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Assessment of morphophysiological and genotypic diversity of endophytic bacteria isolated from rice (*Oryza sativa* L.) plants

Avaliação da diversidade morfofisiológica e genotípica de bactérias endofíticas isoladas de arroz (*Oryza sativa* L.)

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ABSTRACT - Rice production in Brazil incurs high costs due to the significant use of agrochemicals. Some plant growth-promoting rhizobacteria (PGPR) can be used as alternative to fertilizers and phytosanitary products. Thus, the objective of this study was to characterize endophytic bacteria isolated from roots of rice plants. The isolates were characterized based on colony morphology, antibiotic resistance, carbon sources utilization, enzyme activity (catalase, amylase, protease, cellulase, and lipase), inorganic phosphate solubilization, and the 16S-23S rDNA intergenic region. Morphologically, 68% of the isolates presented a rapid growth rate, 46% presented abundant mucus production, and 77% formed viscous colonies. All isolates were resistant to nalidixic acid and 16% presented resistance to streptomycin. The majority (90%) used monosaccharides and disaccharides in carbon source assays. Most of the isolates (95%) were positive for catalase and 63.6% were positive for amylase, protease, lipase, and cellulase activities. Additionally, 59% of them were able to solubilize phosphate. The mean enzymatic index for amylase, cellulase, and protease was 2.8, 3.5, and 1.7 respectively. The similarity analysis revealed high diversity among the isolates, with similarity indices of 70% (based on morphological characteristics) and 60% (based on the intergenic region 16S-23S rDNA). Considering morphophysiological and genotypic characteristics, three promising isolates should be evaluated in studies under field conditions for the potential development of bioproducts to replace industrially manufactured inputs in rice crops.

Keywords: Rice. Plant growth promotion. PCR 16S-23S rDNA. Extracellular enzymes.

RESUMO - O arroz apresenta altos custos de produção no Brasil devido ao elevado uso de agroquímicos. Algumas rizobactérias promotoras de crescimento de plantas (PGPR) podem ser utilizadas como alternativa aos fertilizantes e produtos fitossanitários. Este trabalho teve como objetivo caracterizar bactérias endofíticas obtidas de raízes de arroz. A caracterização dos isolados foi baseada na morfologia da colônia, resistência a antibióticos, uso de fontes de carbono, atividade enzimática (catalase, amilase, protease, celulase e lipase), solubilização de fosfato inorgânico e região intergênica do 16S-23S rDNA. Com base na morfologia, 68% dos isolados apresentaram crescimento rápido, 46% apresentaram produção abundante de muco e 77% apresentaram colônia viscosa. Todos os isolados foram resistentes ao ácido nalidíxico e 16% deles à estreptomicina. A maioria dos isolados (90%) utilizou monossacarídeos e dissacarídeos no ensaio da fonte de carbono. A maioria dos isolados (95%) foi positiva para catalase e 63,6% foram positivas para amilase, protease, lipase e celulase. Além disso, 59% foram capazes de solubilizar o fosfato. A média do índice enzimático para amilase, celulase e protease foi de 2,8, 3,5 e 1,7, respectivamente. A análise de similaridade revelou alta diversidade entre os isolados, com índice de similaridade de 70% (baseado nas características morfológicas) e 60% (baseado na análise da região intergênica 16S-23S rDNA). Com base nas características morfofisiológicas e genotípicas, três isolados promissores devem ser avaliados em estudos futuros em condições de campo, podendo gerar bioprodutos destinados a substituir insumos industrializados na cultura do arroz.

Palavras-chave: Arroz. Promoção de crescimento de plantas. PCR 16S-23S rDNA. Enzimas extracelulares.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most grown grasses worldwide. Approximately 12,000 Mg of rice were produced in Brazil in the 2021/2022 crop season (CONAB, 2022). Rice crops have high cultural and economic importance in Brazil, representing the primary agricultural activity in some Brazilian states (CONAB, 2022). Rice crops present high yields in Brazil, but the crop production costs have increased significantly in recent years, mainly due to the use of chemical fertilizers, which have contributed to approximately 23% of the cost increase (CONAB, 2022). However, plant growth-promoting rhizobacteria (PGPR) may be used as biofertilizers to reduce production costs and environmental impacts, as PGPR is a potential efficient alternative to the application of chemical fertilizers from non-renewable resources (GUIMARÃES; BALDANI, 2013; FERREIRA; KNUPP; MARTIN-DIDONET, 2014, OSÓRIO-FILHO et al., 2014).

