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SOIL SCIENCE

Plant growth-promoting mechanisms and genetic diversity of bacteria strains isolated from *Brachiaria humidicola* and *Brachiaria decumbens*

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Abstract: Plant growth-promoting bacteria (PGPB) have received great interest in recent decades. However, PGPB mechanisms remain poorly understood in forage species. We aimed to evaluate roots endophytic and rhizospheric bacteria strains from *Brachiaria humidicola* and *Brachiaria decumbens*. The strains were evaluated for biological nitrogen-fixing in saline stress (0 to 10.0 g L^{-1} of NaCl), N-acyl homoserine lactones and indole-like compounds (ILC) production, the activity of hydrolytic enzymes, and inorganic phosphate solubilization (IPS) under different C sources. The diversity of strains was assessed by BOX-PCR. About 58% of strains were positive for BNF. High salinity levels reduced the growth and BNF. About 58% produced N-acyl homoserine lactones. The ILC was present in 39% of strains. Cellulase, polygalacturonase, pectate lyase, and amylase production were observed in 77, 14, 22, and 25% of strains, respectively. The IPS was observed in 44, 81, and 87% of isolates when glucose, mannitol and sucrose were used, respectively. Comparing two plant species and niches, the strains associated with *B. humidicola* and root endophytic presented more PGPB mechanisms than others. We found high strain diversity, of which 64% showed similarity lower than 70%. These results can be supporting the bioproducts development to increase forage grasses production in tropical soils.

Key words: Bacteria-plant association, forage grasses, rhizosphere bacterial, root endophytic.

INTRODUCTION

The forage grasses have a primordial importance to worldwide livestock (Capstaff & Miller 2018), mainly to Brazilian production chain because the meat and milk production is an almost exclusively pasture-based system (Cerri et al. 2016). In Brazil, there is a wide diversity of the tropical forage grasses which can be used as pasture (Aguiar et al. 2017).

The genus *Brachiaria* ssp. comprises about 114 million hectares of the pasture in Brazil. *Brachiaria decumbens* (Stapf) R.D.Webster (signalgrass), *B. brizantha* (Stapf) Webster (palisadegrass), *B. humidicola* (Rendle) Morrone & Zuloaga (koroniviagrass) and *B. ruziziensis* (R. Germain & Evrard) Crins (ruzigrass) are the main pasture varieties present in the country (Rezende et al. 2017, Souza et al. 2018). The use of *Brachiaria* spp. grasses for pasture formation was possible due to their high adaptability and tolerance to different soil types and climatic conditions, as well as their easier and more

flexible management (Euclides et al. 2010, Hungria et al. 2016, Souza et al. 2018).

Brazilian livestock sector has faced a strong challenge to become more productive and efficient, in addition to achieving sustainability throughout its production chain. In this scenario, there is a huge demand for low-cost technologies that can increase pasture production. An available alternative for this purpose is the use of plant growth-promoting bacteria (Aguirre et al. 2018, Araujo et al. 2012, Pedreira et al. 2017, Wemheuer et al. 2016). For example, the PGPB improve ecosystem services and contribute to increased primary production through biological nitrogen fixation (BNF), phytohormones and enzymes production, pest and disease control, increased plant tolerance and resistance to abiotic stresses such as drought and salinity (Andrade et al. 2018, Araújo et al. 2014, Oliveira et al. 2017, Wemheuer et al. 2016).

Bacteria can inhabit different niches such as soil, rhizosphere, phyllosphere, inside plant tissues (endophytic) and other. These different habitats contribute to a higher microbial diversity what has a huge potential to find biotechnology properties (Compant et al. 2010, Ferrara et al. 2012, Machado et al. 2013, Souza et al. 2017). Several benefits have been observed from the bacteria-plant association, such as increases in germination and seed emergence, plant growth and production and, consequently, soil healthy (Amaral et al. 2016, Hungria et al. 2016, Kim et al. 2012, Moreira et al. 2014).

The elucidation of the plant growth promoting potential related to bacteria associated these plants, as well as the knowledge of their genetic diversity, can generate biotechnological products (Araujo et al. 2012). It can represent an important alternative for improving the establishment and production of pasture fields. In addition, can contribute to reducing production costs, decreasing mineral fertilizers application and, consequently, mitigating the environmental impacts (Wemheuer et al. 2016).

