latter mechanism is potentially useful for agriculture and industry, especially for pharmaceutical and pesticide production (Santos and Varavallo, 2011).

In addition, microorganisms can potentially substitute chemicals and contribute to environmental sustainability and soil productivity, thus providing strategic alternatives in agricultural and ecological systems because of their biotechnological value (Esitken et al., 2010; Santos and Varavallo, 2011).

Determining the ecological and functional aspects of microbial communities is of great importance because the development of molecular biology techniques has resulted in a considerable increase in information on their diversity, structure and functionality. These new techniques are able to analyze the high complexity of microbial communities, especially because a minority of microbial groups cannot be grown *in vitro* (Bresolin et al., 2010).

The goal of the present study was to isolate and identify the genetic diversity of endophytic and rhizospheric microorganisms from *B. purpurascens* using molecular biology techniques.

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MATERIALS AND METHODS

Butia purpurascens root collection, processing, and microorganism isolation

The roots and rhizosphere soil of an individual *B. purpurascens* (average 10 years old) were collected close to the Rio Verde municipality in the state of Goiás (GO) at the following geographic coordinates: latitude 17° 35' 10.51" S, longitude 50° 59' 13.06" W and 822 m altitude. The chosen plant did not present apparent symptoms of disease. The roots and rhizosphere soil were collected (0-15 cm depth) using a mattock, placed in a plastic receptacle, labeled, and transported to the "Laboratório de Microbiologia Agrícola (IF Goiano – Câmpus Rio Verde)".

For isolation of rhizospheric microorganisms, 10 g of the root fragments was chosen randomly and stirred in sterile peptone water containing 0.1% Tween 80 (Polysorbate 80) for 30 min at 70 rpm at room temperature. A dilution series of the supernatant was prepared in saline solution, and 50 µL aliquots of each dilution were cultured in approximately 20 mL GELP growth medium according to the pour plate technique (Sylvester-Bradley et al., 1982). The plates were incubated at room temperature.

The growth of microorganisms and appearance of $CaHPO_4$ solubilization halos were evaluated following four days of growth. The total and phosphate solubilizing colony forming units (CFU) were quantified, and the necessary dilution corrections were performed to express the results in CFU/g root. $CaHPO_4$ solubilizing colonies were isolated for purification and stored in cold temperatures.

For endophyte isolation, the root fragments were washed in running water to remove the adhering soil and stirred in 1% neutral detergent at 70 rpm for 10 min to decrease the number of epiphytic microorganisms. The fragments were surface sterilized in 70% ethanol solution for 1 min, sodium hypochlorite solution (2.5 active chlorine) for 5 min, and 70% ethanol solution for 30 sand then washed three times in distilled and autoclaved water to remove the excess chemicals. A 500 µL aliquot of water from the last washing was used for evaluation of the sterilization.

Sterilized fragments were cut in approximately 1 cm long fragments and placed in Petri dishes containing potato dextrose agar medium (PDA). The growth of endophytic microorganisms was monitored for 10 days, and the colonization frequency was quantified based on the percentage of fragments with at least one endophyte relative to the total analyzed fragments.

$$\text{Colonization frequency} = \frac{\text{Number of fragments}}{\text{Total fragments}} \times 100$$

Purification and storage of microorganism lines

The bacterial isolates were purified using microbial culture streaking in nutrient agar medium (NA). Purification was confirmed through a Gram staining test. The isolated colonies were cultured in penicillin jars containing NA and stored in cold temperatures. The lines were reactivated every 45 days and stored again.

Sporulating fungi were isolated through monospore purification, and fungi without reproductive structure differentiation were isolated through the removal of fragments from the outer edge of the mycelium; these fragments were placed in penicillin jars containing PDA medium under cold temperatures, and new cultures were established every 60 days.

A replicate of each isolate was stored in an ultrafreezer at an average of -80°C for long-term preservation of the cultures. The bacteria were stored in 80% glycerol (v/v), and fungi were stored in 30% glycerol (v/v), thus establishing a microbial germplasm bank for this tree species.

Molecular identification

Molecular biology methods were used to identify bacterial and fungal isolates and genetic sequencing was performed to determine the genus as well as the species when possible. Extraction, amplification and purification of the microbial DNA were performed at the "Laboratório de Biotecnologia e Ecologia Microbiana do Instituto de Biociências da Universidade Federal de Mato Grosso". DNA samples of each isolate were sent to the "Laboratório Multiusuário Centralizado para Sequenciamento de DNA em Larga Escala e Análise de Expressão Gênica da UNESP" at Jaboticabal for sequencing.