PGPR can colonize the surface of plant roots, and some of these PGPR can

enter plant roots, establishing an endophytic bacterial population throughout the plant, reaching the stem and leaves of several plant species (OSÓRIO-FILHO et al., 2014; HAHN et al., 2014). The plant-bacteria interaction can enhance plant growth through biological nitrogen fixation (BNF), production of phytohormones, phosphate solubilization, ACC deaminase activity, nutrient retention in the rhizosphere, production of siderophores, and plant disease biocontrol (LIU et al., 2014).

Associations of several bacterial genera with rice plants have been reported and investigated, such as the genera *Azospirillum, Bacillus, Burkholderia, Herbaspirillum, Pseudomonas,* and *Rhizobium* (MBAI et al., 2013; GUIMARÃES; BALDANI, 2013; FERREIRA; KNUPP; MARTIN-DIDONET, 2014). However, further studies on rice plant-bacteria interaction are needed, especially focusing on identifying the microbial diversity associated with rice plants (JI; GURURANI; CHUN, 2014; FERREIRA; KNUPP; MARTIN-DIDONET, 2014). Currently, the microbial taxonomy system is based on polyphasic analysis involving morphological, biochemical, and molecular data, combined with evaluations of similarities and differences, as well as the different degrees of similarity among microorganisms (IKEDA et al., 2013).

Generally, the most used physiological and biochemical parameters include antibiotic resistance (HERNÁNDEZ-FORTE et al., 2012), the ability to metabolize different carbon sources (IKEDA et al., 2013), production of enzymes (MBAI et al., 2013), biological nitrogen fixation (GUJRAL et al., 2013), and phosphate solubilization (MAREQUE et al., 2015). These parameters enable the determination of microbial diversity and the selection of potential PGPR (GLICK, 2012).

According to Lupo, Coyne and Berendonk (2012), an intrinsic antibiotic resistance of microorganisms allows them to successfully survive in the soil and colonize plants. The production of hydrolytic enzymes, such as amylases, cellulases, chitinases, proteases, and pectinases, is involved in the pathogen control process, assisting bacteria to penetrate plant tissue and access energy sources (SZILAGYI-ZECCHIN et al., 2014; SILVA et al., 2015). Phosphate solubilization also contributes to the biological control of some fungal species (DINESH et al., 2015). The production of these compounds is connected to bacterial survival and competition within their ecological niche.

Bacterial metabolic diversity also generates high biotechnological interest due to its potential for the development of products of recognized economic value in several areas, such as production of enzymes and polymers (SZILAGYI-ZECCHIN et al., 2014). Large number of studies have indicated that rice endophytic bacteria could be successfully used to promote plant growth (JI; GURURANI; CHUN, 2014; MBAI et al., 2013; FERREIRA; KNUPP; MARTIN-DIDONET, 2014). Additionally, several published studies have shown high genetic diversity among bacteria isolated from rice plants (IKEDA et al.; 2013; ZHAN et al., 2014)

Thus, the objective of this study was to determine the morphophysiological and genotypic diversity of endophytic bacteria isolated from roots of rice plants (*Oryza sativa L*) grown in the Cerrado biome, Brazil.

MATERIAL AND METHODS

Endophytic bacterial isolates were obtained from roots of rice plants grown in the Cerrado Biome, in Goias, Brazil, according to Ferreira, Knupp, and Martin-Didonet (2014). Twenty-two isolates and three standard strains of *Rhizobium tropici* (BR322 and BR520 strains) and *Azospirillum brasilense* (FP2 strain) were used in the present study.

The isolates and standard strains were grown at 30 °C in liquid YM medium under shaking at 140 rpm or on solid YMA medium (2% agar w v^{-1}) containing the antibiotics nalidixic acid (20 μg mL^{-1}) and ampicillin (10 μg ml^{-1}).

Three characteristics were considered to describe the colony morphology of the 22 isolates and standard strains: 1) growth rate = rapid growth (> 24 h; between 1 and 3 days) or very rapid growth (< 24 h); 2) colony consistency = dry, moist, or viscous; 3) mucus production: scanty, moderate, or abundant. Morphological characterization was performed on YMA medium according to Hungria and Silva (2011).