However, the biotechnology potential of the bacterial associated with forage grasses remains poorly understood, mainly in tropical regions. In this context, the aim of this study was to evaluate 36 roots endophytic and rhizospheric bacterial isolates of the *Brachiaria humidicola* (Rendle.) Schweickerdt and *Brachiaria decumbens* Stapf for biotechnology properties related to plant growth promotion. For this, bacterial isolates were evaluated *in vitro* for their ability to perform nitrogen fixation in the different NaCl concentrations, N-acyl homoserine lactones and ILC production, the activity of extracellular enzymes and inorganic phosphate (P) solubilization trait under different carbon sources. Genetic diversity of bacterial isolates was assessed by BOX-PCR fingerprint technique.

MATERIALS AND METHODS

Bacterial Isolates

We evaluated a total 36 pure strains isolated from *Brachiaria* ssp. belongs to the Microbial Genetic and Biotechnology Laboratory, Unit Academic of Garanhuns, Federal University of Pernambuco, Brazil. The experiment consisted for 18 bacterial strains isolated from *Brachiaria humidicola* (Rendle) Schweickerdt and 18 bacterial isolated from *Brachiaria decumbens* Stapf, being nine root endophytic strains and nine rhizospheric strains per forage grass. All bacterial strains were removed from glycerol storage (-20 °C freezer) and re-cultured in TSA 10% (Trypticase Soy Agar) culture medium (4.0 g TSA L^{-1}; 10.0 g agar L^{-1}).

The analyzes were performed in triplicate and the EM303 (*Pseudomonas oryzihabitans*), belongs to the Microbial Genetic and Biotechnology Laboratory strain was used as a positive control. This bacterial isolated was

obtained from soybean tissue and has potential to NBF, IAA (indole acetic acid) and lytic enzymes producer (Kuklinsky-Sobral et al. 2004).

Biological nitrogen fixation test

Biological nitrogen fixation test was performed according to methodology described by Döbereiner et al. (1995). For this, the isolates were inoculated in NFb semisolid culture media (5.0 g L^{-1} of malic acid; 0.5 g L^{-1} of K_2HPO_4; 0.2 g L^{-1} of $MgSO_4$ H_2O; 0.1 g L^{-1} of NaCl; 0.02 g.L^{-1} of $CaCl_2.2H_2O$; 2.0 mL of micronutrient solution (0.4 g L^{-1} of $CuSO4.H_2O$, 1.2 g L^{-1} of $ZnSO_4.H_2O$, 1.4 g L^{-1} of H_3BO_3, 1.0 g L^{-1} of $Na_2MoO_4.2H_2O$, and 1.175 g L^{-1} of $MnSO_4.H_2O$); 2.0 mL of bromothymol blue solution; 4.0 ml of 4 M Fe EDTA; 1.0 mL of the vitamin solution (0.1 g L^{-1} of biotin; 0.02 g L^{-1} of pyridoxine); 4.5 g.L^{-1} of KOH; 1.8 g.L^{-1} of AGAR; pH = 6.8), free nitrogen source, and incubated at 28 °C for 8 days. Isolates positives to growth in NFb media were reinoculated in the same conditions. However, were added different NaCl concentrations into the culture media as follows: 0.1; 2.5; 5 and 10 g L^{-1} and same incubation condition were maintained. Bacterial growth was characterized by the formation of white halo near of the culture medium surface (Leite et al. 2018, Pereira et al. 2012).

ALHs production (Quorum sensing potential activity)

The characterization of quorum sensing signaling molecules was performed by the production of N-acyl homoserine lactones (ALHs), using the *Agrobacterium tumefaciens* NTL4 (pZLR4) as reference which present the gene for the ALHs production. *A. tumefaciens* NTL4 was inoculated linearly in Petri dishes together with the evaluated bacterial strains. Perpendicularly to the same plate was added 10.0 µg mL^{-1} of Luria Bertani medium (LB-Agar) containing the X-gal chromogen (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside). Then the plates were incubated at 28 °C for 48 h. The presence of the *A. tumefaciens* colonies with bluish color is indicative of the ALH production (Grönemeyer et al. 2012, Leite et al. 2014).