Bacteria

The extraction of total genome DNA from purified bacteria was performed according to Cheng and Jiang (2006), and the evaluation of bacterial genetic diversity was performed by enterobacterial repetitive intergenic consensus (ERIC)-PCR using the oligonucleotides ERIC1 (5'-ATGTAAGCTCCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAA GTGACTGGGGTGAGCG-3') (Tian-Xing et al., 2011). ERIC-PCR was conducted in a final volume of 25 µL that contained 3 µL DNA, 14.45 µL MilliQ water, 2.5 µL 10x PCR buffer, 0.75 µL $MgCl_2$, 2 µL dNTP, 1 µL primer ERIC1, 1 µL primer ERIC2 and 0.3 µL Taq polymerase (Invitrogen). The PCR program consisted of an initial denaturation at 94°C for 2 min, followed by 30 cycles at 94°C for 1 min, 50°C for 1 min 30 s, and 68°C for 4 min, and final extension at 68°C for 10 min.

ERIC-PCR products were separated by electrophoresis in 1.2% agarose gel, stained with ethidium bromide in tris-borate-EDTA (TBE) 0.5x buffer. The amplicon size was determined using the 123 bp DNA Ladder molecular weight marker (Sigma-Aldrich, Inc.). Images were recorded using a gel documentation system (Loccus Biotecnologia, Brasil) for subsequent analysis. Diversity was evaluated through visual assessment of the gel and considered all visible amplicons. All of the isolates of the same species with the same amplification profile were considered, and one isolate representative of each morphotype was selected for molecular identification.

Amplification of gene 16S rDNA was performed using primers 27F (5′-AGA GTT TGA TCM TGG CTCAG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) (Weisburg et al., 1991). The reaction was conducted in a final volume of 25 µL that contained 1 µL DNA, 17.45 µL MilliQ water, 2.5 µL PCR 10x buffer, 0.75 µL $MgCl_2$, 2 µL dNTP, 1 µL primer 27 F, 1 µL primer 1492 R and 0.3 µL Taq polymerase (Invitrogen). The PCR program consisted of an initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 40 s, 58°C for 35 s, and 72°C for 1 min 20 s, and final extension at 72°C for 10 min.

The amplification products were purified (Dunn and Blattner, 1987), and the DNA was quantified using 1 µL of the product in 0.8% agarose gel electrophoresis. Sequencing was performed according to the Sanger method using the Kit Big Dye from ABI3100 Applied Biosystems. 16S sequences were compared with known sequences from the GenBank database (http://www.ncbi.nlm.nih.gov) through a BLASTn similarity search.

Fungi

The fungi were grown in BD liquid medium (400 mL potato infusion, 20 g dextrose) at room temperature for seven days. Fungal mycelia were washed in distilled water using a sieve for complete removal of culture medium. The sample was then dried with a paper towel and stored in a freezer at -20 °C for further analysis.

The extraction of the fungal genomic DNA was performed using a DNA extraction kit according to the manufacturer's instructions

Table 1. Community of rhizospheric microorganisms capable of $CaHPO_4$ solubilization *in vitro* isolated from *B. purpurascens* at Rio Verde, GO.

Microorganism	Population density
Total rhizospheric microorganisms (CFU/g root)	$1.39x10^6$
$CaHPO_4$ solubilizing rhizospheric microorganisms (CFU/g root)	$6.66x10^4$
Number of $CaHPO_4$ solubilizing bacteria	79
Number of $CaHPO_4$ solubilizing fungi	8
% solubilizing agents of rhizospheric microorganisms in total community	4.79

(Axygen Biosciences, USA). Morphotyping validation was determined through a genetic variability analysis using the inter-simple sequence repeat (ISSR) amplification molecular marker.

The amplification of the ISSR molecular marker was performed using oligonucleotide BH1 (5' - GTG GTG GTG GTG GTG - 3') (Smith and Bateman, 2002). The reaction was conducted in a final volume of 25 µL that contained 4 µL DNA, 14.45 µL MilliQ water, 2.5 µL PCR 10x buffer, 0.75 µL $MgCl_2$, 2 µL dNTP, 1 µL primer BH1 and 0.3 µL Taq polymerase (Invitrogen). The PCR program consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min, and final elongation at 72°C for 10 min (performed using an AmpliTherm Thermal Cycler).