The ability of the 22 bacterial isolates and standard strains to metabolize different carbon sources was assessed in minimal medium by separately adding 14 carbon sources: maleic acid, malic acid, nicotinic acid, succinic acid, arabinose, glycerol, glucose, fructose, inositol, mannitol, mannose, sucrose, sorbitol, and trehalose. Carbon sources were used at a concentration of 10 mM L^{-1}. The assays were conducted in deep-well plates containing 1 mL of the medium under agitation at 28 °C for 72 h. An aliquot of 200 μL of each culture was transferred to Elisa plates after incubation to assess bacterial growth through optical density at 600 nm, using a microplate spectrophotometer (Epoch; BioTek Instruments, Winooski, USA).

The 22 isolates and the three standard strains were inoculated on solid YMA medium containing ampicillin, chloramphenicol, streptomycin, and tetracycline at different concentrations (30 μg mL^{-1}, 50 μg mL^{-1}, 100 μg mL^{-1}, and 200 μg mL^{-1}). The plates were incubated at 30 °C for 72 h in the dark to prevent antibiotic photodegradation. The bacterial growth in the different antibiotic concentrations was classified into four types: no growth (0); growth at 30 μg mL^{-1} (1); growth at 50 μg mL^{-1} (2); growth at 100 μg mL^{-1} (3); and growth at 200 μg mL^{-1} (4).

The enzyme catalase was evaluated according to Nakamura et al. (2012). The capacity to produce extracellular enzymes (amylases, proteases, lipases, and cellulase) was assessed as described by Cappuccino and Sherman (2014). Cellulolytic and lipolytic activities were determined according to Melo et al. (2018).

Two solid media were utilized in the phosphate solubilization assay: Pikovskaya (PVK) medium for basic phosphate and NBRIP medium for acid phosphate. Both media were utilized at pH 7.0, and the plates were incubated for 168 h.

The isolates and the standard strains were grown for 24 h in liquid YM medium, as previously described. DNA was extracted according to Ausubel et al. (1999). Polymerase chain reaction (PCR) and primers for the intergenic spacer region (16S-23S rDNA) were performed as recommended by Tokajian et al. (2016).

Enzymatic activity and phosphate solubilization were determined through enzymatic index (EI) and solubilization index (SI), respectively, which are based on semiquantitative measurements. EI and SI were calculated by the ratio between the diameters of the degradation halo and the colony (HANKIN; ANAGNOSTAKIS, 1975). Isolates with EI ≥ 2 were considered good enzyme producers (LEALEM; GASHE, 1994). Regarding the ability to solubilize phosphate, the isolates were classified as low SI (SI < 2), moderate SI ($2 \leq$ SI < 4), and high SI (SI > 4) (HARA; OLIVEIRA, 2005).

Biochemical and molecular data were separately subjected to cluster analysis. The data were used to generate a binary matrix (presence/absence) to evaluate the similarity among the 22 isolates and the 3 standard strains. Similarity indices were estimated using the Jaccard similarity coefficient (J) and clustering was performed through the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), using the NTSYSpc 2.02i Applied Biostatistcs software (IKEDA et al., 2013). The assays were conducted in triplicate, and the results represented the mean of the replicates.

RESULTS AND DISCUSSION

The characterization of the bacterial isolates based on the growth rate revealed that 68% of them had rapid growth rates (between 1 and 3 days to grow), whereas 32% had very rapid growth rates (< 24 h) (Table 1).

Table 1. Morphological characteristics of 22 endophytic bacterial isolates obtained from roots of rice (*Oryza sativa* L.) and standard strains: *Rhizobium tropici* (BR322 e BR520) and *Azospirillum brasilense* (FP2). The bacterial cells were growth in YMA solid medium for 48 h.

Isolates	Morphological characters		
	Growth speed	Colony consistency	Mucus production
R18	Fast-growing	Dry	Scanty
R20	Fast-growing	Dry	Scanty
R21A	Fast-growing	Dry	Scanty
R62A	Fast-growing	Dry	Scanty
R62B	Fast-growing	Dry	Scanty
R64	Fast-growing	Dry	Scanty
R21B	Fast-growing	Moist	Moderate
FP2	Fast-growing	Moist	Scanty
R130	Fast-growing	Viscid	Abundant
R141	Fast-growing	Viscid	Abundant
R145	Fast-growing	Viscid	Abundant
R23	Fast-growing	Viscid	Moderate
R50	Fast-growing	Viscid	Moderate
R59	Fast-growing	Viscid	Moderate
R68	Fast-growing	Viscid	Moderate
R139	Fast-growing	Viscid	Moderate
BR322	Fast-growing	Viscid	Moderate
BR520	Fast-growing	Viscid	Moderate
R136A	Very-fast-growing	Viscid	Abundant
R136B	Very-fast-growing	Viscid	Abundant
R147A	Very-fast-growing	Viscid	Abundant
R147B	Very-fast-growing	Viscid	Abundant
R148	Very-fast-growing	Viscid	Abundant
R158	Very-fast-growing	Viscid	Abundant
R161	Very-fast-growing	Viscid	Abundant