Indole-like compounds (ILC) production

Bacterial strains were evaluated for indole acetic acid (ILC) production. To order, the bacterial isolates were inoculated in liquid TSA medium, with and without L-tryptophan (5.0 mM) addition. The strains were incubated under shaking at 120 rpm, at 28 °C, in the absence of light, for 24 h. Then, bacterial cultures were centrifuged at 12,000 g for 5 min, after1.5 mL of supernatant was transferred to clean microtube, and 0.5 mL of Salkowski reagent (2.0% $FeCl_3$ 0.5 M in 35.0% perchloric acid) was added. Samples were incubated in the dark room at 28 °C for 30 min (Crozier et al. 1988, Pereira et al. 2012). The ILC quantification was performed in a spectrophotometer at an optic density of 530 nm.

Lytic enzymes production

The lytic enzymes production (cellulase, amylase, pectate lyase and polygalacturonase) was evaluated according to Stamford et al. (2001), Alves et al. (2002) and Carrim et al. (2006), respectively. In all enzymatic assays the incubations were performed at 28 °C for 72 h. The enzymatic activity was evaluated by the presence of bacterial growth halo in the culture medium. While the enzymatic index was determined by the ratio between diameter of the growth halo and the diameter of the bacterial colony.

Inorganic phosphate solubilizing test

The inorganic phosphate solubilizing test was performed according to Verma et al. (2001). The bacterial strains were inoculated in solid culture medium containing insoluble phosphate source (10 g L^{-1} glucose, 5 g L^{-1} NH_4Cl, 1 g L^{-1} NaCl, 1 g L^{-1}

$MgSO_4.7H_2O$, 4 g L^{-1} $CaHPO_4$, 15 g L^{-1} agar, pH 7.2). Addition, the bacterial isolates that solubilized phosphate in the media containing glucose were also tested in others culture media, but using different C sources, such as sucrose and mannitol (10 g L^{-1}). Incubations occurred at 28 °C for 72 h. The inorganic phosphate solubilization index were estimated by the ratio between halo hydrolysis diameter and the bacterial colony diameter.

Bacterial genetic variability

The bacterial isolates genetic variability was analyzed by BOX-PCR fingerprint technique (Versalovic et al. 1994). For this, the isolates genomic DNA was extracted using the Genomic DNA Purification kit (Fermentas, Waltham, Massachusetts, EUA) according to the manufacturer's instructions. A PCR reaction was performed using the primer BOXA1R (5´-CTACGGCAAGGCGACGCTGACG-3´), in a final volume of 25 µL containing ~10.0 ng of the DNA template; 1.0 µM of primer; 1.0 mM of each dNTPs; 1x of DMSO (dimetilsufoxamida); 1x of Taq Buffer, 3.5 mM of $MgCl_2$ and 0.08 U of Taq DNA polimerase (Fermentas, Fermentas, Waltham, Massachusetts, EUA).

Amplification reactions were performed with initial denaturation at 95 °C for 2 min, 35 denaturation cycles at 9 °C for 2 min, 92 °C for 30 s, annealing at 50 °C for 1 min and extension at 65 °C for 1 min, followed by a final extension at 65 °C for 10 min. After amplification, the reactions were evaluated using agarose gel electrophoresis (1.5%, v/v) in 1x TAE buffer (40.0 mM Tris-acetate, 1.0 mM EDTA) and stained with Blue green loading dye (LGC Bio, Cotia, São Paulo, Brazil). Agarose gel was photographed and analyzed using Gel Analyzer version 2010a.

Statistical analysis

The isolates groups (roots endophytic and rhizospheric) and qualitative variables were verified by the chi-square test (X^2). Quantitative variables were analyzed by orthogonal contrast and statistical significance between contrast was verified by *t*-test. The comparison between the different C sources (sucrose and mannitol), used in the inorganic phosphate solubilization test, were evaluated by the Tukey test at the 5.0% probability level, both using SISVAR 5.6 software.

Binary matrix of the DNA band profiles was used to verify the bacterial genetic variability. For this, a dendrogram was constructed based on Jaccard coefficient similarity and clusters were grouped using the UPGMA algorithm (Unweighted Pair-Group Method with Arithmetical Average), using PAST 2.15 software.

RESULTS AND DISCUSSION

The 36 bacterial isolates selected were positive for one or more plant growth-promoting (PGP) mechanisms (Table I). Bacterial growth in culture media without free nitrogen source was verified in 50% of the isolates. The increase of the salt concentration in the semisolid NFb media (0.1; 2.5; 5 and 10 g L^{-1} of NaCl) affected bacterial growth and BNF capacity, with decreases in percentages of 100; 77; 55.5 and 16%, respectively. However, no significant differences were observed between isolates in the comparison between species, plant species and niches. In general rhizosphere bacteria showed higher salt tolerance in the culture medium than root endophytic isolates (Table I).