The ISSR-PCR products were subjected to electrophoresis in 1.2% agarose gel in buffer TBE 0.5x. The amplicon size was determined using a 123 bp DNA Ladder molecular weight marker (Sigma-Aldrich, Inc.). Images were recorded using a gel documentation system (Loccus Biotecnologia, Brazil) for subsequent analysis. Diversity was evaluated through a visual assessment of the gel that considered all of the visible amplicons. Isolates with the same amplification profile were considered as belonging to the same morphotype, and a representative line of each morphotype was selected for molecular identification.

Identification was performed through partial sequencing of the rDNA internal transcribed spacer (ITS) region from representatives of each morphotype group. Oligonucleotides ITS 4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and ITS 5 (5`-GGA AGT AAA AGT CGT AAC AAG G-3') (White et al., 1990) were used for amplification of the 18S-28S intergene region. The reaction was conducted in a final volume of 35 µL that contained 22.35 µL MilliQ water, 3.5 µL buffer PCR 10x, 1.5 µL $MgCl_2$, 2.8 µL dNTP, 1 µL primer ITS 4, 1 µL primer ITS 5 and 0.3 µL Taq polymerase (Invitrogen). The PCR program consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 1 min, and final elongation at 72°C for 10 min, using an AmpliTherm Thermal Cycler.

The amplification products were purified (Dunn and Blattner, 1987), and DNA was quantified using 1 µL of the product in 0.8% agarose gel electrophoresis. Sequencing was performed according to the Sanger method using the Kit Big Dye and ABI3100 Applied Biosystems. The ITS sequences were compared with known sequences from the GenBank data base (http://www.ncbi.nlm.nih.gov) through a BLASTn similarity search (Altschul et al., 1990).

Data analysis

The relative frequency of the observed species was calculated by dividing the number of isolates of each species by the total number of isolates. The Shannon diversity index (H') was calculated according to Kumar and Hyde (2004).

RESULTS

Isolation

A community of microorganisms capable of $CaHPO_4$ solubilization *in vitro* was isolated from the *B. purpurascens* rhizosphere, and 79 bacteria and eight fungi were obtained (Table 1).

The colonization frequency of the root fragments by endophytes was 100% after 10 days of incubation; 66 bacteria and 29 fungi were isolated, which constituted the endophyte bank set for use in future experiments.

Thirty-seven endophytic and rhizospheric fungi were grouped in 26 morphotypes that were differentiated by macroscopic characteristics (outer edge, type and color of mycelium) and microscopic characteristics (differentiation of reproductive structures).

Molecular identification

The molecular identification of 66 endophytic and 79 rhizospheric bacteria was performed; 29 endophytic and eight rhizospheric fungi were identified, and 145 endophytic and rhizospheric bacteria lines were identified. The bacteria belonged to 17 genera distributed over 38 taxa belonging to phylum Proteobacteria and classes α, β and γ (65.5%), phylum Firmicutes and class Bacilli (33.8%), and phylum Actinobacteria and class Actinobacteria (0.7%). The predominant genera were *Bacillus* (31.0%), *Enterobacter* (20%), *Pseudomonas* (13.8%), and *Agrobacterium* (8.3%). The remaining genera presented relative frequencies lower than 6.3% (Table 2).

Sixty-six endophytic bacterial lines were identified that belonged to 14 genera distributed over 27 taxa belonging to phylum Proteobacteria and classes α, β and γ (78.8%), phylum Firmicutes and class Bacilli (19.7%), and phylum Actinobacteria and class Actinobacteria (1.5%). The predominant genera were *Enterobacter* (28.8%), *Pseudomonas* (18.2%), *Bacillus* (15.2%), and *Agrobacterium* (9.1%). The remaining genera presented relative frequencies lower than 6.2% (Table 2).

Seventy-nine (79) rhizospheric bacterial lines were identified belonging to 12 genera distributed over 25 taxa

Table 2. Molecular identification of endophytic and rhizospheric bacteria isolates from *B. purpurascens*, based on sequencing of the 16S region.