Considering the morphological parameters, the isolates predominantly presented a rapid growth rate, viscous colony consistency, and abundant mucus production. Bacterial growth rate is an important criterion in taxonomic studies and can be useful for distinguishing endophytic microorganisms (DURÁN et al., 2013). Rapid growth and metabolic diversity

are connected to the success of bacteria in colonizing and surviving in the rhizosphere (BADRI et al., 2013; HERNÁNDEZ-FORTE et al., 2012). Hernández-Forte and Nápoles-García (2017) reported a mean growth rate of 48 h for most of the evaluated rice isolates, similar results to that found in the present study (Table 1).

The mucus production assay showed a high production level, with 46% of the isolates exhibiting abundant mucus, 27% moderate, and 27% scanty. Only five isolates (R23, R50, R59, R68, and R139) presented similarity to *Rhizobium tropici* (standard strains BR322 and BR520), characterized by rapid growth, viscous colony, and moderate mucus production (Table 1). The isolate R21B showed a similar moist colony consistency to that of *Azospirillum brasilense* (control strain FP2). Considering the evaluated colony characteristics, a predominance of isolates with viscous mucus was found, indicating a high content of exopolysaccharides, which are important components for bacterial survival under environmental stresses (LIU et al., 2013).

Two isolates (R148 and R161) that presented very rapid growth rate were able to grow in 86% of the tested carbon sources, whereas isolates R136B, R147A, R147B, R21B, and R62B, as well as the standard strains BR322 and BR520 did not utilized carbon sources (Table 2). These results showed that most of the evaluated rice isolates can grow on a wide range of carbon sources, indicating a high metabolic diversity. Overall, 90% of the isolates preferentially utilized monosaccharides and disaccharides as carbon sources. Approximately 59% of the isolates presented lower ability to metabolize acidic carbon. Additionally, more than half of the isolates (63.6%) were positive for amylase, protease, lipase, and cellulase activities (Table 2). Eight isolates (R21B, R136A, R139, R147A, R147B, R148, R158, and R161) were negative for these extracellular enzymes (Table 2).

Table 2. Ability to use different carbon sources and enzymatic activity of 22 endophytic bacterial strains isolated from roots of rice (*Oryza sativa* L.) and control strains: *Rhizobium tropici* (BR322 and BR520) and *Azospirillum brasilense* (FP2) grown in YMA solid medium.

		Isolates																								
		R18	R20	R21A	R21B	R23	R50	R59	R62A	R62B	R64	R68	R130	R136A	R136B	R139	R141	R145	R147A	R147B	R148	R158	R161	BR322	BR520	FP2
Carbon sources	Maleic acid	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-
	Malic acid	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+
	Nicotinic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	Succinic acid	-	-	-	-	+	+	+	-	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+
	Arabinose	+	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-
	Frutose	-	-	-	-	+	+	+	-	-	-	+	-	+	-	-	+	-	-	-	+	-	+	-	-	-
	Glycerin	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+
	Glucose	+	-	-	-	-	+	+	-	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-
	Inositol	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
	Manitol	-	-	+	-	+	+	+	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+
	Mannose	-	-	-	-	+	-	+	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+	+	+	+
	Sucrose	-	+	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Sorbitol	+	+	-	-	+	+	+	-	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+
	Trehalose	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Enzyme activity	Amilase	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	Cellulase	+	+	+	-	-	-	-	+	-	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-
	Acid phosphate	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
	Basic phosphate	-	-	-	-	+	+	+	-	+	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	-
	Lipase	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
	Protease	+	+	+	-	-	-	+	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-

(-) negative results; (+) positive results

Strains with a very rapid growth rate had better performance in assimilating carbon sources, as previously reported in the literature (HERNÁNDEZ-FORTE et al., 2012; HERNÁNDEZ-FORTE; NÁPOLES-GARCÍA, 2017). Overall, the present study showed that 90% of the rice isolates preferentially utilized monosaccharides and disaccharides as carbon sources, whereas approximately 59% of the isolates presented lower metabolism of acidic carbon. Similar results were reported by Ikeda et al. (2013), who found that approximately 96% of the isolates utilized sugars as carbon sources. The ability of endophytic bacteria to utilize carbon sources have been applied in several studies, showing the complexity of the microbiota colonizing internal plant tissues (MAREQUE et al., 2015).