High levels of soluble salts may have toxic effects on microbial cells, decreasing bacterial activity, which may impact crucial ecosystem services provided by soil microorganisms, such

Table I. ***In vitro*** **PGP mechanisms of the 36 bacterial isolates associated with** ***Brachiaria humidicola*** **(Rendle.) Schweickerdt and** ***Brachiaria decumbens*** **Stapf. in the root and rhizospheric niche.**

Isolates ID	***Brachiaria humidicola*** **(Rendle.) Schweickerdt (root)**														
	NFb (% NaCl)					ALHs	IAA		IE				IS		
	0.0	0.1	2.5	5.0	10.0		CLT	SLT	CE	PO	PE	AM	GL	MA	SA
UAGB1	+	+	+	+	-	+	35.52	5.47	0.00	0.00	4.26	1.81	3.83	1.36	1.66
UAGB2	+	+	+	+	-	-	0.00	0.00	0.00	0.00	0.00	0.00	3.28	2.19	1.60
UAGB3	-	-	-	-	-	-	0.00	0.00	0.93	0.00	0.00	0.00	0.00	0.00	0.00
UAGB4	-	-	-	-	-	-	29.53	0.00	1.19	0.00	0.00	0.00	0.00	0.00	0.00
UAGB5	-	-	-	-	-	+	0.00	0.00	0.91	0.00	0.00	0.00	0.00	0.00	0.00
UAGB6	-	-	-	-	-	+	26.38	2.40	1.20	0.68	2.20	1.20	1.59	2.74	1.67
UAGB7	-	-	-	-	-	-	63.04	5.03	1.08	0.00	0.00	0.00	4.26	3.64	6.54
UAGB8	-	-	-	-	-	-	38.11	14.78	1.00	0.00	0.00	0.00	3.08	3.64	3.29
UAGB9	+	+	+	+	-	+	30.05	5.25	1.00	0.00	0.00	0.00	3.14	4.66	4.32
	Brachiaria humidicola **(Rendle.) Schweickerdt (rhizospheric)**														
UAGB10	+	+	+	+	-	+	13.48	9.21	1.20	0.00	4.86	1.09	0.00	0.00	0.00
UAGB11	-	-	-	-	-	+	0.00	0.00	0.91	0.96	3.36	1.49	2.80	0.00	0.00
UAGB12	-	-	-	-	-	+	0.00	0.00	1.30	0.00	3.43	1.48	0.00	0.00	0.00
UAGB13	+	+	+	+	+	-	16.07	2.79	0.96	0.94	0.00	0.00	0.00	0.00	0.00
UAGB14	+	+	+	-	-	+	0.00	0.00	0.97	0.00	0.00	1.49	0.00	0.00	0.00
UAGB15	+	+	+	+	-	-	0.00	0.00	1.05	0.00	0.00	0.00	0.00	0.00	0.00
UAGB16	-	-	-	-	-	-	0.00	0.00	1.10	0.00	0.00	2.62	0.00	0.00	0.00
UAGB17	-	-	-	-	-	+	0.00	0.00	1.12	0.00	0.00	1.92	0.00	0.00	0.00
UAGB18	-	-	-	-	-	+	0.00	0.00	1.06	0.00	2.54	1.20	2.16	2.11	2.12
	Brachiaria decumbens **Stapf. (root)**														
UAGB19	+	+	-	-	-	-	0.00	0.00	1.18	0.00	0.00	0.00	2.31	3.30	3.47
UAGB20	-	-	-	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00	1.40	4.00	4.50
UAGB21	+	+	+	+	-	+	0.00	0.00	1.24	0.64	0.00	0.00	2.58	4.00	5.38
UAGB22	-	-	-	-	-	-	64.72	10.94	0.95	0.00	0.00	0.00	5.00	0.00	0.00
UAGB23	+	+	-	-	-	+	0.00	0.00	1.16	0.00	0.00	0.00	0.00	0.00	0.00
UAGB24	-	-	-	-	-	+	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
UAGB25	+	+	+	-	-	+	0.00	0.00	0.00	0.00	8.27	0.00	0.00	0.00	0.00
UAGB26	+	+	-	-	-	-	0.00	0.00	0.78	0.63	7.55	0.00	0.00	0.00	0.00
UAGB27	-	-	-	-	-	+	0.00	0.00	1.09	0.00	0.00	0.00	0.00	0.00	0.00
	Brachiaria decumbens **Stapf. (rhizospheric)**														
UAGB28	-	-	-	-	-	+	28.19	3.22	0.00	0.00	0.00	0.00	3.14	3.26	6.35
UAGB29	+	+	-	-	-	+	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
UAGB30	+	+	+	+	-	+	0.00	0.00	1.08	0.00	0.00	0.00	1.61	4.25	5.55
UAGB31	-	-	-	-	-	+	10.29	0.00	1.05	0.00	0.00	0.00	0.00	0.00	0.00
UAGB32	-	-	-	-	-	-	11.46	2.70	0.98	0.00	0.00	0.00	0.00	0.00	0.00
UAGB33	+	+	+	-	-	+	0.00	0.00	0.96	0.00	0.00	0.00	0.00	0.00	0.00
UAGB34	+	+	+	+	+	-	27.03	1.93	1.05	0.00	0.00	0.00	1.49	0.00	2.44
UAGB35	+	+	+	-	-	+	59.63	16.68	1.08	0.00	0.00	0.00	6.08	3.79	4.50
UAGB36	+	+	+	+	+	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