Isolate	Environment/Quantity	GenBank	GenBank accession number	ID (%)	Relative frequency (%)
BP1EB	E (1)	*Herbaspirillum rubrisubalbicans*	AB681857	98	0.7
BP60EB	E (6)/R (6)	*Pseudomonas putida*	JQ701740	99	8.3
BP61EB	E (1)	*Enterobacter oryzae*	JF513179	99	0.7
BP276EB	E (1)	*Burkholderia vietnamiensis*	KF114029	99	0.7
BP321EB	E (6)/R (6)	*Agrobacterium tumefaciens*	AB826000	99	8.3
BP324AEB	E (2)/R (1)	*Citrobacter amalonaticus*	KC689293	99	2.1
BP336EB	E (2)	*Pseudomonas plecoglossicida*	KC469709	99	1.4
BP342EB	E (1)/R (1)	*Burkholderia tropica*	HQ023259	97	1.4
BP3EB	E (2)	*Rhizobium huautlense*	KC355318	99	1.4
BP7EB	E (4)/R (4)	*Pantoea cypripedii*	JX556216	98	5.5
BP59EB	E (10)/R (6)	*Enterobacter asburiae*	EU221358	99	11.0
BP46EB	E (2)/R (5)	*Bacillus subtilis*	KF241533	99	4.8
BP318EB	E (3)/R (2)	*Pectobacterium cypripedii*	JF430157	99	3.5
BP38EB	E (1)	*Microbacterium oxydans*	EU714340	96	0.7
BP42EB	E (1)	*Enterobacter cancerogenus*	HQ683957	98	0.7
BP35EB	E (1)	*Bacillus methylotrophicus*	JF899259	99	0.7
BP339BEB	E (1)	*Paenibacillus illinoisensis*	HQ683877	96	0.7
BP58EB	E (1)	*Bacillus megaterium*	KC609020	99	0.7
BP343EB	E (6)/R (20)	*Bacillus pumilus*	GU969599	99	17.9
BP371EB	E (2)	*Pseudomonas mosselii*	KC293833	98	1.4
BP356EB	E (1)	*Achromobacter insolitus*	JQ659574	99	0.7
BP15EB	E (2)/R (1)	*Brevibacillus agri*	HM629394	99	2.1
BP23EB	E (5)/R (2)	*Enterobacter cloacae*	HM854373	98	4.8
BP261EB	E (1)	*Enterobacter aerogenes*	KF358443	97	0.7
BP323EB	E (1)/R (2)	*Enterobacter ludwigii*	KF475838	98	2.1
BP334EB	E (1)/R (1)	*Pseudomonas denitrificans*	NR_102805	99	1.4
BP8RB	E (1)/R (1)	*Pseudomonas* sp.	AB461802	96	1.4
BP188RB	R (3)	*Klebsiella pneumoniae*	KC153122	99	2.1
BP5RB	R (1)	*Bacillus safensis*	KF318396	99	0.7
BP61RB	R (4)	*Yokenella regensburgei*	AB681877	99	2.8
BP35RB	R (1)	*Bacillus altitudinis*	KC414719	99	0.7
BP44RB	R (1)	*Bacillus aerophilus*	KC414720	99	0.7
BP55RB	R (6)	*Bacillus amyloliquefaciens*	KF483660	99	4.1
BP57ARB	R (1)	*Bacillus cereus*	KF475814	99	0.7
BP16RB	R (1)	*Pantoea* sp.	CP002433	96	0.7
BP187RB	R (1)	*Serratia nematodiphila*	HF585498	99	0.7
BP212RB	R (1)	*Klebsiella variicola*	JX968498	99	0.7
BP54RB	R (1)	*Burkholderia* sp.	KF059273	95	0.7

E = endophytic/R = rhizospheric; ID (%) = % identity.

that belong to phylum Proteobacteria and classes α, β and γ (54.4%) and phylum Firmicutes and class Bacilli (45.6%). The predominant genera were *Bacillus* (44.3%), *Enterobacter* (12.7%), *Pseudomonas* (10.1%), and *Agrobacterium* (7.6%). The remaining genera presented relative frequencies lower than 6.4% (Table 2).

The morphotype grouping of rhizospheric and endophytic fungal isolates resulted in the formation of 26 groups based on macroscopic characteristics. Identical profiles were detected following molecular identification of a representative of each group through amplification of the ISSR molecular marker (data not shown), which

Table 3. Molecular identification of endophytic and rhizospheric fungi isolates from *B. purpurascens* based on the ITS region sequencing.