Catalase activity was found in all evaluated isolates, except for R145 (Table 2). Thus, these isolates can assist plants in mitigating oxidative stress in the environment and are present in a wide range of endophytic bacteria obtained from grasses (MBAI et al., 2013; IKEDA et al., 2013; MAREQUE et al., 2015). Kenia, Mbai et al. (2013) evaluated 73 endophytic bacteria obtained from cultivated rice and reported that all of them were positive for catalase activity.

The present study found that 63.6% of the isolates were positive for amylase, protease, lipase, and cellulase activities (Table 2). Only eight isolates (R21B, R136A, R139, R147A, R147B, R148, R158, and R161) were negative for these extracellular enzymes. Szilagyi-Zecchin et al. (2014) found the presence of hydrolytic enzymes in bacterial isolates from maize roots, which may be involved in antibiosis activity against phytopathogens. The presence of hydrolytic enzymes such as cellulase and protease was also found in endophytic bacteria associated with sorghum plants (MAREQUE et al., 2015).

The antibiotic resistance assay showed that only two isolates (R21B and R59) had intrinsic resistance to all tested antibiotics at the highest concentration (200 µg mL^{-1}) and were thus classified as type 4 (Table 3). Isolates R18, R20, and R62B were highly susceptible to ampicillin (type 2; concentration of 50 µg mL^{-1}), chloramphenicol, streptomycin, and tetracycline (type 1; concentration of 30 µg mL^{-1}) (Table 3). Seven isolates (R23, R50, R68, R141, R145, R158, and R161) showed similar results to those found for *R. tropici* (BR322 and BR520), characterized by resistance to ampicillin, chloramphenicol, and tetracycline (type 3 - 100µg mL^{-1}). All isolates were resistant to nalidixic acid and only 16% showed resistance to streptomycin at 200 µg mL^{-1} (type 4) (Table 3).

Considering that intrinsic resistance is the innate ability of bacterial species to withstand an antimicrobial agent through their inherent structural or functional characteristics (COSTA et al., 2014), only two isolates (R21B and R59) presented intrinsic resistance to all antibiotics at the highest concentration (200 µg mL^{-1}). These results indicate that these isolates are highly adaptable strains to different conditions, including rhizosphere conditions, making them promising competitors with the indigenous microflora. Additionally, all isolates were resistant to nalidixic acid and only 16% presented resistance to streptomycin. Similar results were found by Hernández-Fortes et al. (2012) in *Rhizobium* isolates obtained from *Canavalia ensiformis* nodules, with 100% of the isolates showing resistance to nalidixic acid and 25% showing resistance to streptomycin.

Susceptibility or resistance in some soil bacteria has been considered an indicator of survival and competition for nutrients in the rhizosphere, which is a region of intense metabolic activity (COSTA et al., 2014). Resistance to more than one antibiotic indicates that bacteria have a molecular strategy to inactivate the activity of antibiotic compounds (MELO, 1999). Furthermore, the mucus production may hinder the antimicrobial agent assimilation by preventing the passage of substances into the bacterial cell (MARTINS et al., 1997).

The mean solubilization index (SI) found for the isolates in NBRI-P medium (acid phosphate) was 2.3, whereas the highest SI was 2.8 (R161) (Table 3). Six isolates (R23, R50, R59, R68, R141, and R161) presented moderate SI ($2 \leq SI < 4$) in NBRI-P medium, whereas five isolates (R62B, R136A, R147A, and R147B) showed low SI ($SI < 2$) (Table 3). Regarding the Pikovskaya medium (basic phosphate), the mean SI (1.3) was lower compared to that found in NBRI-P medium, whereas the highest SI found was 1.5 (isolates R50, R59, and R161) (Table 3). Therefore, all isolates showed low SI ($SI < 2$) in this basic medium. No isolates exhibited high SI ($SI > 4$) in both tested media (Table 3). The standard strains BR 322 and BR 520 (*R. tropici*) showed phosphate solubilization activity (Tables 2 and 3) in both media. However, the standard strain FP2 (*A. brasilense*) showed no phosphate solubilization activity under the tested conditions (Tables 2 and 3).