NFb – culture media without N source (semisolid); ALHs - Quorum sensing production; IAA – Indole acetic acid production (µg mL^{-1}); CTL – IAA production via L-Tryptophan-dependent; SLT - IAA production via L-Tryptophan-independent; IE – enzymatic index; CE – cellulase enzyme; PO – Polygalacturonase enzyme; PE - Pectate lyase enzyme; AM – Amylase enzyme; IS – P solubilization index; GL – Glucose source; MA - Mannitol source; SAC - Sucrose source.

as biological nitrogen fixation (Egamberdieva & Kucharova 2009).

Pereira et al. (2012) evaluated the effect of salinity on the PGP mechanisms of bacteria isolated from sugarcane plants. The authors reported that the bacterial strains were able to grow and produce indole acetic acid at a concentration of up to 10 g L^{-1} NaCl in the culture media. However, the growth rate was reduced and BNF was completely inhibited at 25 and 50 g L^{-1} NaCl concentrations. In general rhizosphere bacteria have a higher capacity to fix nitrogen in high salt concentrations of endophytic bacteria. This is because rhizosphere isolates have greater ability to adapt to environmental changes (Ferrara et al. 2012, Richardson et al. 2009, Santos et al. 2012).

ALHs and quorum sensing production were detected in 58% of the bacterial isolates, no distinction between plant species and niches was verified (Table I). The ALHs production, auto-inducers and chemical signal chemical are related to the ability of intercellular communication, mobility regulation, horizontal gene transfer, enzyme production, biofilm formation and stress tolerance (Pinton et al. 2010).

The biofilms provide protection to microbial populations against environmental stresses, such as drought and salinity. Ability to form biofilm is a desired feature in bacterial strains intended for inoculant composition. Biofilms have helped to maintain the inoculum population in the soil or seed, but the bacteria-plant interaction may be beneficial or deleterious (Grönemeyer et al. 2012).

Indole-like compounds biosynthesis was identified in 39% of bacterial strains. This phytohormone was detected in the concentration ranging from 10 to 65 µg mL^{-1}. The ILC production by metabolic pathway L-tryptophan-independent was observed in the 86% of isolates, however ILC concentration was lower and ranged from 2 to 16.5 µg mL^{-1} (Table I).

Bacteria associated with plants can produce ILC by different pathways. The more known route in PGPBs for indole-like molecules synthesis is the via IPyA (indole pyruvic acid) in which the bacteria use the amino acid L-tryptophan as a precursor.

However, some bacterial can synthesize ILC by L-tryptophan-independent metabolic routes, however the production rate is lower (Richardson et al. 2009). Auxins synthesis by microorganisms associated with plants promotes root growth and root elongation, consequently, increasing the absorption of water and nutrients and thus potentiating the development of plants (Araujo et al. 2012, Machado et al. 2013).

Enzymatic production was observed in 78, 14, 22 and 25% of the bacterial strains for the enzymes cellulase, polygalacturonase, pectate lyase and amylase, with enzymatic indexes of 1.3, 1.8, 8.3 and 2.7, respectively (Table I).

The lytic enzymes produced by bacteria act in the degradation of several organic compounds and have great potential of application in the production of tissues, food and paper, besides use in agriculture. In the latter case, lytic enzyme-producing bacterial strains can more easily colonize the plant and act through its PGP mechanism (Compant et al. 2010).