Isolate	Environment/Quantity	GenBank	GenBank accession number	ID (%)	Relative frequency (%)
BP328EF	E (5)	*Codinaeopsis* sp.	EF488392	99	13.5
BP364EF	E (1)	*Bionectria ochroleuca*	HQ607832	99	2.7
BP16EF	E (1)	*Penicillium purpurogenum*	GU566198	97	2.7
BP5EF	E (8)	*Gibberella moniliformis*	JN232122	99	21.6
BP375EF	E (2)	*Phomopsis* sp.	EF488379	99	5.4
BP314BEF	E (2)	*Fusarium proliferatum*	HQ332533	99	5.4
BP14EF	E (1)	*Fusarium oxysporum*	AY669123	99	2.7
BP329BEF	E (1)	*Periconia macrospinosa*	FJ536208	99	2.7
BP33EF	E (1)	*Hamigera insecticola*	JQ425375	98	2.7
BP40EF	E (1)	*Talaromyces amestolkiae*	JX965247	99	2.7
BP55EF	E (1)/R (2)	*Fusarium concentricum*	HQ379635	99	8.1
BP332EF	E (1)	*Viridispora diparietispora*	JN049838	97	2.7
BP341EF	E (4)	*Diaporthe* sp.	EF423549	95	10.8
BP196RF	R (2)	*Ceratocystis paradoxa*	JQ963886	99	5.4
BP62RF	R (1)	*Curvularia affinis*	EF187909	99	2.7
BP191RF	R (1)	*Neodeightonia phoenicum*	HQ443209	99	2.7
BP202RF	R (1)	*Hypocreales* sp.	HQ248209	98	2.7
BP192RF	R (1)	*Aspergillus brasiliensis*	JQ316521	97	2.7

E = Endophytic/R = rhizospheric; ID (%) = % identity.

allowed for the identification of 18 identical profiles.

Morphological groups were identified through sequencing of the ITS region of the large subunit rRNA. Eighteen taxa were identified with high identity using the ITS region identity above 97% as a similarity criterion at the species level (O'Brien et al., 2005). The sequences were then compared with the deposited sequences at the GenBank.

Molecular analysis of the groups revealed taxa from the phylum Ascomycota and belonging to the three classes Sordariomycetes (83.8%), Eurotiomycetes (10.8%) and Dothideomycetes (5.4%) as well as 16 genera, predominantly *Gibberella* (20.5%), *Fusarium* (15.4%), *Codinaeopsis* (12.8%), *Diaporthe* (10.3%), *Ceratocystis* (7.7%), *Phomopsis* and *Hypocreales* (5.1%), *Curvularia, Bionectria, Penicillium, Periconia, Hamigera, Talaromyces, Viridispora, Neodeightonia* and *Aspergillus* (2.6%).

Twenty-nine (29) endophytic fungal lines were identified that belonged to 12 genera distributed over 13 taxa in the classes Sordariomycetes (89.7%) and Eurotiomycetes (10.3%). The predominant genera were *Gibberella* (27.6%), *Codinaeopsis* (17.2%), *Fusarium* (13.8%), *Diaporthe* (13.8%), *Phomopsis* (6.9%), *Bionectria, Penicillium, Periconia, Hamigera, Talaromyces* and *Viridispora* (3.4%) (Table 3).

Eight rhizospheric fungal lines were identified that belonged to six taxa in the classes Sordariomycetes (62.5 %), Dothideomycetes (25.0%) and Eurotiomycetes (12.5 %) and six genera, predominantly *Fusarium and Ceratocystis* (25%), *Curvularia, Neodeightonia, Hypocreales* and *Aspergillus* (14.3%) (Table 3).

Diversity

The Shannon-Wiener H' diversity indexes for the microbial populations of *B. purpurascens* were 2.97 for endophytic and 2.73 for rhizospheric bacteria and 2.23 for endophytic and 1.73 for rhizospheric fungi.

DISCUSSION

Interactions between plants and microorganisms are complex, can influence the establishment of plant communities, and can change ecosystem properties. These relations are interesting for researchers, and related research results may clarify interaction mechanisms and have relevant ecological and biotechnological applications. The present study is the first on the taxonomic and genetic diversity of endophytic and rhizospheric growable microorganisms from *B. purpurascens* from the Brazilian Cerrado.

Microbial diversity can be defined in terms of taxonomic, genetic, and functional diversity and used in

the characterization of species richness and real communities. The metabolic versatility of a population is based on its genetic variability and capacity to interact with other populations of species with functional diversity (Jha et al., 2010).