Considering that the SI was, on average, 2.05 in acid phosphate medium and 1.3 in basic phosphate medium and that phosphorus (P) is an essential nutrient to plants, the utilization of bacteria that can solubilize inorganic phosphate to organic phosphate may be a sustainable alternative for supplying P to plants (ALORI; GLICK; BABALOLA, 2017). Several genera have been described as P-solubilizing agents, including some bacterial genera such as *Bacillus, Pseudomonas, Burkholderia,* and *Rhizobium* (MAREQUE et al., 2015), and some of them have been reported in association with rice plants (MBAI et al., 2013).

The mean enzymatic index (EI) for amylase, cellulase, and protease was 2.8, 3.5, and 1.7, respectively. The isolate R136B showed the highest EI (4.5) for cellulase activity, whereas the isolates R18 and R62A showed the highest EI for amylase and protease, 3.7 and 2.5, respectively. Hydrolytic enzyme-producing microorganisms have more promising and sustainable functions in controlling phytopathogens than chemicals fungicides (JADHAV; SAYYED, 2016). Thus, the isolates R136B, R18, and R62A may be considered promising microorganisms to be used as biocontrol agents in field experiments.

Table 3. Resistance to antibiotics (Ampicillin=AMP, Chloramphenicol=CHL, Streptomycin=STR, and Tetracycline=TET), Enzymatic index (amylase=AM, cellulase=CE, lipase=LP, and protease=PR), and Solubilization P index for 22 rice isolates and control strains: *Rhizobium tropici* (BR322 and BR520 strains) and *Azospirillum brasilense* (FP2 strain).

Bacteria	Resistence Antibiotic				Enzimatic Index				Solubilization P Index	
	AMP	CHL	STR	TET	AM	CE	LP	PR	ACID (NBRIP)	BASIC (PVK)
R18	1	0	0	0	3.7±0	4.1 ± 0.35	-	1.6±0	-	-
R20	1	0	0	0	3.2 ± 0.33	3.1 ± 0.2	-	1.7 ± 0.06	-	-
R21A	0	1	0	0	2.1 ± 0.75	2.9 ± 0.14	-	2.0 ± 0.07	-	-
R21B	4	4	4	4	-	-	-	-		-
R23	4	4	2	4	-	-	1.2 ± 0.07	-	2.4 ± 0.19	1.3±0
R50	4	4	0	4	-	-	1.2 ± 0.01	-	2.5 ± 0.25	1.5 ± 0.19
R59	4	4	4	4	-	-	1.1 ± 0.07	1.5 ± 0.15	2.6 ± 0.34	1.5 ± 0.12
R62A	0	1	0	0	3.0 ± 0.06	3.2±0	-	2.5 ± 0.05	-	-
R62B	1	0	0	0	1.4 ± 0.11	-	-	-	1.1 ± 0.04	-
R64	0	0	0	0	3.2 ± 0.07	3.5 ± 0.14	-	1.6 ± 0.13	-	-
R68	4	4	0	4	3.4 ± 0.07	-	1.2±0	-	2.7 ± 0.50	1.4 ± 0.07
R130	4	2	0	2	-	3.7±0	-	1.3 ± 0.12	-	1.2 ± 0.07
R136A	0	2	0	1	-	-	-	-	1.8 ± 0.09	-
R136B	0	2	0	1	-	4.5 ± 0.08	-	1.6 ± 0.2	-	-
R139	0	4	0	4	-	-	-	-	-	-
R141	4	4	0	4	-	-	-	-	-	-
R145	4	4	0	4	-	-	1.1±0	1.6 ± 0.05	2.1±0	1.4 ± 0.16
R147A	0	2	0	1	-	3.0±0	-	-	-	1.1 ± 0.09
R147B	0	2	0	2	-	-	-	-	1.8 ± 0.16	1.2 ± 0.03
R148	4	2	3	4	-	-	-	-	1.8 ± 0.09	1.2 ± 0.06
R158	4	4	0	4	-	-	-	-	1.9 ± 0.25	1.2 ± 0.04
R161	4	4	3	4	-	-	-	-	2.8 ± 0.16	1.5 ± 0.07
BR 322	4	3	4	3	-	-	-	-	1.6 ± 0.08	1.2 ± 0.07
BR 520	4	4	2	3	-	-	-	-	1.6 ± 0.13	1.2 ± 0.07
FP2	4	1	4	0	-	-	-	-	-	-

Resistence of all antibiotics types: 0 - growth absence; 1- growth at 30 µg mL^{-1}; 2- growth at 50 µg mL^{-1}; 3- growth at 100 µg mL^{-1} and 4- growth at 200 µg mL^{-1}. Absence of activity (-); SD- standard deviation. Medium for P solubilization assays: NBRIP medium and Pikovskaya.