Inorganic phosphate solubilizing (IPS) was observed in 44% of isolates in the culture medium containing glucose as carbon source. In the culture media containing mannitol and sucrose, 81% and 87.5% of the bacterial strains were positive for the IPS test. The highest solubilization rates were 6.1, 4.7 and 6.5, in the glucose, mannitol and sucrose respectively (Table I). Some microorganisms present in soils and/or associated with plants play an important role in the phosphorus cycling, hydrolyzing P inorganic forms, making it susceptible of

assimilation by the plants, from the action of hydrolytic enzymes, mainly acid phosphatases (Leite et al. 2014, Santos et al. 2012, Verma et al. 2001).

The inorganic phosphate solubilizing *in vitro* by bacteria can occur in media containing different carbon sources and supplemented with insoluble phosphate forms. In this work, it was observed that the ability to solubilize inorganic phosphate was dependent on the carbon source added to the culture medium.

Barroso et al. (2006) reported greater effect of carbon source on the inorganic phosphate solubilization *in vitro* by *Aspergillus niger*. The maltose, sucrose, glucose, mannose and fructose were the carbon sources which most stimulated citric acid production, consequently, increasing the IPS in the culture media. In this work, sucrose was the carbon source that most promoted inorganic phosphate solubilization (Table II).

A high heterogeneity of PGP mechanisms was observed, when evaluated according to the plant species and niche (Table III). Analysis of orthogonal contrasts showed that *B. humidicola* bacterial isolates had higher potential for PGP mechanism expression than *B. decumbens* isolates. The root endophytic isolates synthesized higher ILC concentrations in the presence of L-tryptophan and had the highest phosphate solubilization index in media containing glucose and mannitol as carbon source. While the rhizosphere isolates were distinguished in the enzymatic synthesis of cellulase and amylase, and in the solubilization of phosphate (Table III).

Considering the forage grass species, the root bacterial isolates of *B. humidicola* produced higher ILC L-tryptophan-dependent concentrations and presented greater ability to perform phosphate solubilization at different carbon sources. While the rhizosphere bacterial isolates had the highest enzymatic indexes for all enzymes evaluated.

For niches, the rhizosphere isolates of *B. decumbens* showed highest ILC concentrations by L-tryptophan pathway and the solubilization of phosphate, mainly in sucrose as carbon source in the culture media. The roots isolates also presented highest enzymatic indices for polygalacturanase and pectate lyase enzymes (Table III).

Endophytic niches, such as root and rhizosphere are physically, chemically and biologically distinct. In the rhizosphere there is action of the root exudates, which modify the nutritional quality of the niche, in comparison with the bulk soil. In this niche there is a greater competition between different genera and species of microorganisms (Compant et al. 2010, Machado et al. 2013, Souza et al. 2017). In contrast, the root endophytic niche is more stable and uniform, with less competition between microorganisms and greater availability of nutrients. In this way, bacteria from distinct niches present different mechanisms to promote plant growth according to their adaptive capacity, directly reflecting their potential to influence the

Table II. Average of inorganic phosphate solubilization index, in vitro, with different C sources in the culture media (glucose, mannitol and sucrose). Isolates associated with *Brachiaria humidicola* (Rendle.) Schweickerdt and *Brachiaria decumbens* Stapf. from root and rhizosphere niche.

Carbon source	Glucose	Mannitol	Sucrose
Solubilization index (average)	2.98 C	3.30 B	3.81 A
Number of positive isolates	16	13	14

Means followed by the same letter do not differ by Tukey test at 5% probability.

Table III. Comparison between groups of averages by orthogonal contrasts for the PGP mechanisms of 36 bacterial isolates associated with *Brachiaria humidicola* (Rendle.) Schweickerdt and *Brachiaria decumbens* Stapf. in endophytic root (ER) and rhizosphere (RI) niches.