Factors such as the type of soil, climate and plant species may affect the microbial community, and it is important to understand interactions between plants and microorganisms because they play a fundamental ecological role in nutrient recycling and plant growth promotion (Kumar et al., 2012).

Jha et al. (2010) studied the *Jatropha curcas* rhizosphere in five different soil conditions and locations at the Indian state of Gujarat and observed Shannon (H') indexes between 0.96 and 0.92, which indicated that the plants generate a strong selection pressure on the rhizosphere under the studied conditions and are associated with bacteria that provide beneficial effects for plant growth and health, thus resulting in low bacterial diversity. In the present study, the rhizospheric bacteria presented high H' (2.73), indicating higher bacterial diversity at the rhizosphere.

A value of H' = 1.41 was reported for halotolerant bacteria from *Suaeda fruticosa* collected from a salt desert, indicating that the community of organisms residing at a given niche is specific and dependent on physical and environmental factors (Goswami et al., 2014). Based on the bacterial diversity indexes observed for *B. purpurascens*, it can be concluded that the endophytic (H'= 2.97) and rhizospheric (H'= 2.73) species diversity was higher for this species than for *S. fruticosa*, indicating lower selection pressure.

In a similar study by Alberto (2013) of the endophytic fungi from *Anacardium othonianum* from the Cerrado of Goiás state, an H' value of 1.82 was observed, which is lower than the value observed in the present study. Higher diversity (H' = 2.99) was observed by Bezerra et al. (2013) in a study performed with *Cereus jamacaru* in Brazilian tropical dry forest between September and November.

The microbial diversity observed in the present study indicated differences in the endophytic and rhizospheric communities because some of the described genera were only detected in one environment. Kumar et al. (2012) studied the bacteria associated with the roots of *Ajuga bracteosa*, a medicinal plant traditional in India and China, from the Kangra Valley in India and found the phyla Proteobacteria (69.9%), Firmicutes (24.4%) and Actinobacteria (5.7%). The first two were also observed in the *B. purpurascens* rhizospheric community in the present study.

Using 16S rRNA sequence analysis, a recent study performed on soil from three Cerrado regions of Minas Gerais, Brazil identified a bacterial community formed by genera such as *Bacillus, Klebsiella, Enterobacter, Pantoea, Escherichia* and *Leuconostoc* (Mesquita et al., 2013).

At Rio Grande do Sul state, corn roots and rhizospheric soil were found in association with 21 genera of rhizobacteria, with *Klebsiella* and *Burkholderia* the dominant genera (Arruda et al., 2013). This result is in contrast with the present study, where *Bacillus* and *Enterobacter* were found to be the dominant genera of rhizospheric bacteria and *Enterobacter* was found to be the dominant genus of endophytic bacteria.

Madhaiyan et al. (2013) studied endophytic bacteria isolated from *Jatropha curcas* roots from Indonesia, China and India and observed a predominance of strains from genus *Enterobacter*, such as the species *E. oryzae, E. asburiae, E. cancerogenus, E. cloacae, E. aerogenes* and *E. ludwigii,* which were also found in the present study, with several only found in the endophytic environment and others found in both environments.

The genus *Pseudomonas* is known for its habitat and physiological diversity, and certain strains can degrade recalcitrant compounds, which makes these species important for the bioremediation of natural and xenobiotic pollutants. In addition, this genus is able to stimulate plant growth through different mechanisms (Patel et al., 2011; Behera et al., 2014).

The genus *Bacillus* possesses remarkable properties because of several mechanisms, and it produces a wide range of compounds beneficial for the promotion of plant growth through phosphate solubilization, indole-3-acetic acid (IAA) production and nitrogen fixation (Idris et al., 2007; Kavamura et al., 2013) and for biological control of many common phytopathogens, such as through the production of hydrogen cyanide (HCN), siderophores, hydrolytic enzymes and antibiotics (Kumar et al., 2012).

Bacillus pumilus is known for the production of enzymes such as proteases, which are widely used in the food, chemical, detergent and leather industries, as well as the production of antifungals, antibiotics and chitinase, which are used in the biological control of plant diseases (Zhang et al., 2010). Higher relative frequencies were observed for this species, and it is easily found in the *B. purpurascens* roots and rhizosphere.

Yadav et al. (2013) observed the capacity for *in vitro* phosphate solubilization in *Brevibacillus* sp. strains, which is a thermotolerant bacteria isolated from deposits in India. In the present study, this genus was also isolated as an endophyte and at the rhizosphere.