Cluster analysis was applied to the biochemical and molecular datasets, identifying 11 clusters with a similarity of approximately 70% for the biochemical dataset (Figure 1).

The results of this analysis indicate that these isolates from rice roots have high phenotypic diversity, as 6 (C3, C7, C8, C9, C10, and C11) of the 11 clusters presented unique characteristics among the isolates (Figure 1). Cluster C5 contained a subgroup with two isolates (R147A and R147B), which showed 100% similarity to each other. Isolates R147A and R147B showed 85% and 95% similarity to the *R. tropici* strains (BR322 and BR520), respectively, denoting that these isolates share similar biochemical characteristics with rhizobial strains. C1, C2, and C3 grouped isolates with 35% similarity to the strains BR 322 and BR 520. C4 and C5 clusters presented 47% similarity to the *A. brasilense* strain (FP2). Overall, the rice isolates (C1 to C5 clusters) showed low biochemical similarity with the rhizobial cluster.

The dendrogram of PCR targeting the 16-23S rDNA intergenic spacer region showed high diversity, represented by 10 clusters with 60% similarity (Figure 2).

The largest cluster C1 was formed by three subgroups with similarity of 100%. C2 cluster had 100% similarity compared to standard strains (BR 322 and BR 520) (Figure 2). The C4 cluster was composed of isolates that also showed high similarity based on their biochemical dataset (Figure 1), suggesting that these isolates may belong to the same taxonomic cluster. The isolates R62B (C2), R145 (C5), R130 (C8), R148 (C9) and R139 (C10) were individual clusters in the dendrogram of the 16S-23S rDNA intergenic region (Figure 2), such as seen in the dendrogram to biochemical dataset (Figure 1).

The largest cluster (C1) consisted of three subgroups with 100% similarity. Cluster C2 had 100% similarity to the standard strains BR 322 and BR 520 (Figure 2). Cluster C4 encompassed isolates that presented high similarity in the cluster analysis for the biochemical dataset, indicating that

these isolates may belong to the same taxonomic cluster. Isolates R62B (C2), R145 (C5), R130 (C8), R148 (C9), and R139 (C10) represented individual clusters in the dendrogram of the 16S-23S rDNA intergenic region (Figure 2), which is consistent with the dendrogram for the biochemical dataset (Figure 1).

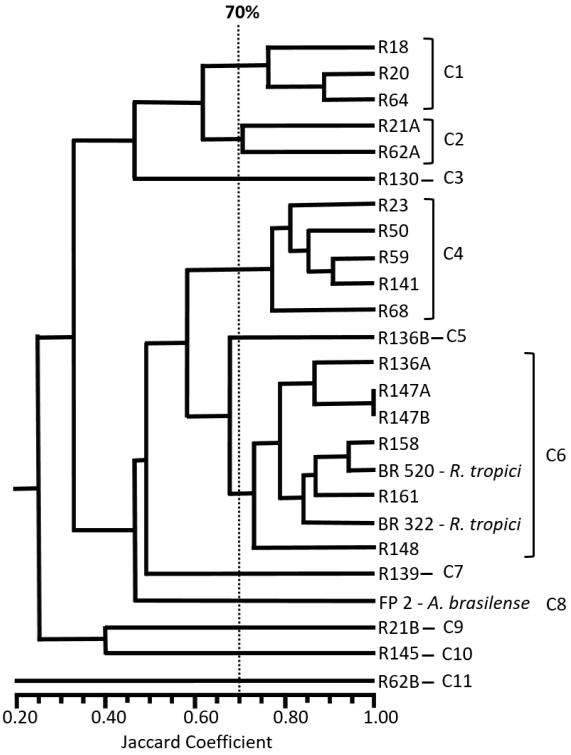

Figure 1. Similarity dendrogram created by the NTSYS-pc® 2.10 based on the biochemical characterization defined by the UPGMA algorithm by Jaccard coefficient using bacteria isolates from rice plant and three standard strains: *Rhizobium tropici* (BR 322 and BR 520) and *Azospirillum brasilense* (FP2). It was considered at 70 % similarity as cut-off point for the clustering of the isolates.