Average	IAA		IE				IS		
	CLT	SLT	CE	PO	PE	AM	GL	MA	SA
B. humidicola	14.01	5.31	0.94	0.14	1.15	0.80	1.34	2.54	2.65
B. decumbens	11.18	5.91	0.70	0.07	0.88	0.00	1.31	2.83	4.02
Nicho ER	15.96	5.92	0.76	0.11	1.24	0.17	1.69	2.95	3.24
Nicho RIZ	9.23	5.22	0.88	0.11	0.79	0.63	0.96	2.24	3.49
B. humidicola - ER	24.74	5.08	0.81	0.08	0.72	0.34	2.13	3.04	3.18
B. humidicola - RIZ	3.28	6.00	1.08	0.21	1.58	1.26	0.55	1.06	1.06
B. decumbens - ER	7.19	10.94	0.71	0.14	1.76	0.00	1.25	2.83	3.34
B. decumbens - RIZ	15.18	4.90	0.69	0.00	0.00	0.00	1.37	2.83	4.71
Total	12.60	5.57	0.82	0.11	1.01	0.40	1.33	2.68	3.34
***Brachiaria humidicola* (Rendle.) Schweickerdt *vs* *Brachiaria decumbens* Stapf**									
T test	2.49*	-0.23^{ns}	9.02**	7.93**	0.9^{ns}	39.21**	0.34^{ns}	-1.58^{ns}	-13.45**
Root *vs* Rhizospheric									
T test	5.95**	0.28^{ns}	-4.47**	0.32^{ns}	1.53^{ns}	-22.71**	8.37**	3.90**	-2.38*
***Brachiaria humidicola* (Rendle.) Schweickerdt - Root *vs* Rhizospheric**									
T test	16.17**	-0.27^{ns}	-10.03**	-11.72**	-6.52**	-22.67**	12.94**	9.38**	24.00**
***Brachiaria decumbens* Stapf - Root *vs* Rhizospheric**									
T test	-4.35**	1.04^{ns}	0.42^{ns}	10.15**	3.09**	0.0^{ns}	-0.91^{ns}	0.00^{ns}	-7.24**

IAA – Indole acetic acid production (μg mL^{-1}); CTL – IAA production via L-Tryptophan-dependent; SLT - IAA production via L-Tryptophan-independent; IE – enzymatic index; CE – cellulase enzyme; PO – Polygalacturonase enzyme; PE - Pectate lyase enzyme; AM – Amylase enzyme; IS – P solubilization index; GL – Glucose source; MA - Mannitol source; SAC - Sucrose source; ^{ns} - no significant; * and ** - significant at 5% and 1% of probability by the t test.

plant development and growth over the natural environment (Compant et al. 2010, Ferrara et al. 2012).

The BOX-PCR technique evidenced that 64% of bacterial isolates had genetic similarity of less than 70% (Figure 1). However, UAGB5 and UAGB6, UAGB8 and UAGB9 root endophytes and UAGB 12 and UAGB 14 isolates from the rhizosphere of *B. humidicola* presented high similarity to each other, reaching 100% in both evaluated niches (Figure 1).

The microbial diversity in the plants is influenced by the differences in the quantity and chemical composition of the root exudates, which can be modified by the genotype, phenological and nutritional plant state, among other (Murphy et al. 2016, Oliveira et al. 2017). Despite the distinction between niches, there are microorganisms able to migrate between them, adapting the conditions of each niche, having as the main causes of migration the environmental conditions and nutritional status of plants (Luvizotto et al. 2010).

The positive effects of inoculating bacteria with PGP mechanisms in non-leguminous plants may be numerous (Hungria et al. 2016, Lima et al. 2018). These effects may occur by direct influence with the production of plant growth regulators (phytohormones) and the solubilization of inorganic phosphate, or by