Ascomycetes are referred to as producers of active antimicrobial compounds. In a study of *Vitis labrusca* in the São Paulo region, Brazil, 48.14% of the endophyte fungi isolates belonged to phylum Ascomycota (Brum et al., 2012). In the present study, all of the isolates belonged to this phylum, suggesting that they may be involved in the protection of the host plant against fungal diseases.

Alberto (2013) studied the endophytic fungi community of *A. othonianum* and isolated genera *Penicillium*, *Fusarium, Periconia*, *Bionectria* and *Diaporthe*, some of which were also found in the present study. This result

indicates that these genera are common to plant hosts from locations such as the Brazilian Cerrado.

The fungus *Penicillium purpurogenum* is of great interest for the food industry because it produces a red pigment through the combined effects of pH and temperature. These pigments are frequently more stable and soluble than pigments from plant or animal sources; in addition, they may present high productivity and are used as additives, color intensifiers, antioxidants, etc. (Méndez et al., 2011). This species was also detected in the present study.

The endophytic fungi from cacti of the Brazilian tropical dry forest that were isolated by Bezerra et al. (2013) belonged to genera *Fusarium* and *Aspergillus*. Srinivasan et al. (2012) also isolated these genera from salt-affected soils in India. Similarly, in the present study, these genera were also detected in *B. purpurascens* in both endophytic and rhizospheric environments.

Genus *Fusarium* produces a wide variety of biologically active secondary metabolites, including mycotoxins and fumonisins, which are detrimental for humans and animals (Matić et al., 2013). Although it is known for its pathogenicity, it also includes non-pathogenic species that are effective for biological control of phytopathogenic fungi. However, species such as *Fusarium oxysporum* can produce toxins and cause plant diseases, thereby invalidating the use of *Fusarium* for biocontrol because of the risks of food contamination (Brum et al., 2012). In the present study, the phytopathogenic and biocontrol capacities of this genus were not evaluated.

Fungus *Gibberella moniliformis* corresponds to the *Fusarium verticillioides* anamorphic phase. It commonly colonizes corn plants and can act as endophyte or pathogen depending on environmental and genetic factors which are not well understood (Desjardins et al., 2007). *Fusarium verticillioides* can cause diseases such as root rot and stem and grain wilting, and it is a highly toxigenic species because it produces of mycotoxins (Covarelli et al., 2012).

Aspergillus brasiliensis can produce low cost microbial enzymes through solid state fermentation, and these enzymes are important for various industries; for example, naringinase is an enzyme with a high potential for the biotransformation of steroids and antibiotics and especially for glycoside hydrolysis and it has the potential for use in the pharmaceutical and food industries (Shanmugaprakash et al., 2014).

Chen et al. (2013) observed that the wide spectrum endophytic fungus *Phomopsis* isolated from the internal bark of *Bischofia polycarpa* can accelerate litter decomposition through lignin degradation by increasing the concentration of soil nutrients, promoting plant growth, and inducing an increase in plant defenses.

The *B. purpurascens* palm tree possesses an assembly of heterotrophic endophytic and rhizospheric microorganisms that were previously unknown. The present study is the first report of the microbial diversity associated with this tree species, and this study enabled the establishment of a microbial species bank that can be used to identify these microorganisms, assist in future research to develop processes and/or products, contribute to the improvement of soil quality, and promote plant growth and productivity.

Conclusions

The rhizosphere and roots of *B. purpurascens* harbor a diverse set of growable organisms, including fungi, bacteria and actinobacteria. Fourteen endophytic and 12 rhizospheric bacterial genera belonging to three phyla (Proteobacteria, Firmicutes and Actinobacteria) and 11 endophytic and six rhizospheric fungal genera (exclusively from phylum Ascomycota) were detected in *B. purpurascens* endophytic and rhizospheric environments. The most frequently isolated genera were *Enterobacter* and *Pseudomonas* for the endophytic bacteria, *Gibberella* and *Codinaeopsis* for the endophytic fungi, *Bacillus* and *Enterobacter* for the rhizospheric bacteria, and *Ceratocystis* for the rhizospheric fungi. A difference was observed between the endophytic and rhizospheric microbial communities of *B. purpurascens,* with certain microorganisms only present in one of the environments. Further studies with a larger number of individuals are required to confirm this result.

Conflict of interests

The authors did not declare any conflict of interest.

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