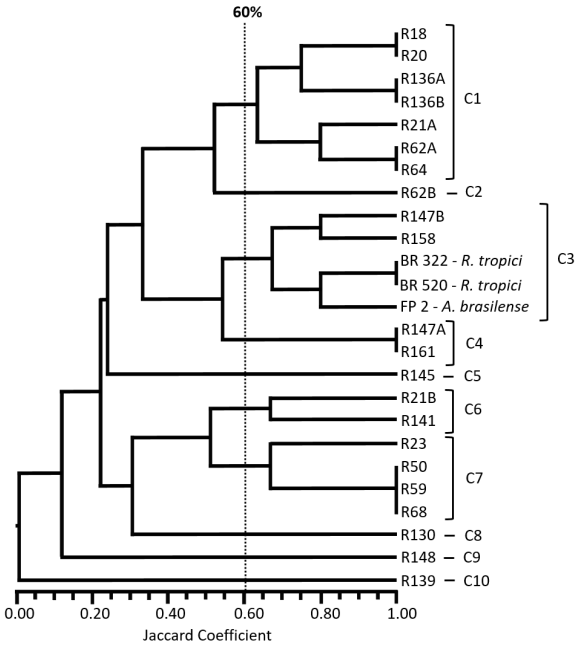

Figure 2. Similarity dendrogram created by the NTSYS-pc® 2.10 software based on the molecular characterization defined by the UPGMA algorithm by Jaccard coefficient using bacteria isolates from rice plant and three standard strains: *Rhizobium tropici* (BR 322 and BR 520) and *Azospirillum brasilense* (FP2). It was considered at 60 % similarity as cut-off point for the clustering of the isolates.

The PCR dendrogram for the 16-23S rDNA intergenic spacer indicate a genotypic diversity among bacterial populations isolated from rice plants grown in the Cerrado biome. Isolates R130, R148, and R139 presented the greatest genotypic distance from the other isolates. The clustering enabled to identify that only a few isolates were closely related to the family Rhizobiaceae, as found in the other assays. Several studies have reported the effectiveness of estimating bacterial diversity associated with grasses and legumes through analyses of the intergenic spacer region (DINESH et al., 2015). This is possible due to variations in the length of this region, which can be used to distinguish bacterial strains and taxonomically related species (CARDOSO et al., 2017).

Bacteria are considered growth-promoting rhizobacteria (PGPR) when they present at least one trait related to the improvement of plant growth, such as hydrolytic enzyme production, phosphate solubilization, phytohormone or siderophore production, or biological nitrogen fixation (GLICK, 2012). Therefore, 19 of the evaluated isolates exhibited hydrolytic enzyme activity and/or phosphate solubilization, which can characterize them as PGPR. Notably, isolates R59, R68, and R141 showed positive activity for two different hydrolytic enzymes and were able to solubilize inorganic phosphate under both acidic and basic conditions.

Furthermore, several studies have focused on characterizing the bacterial diversity associated with grasses and selecting these microorganisms for technological application in agriculture (IKEDA et al., 2013; JI; GURURANI; CHUN et al., 2014; MAREQUE et al., 2015; HERNÁNDEZ-FORTE; NÁPOLES-GARCÍA, 2017). Endophytic bacteria from roots of rice plants grown in the Cerrado biome in Brazil have a high potential to be used in several technological processes. Additionally, these isolates have some traits that indicate their potential as PGPR. Further bioprospecting studies involving these isolates may be a great strategy to reduce crop production costs and provide a sustainable alternative for the development of bioinoculants for agricultural use.

CONCLUSIONS

Most of the bacterial isolates from roots of rice plants utilized monosaccharides and disaccharides as carbon sources, formed viscous colonies, showed catalase, amylase, protease, lipase, and cellulase activities, and were resistant to nalidixic acid.

Approximately half of the isolates presented a rapid growth rate, abundant mucus production, and phosphate solubilization, whereas a few of them were resistant to streptomycin.

The mean enzymatic index for amylase, cellulase, and protease was 2.8, 3.5, and 1.7, respectively. Similarity analysis revealed high diversity among the isolates, with similarity indices of 70% (based on morphological characteristics) and 60% (based on analysis of the intergenic region 16S-23S rDNA).

Considering morphophysiological and genotypic characteristics, three promising isolates (R59, R68, and R141) are qualified for further studies under field conditions.

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