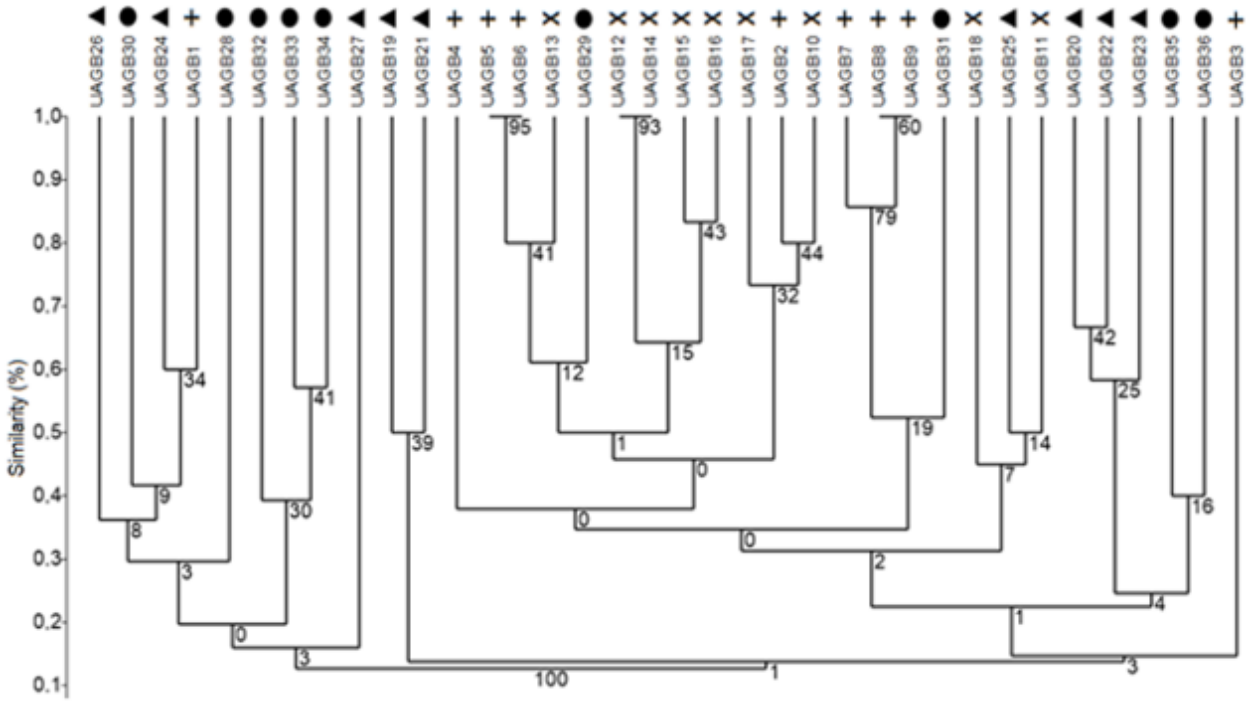

Figure 1. Dendrogram of genetic similarity using BOX gene based on the Jaccard Coefficient and grouped by the algorithm Unweighted Pair-Group Method with Arithmetical Average (UPGMA) of the 36 isolates associated with *Brachiaria humidicola* (Rendle.) Schweickerdt and *Brachiaria decumbens* Stapf. from root and rhizospheric niche. + = root niche isolates of *B. humidicola*; × = rhizospheric niche isolates of *B. humidicola*; ▲ = root niche isolates of *B. decumbens*; • = rhizospheric niche isolates of *B. decumbens*.

indirect influence, such as the suppression of pathogens by production of siderophores or antibiotics (Andreote et al. 2014).

The inoculation of *Burkholderia phytofirmans*, a bacterial strain with PGP mechanisms in seeds of *Panicum virgatum* (Alamo cultivar) was able to increase seed germination speed index, as well as root length by 33% and shoot length by 35.6% after 30 days of emergency (Kim et al. 2012). Inoculation of *B. brizantha* with diazotrophic bacteria of genus *Bacillus* spp. showed an increase in forage mass, leaf area, leaf green intensity, leaf number and tillers in the average of the three cuts (Araujo et al. 2012)

The knowledge of the biotechnological potential of bacteria associated to forage grasses in different colonization niches aims at the development of inoculant, able to reduce the use of fertilizers, as well as to promote the vegetal growth, thus increasing the forage production (Araújo et al. 2014, Hungria et al. 2016, Machado et al. 2013).

CONCLUSION

All bacterial isolates were positive for one or more PGP mechanisms, regardless of grass species or niche. The bacterial isolates were able to fix nitrogen at high salt concentrations, besides producing ALH molecule and ILC with and without L-tryptophan, and synthesize extracellular enzymes (cellulase, polygalacturonase, pectate lyase and amylase). Carbon source has strong effect on inorganic phosphate solubilization *in vitro*. Bacterial isolates from *B. humidicola* (Rendle.) Schweickerdt showed higher number of biotechnological characteristics. High

genetic diversity was observed, with 64% of the isolates below 70% of similarity, without the formation of similar clusters by plant species and colonization niche. Considering the PGP mechanisms, the strains with the highest potential for inoculant formulation were: UAGB1-UAGB6-UAGB9-UAGB10-UAGB21-UAGB34-UAGB35 (10–12 PGP mechanisms). The knowledge of the biotechnological potential and genetic diversity of bacteria associated with forage grasses becomes an important tool for the development of bioproducts, being an alternative for pasture management and yield, as well as reducing costs and environmental impacts.

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