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PHYSIOLOGICAL CHARACTERIZATION OF DIAZOTROPHIC BACTERIA ISOLATED FROM *Brachiaria brizantha* RHIZOSPHERE[1]

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ABSTRACT - The objective of this study was to evaluate the ability of diazotrophic bacteria isolated from *Brachiaria brizantha* rhizosphere to grow at different pH values and salt concentrations, to produce indoleacetic acid (IAA), and to solubilize phosphate. Both acidity and salinity tolerance tests were performed on modified solid FAM medium. Acidity resistance tests were performed at pH values of 4.0, 5.0, 6.0, and 7.0, while, salt resistance was evaluated at concentrations of 0.01, 5.02, 9.99, 14.96, 19.98, 24.95, 29.98, 34.94, 39.97, 44.94, and 49.96 gL^{-1} NaCl. For the IAA production assay, bacterial strains were cultivated in liquid DYGS medium both in the absence and presence of tryptophan (Trp). In addition, phosphorus solubilization assay was performed in GL liquid medium. All strains grew at every pH value tested, and a high diversity was observed after salt resistance, IAA production, and phosphate solubilization testing. Strains UNIFENAS 100-51, UNIFENAS 100-52, UNIFENAS 100-60, UNIFENAS 100-63, and UNIFENAS 100-65 were those with the best growth at the highest salt concentrations. Furthermore, in the presence of Trp, strains UNIFENAS 100-63 and UNIFENAS 100-69 were the ones with the highest IAA production. Strain UNIFENAS 100-52 showed the best response to the *in vitro* phosphate solubilization assay. Based on these results, it can be seen that studies related to the physiological and metabolic characteristics of diazotrophic bacterial strains are important to ensure greater success in the field.

Keywords: Plant growth-promoting bacteria. Soil microorganisms. Sustainability in animal production.

CARACTERIZAÇÃO FISIOLÓGICAS DE BACTÉRIAS DIAZOTRÓFICAS ISOLADAS DA RIZOSFERA DE *Brachiaria brizantha*

RESUMO - O objetivo do estudo foi analisar a capacidade das bactérias diazotróficas, isoladas da rizosfera de *Brachiaria brizantha*, crescer em meios com diferentes valores de pH e concentrações salinas, de produzir ácido 3-indol acético (AIA) e solubilizar fosfato. Os testes de acidez e salinidade foram realizados em meio FAM sólido. Para acidez foram usados os valores de 4.0, 5.0, 6.0 e 7.0. Para salinidade foram utilizadas as seguintes concentrações: 0.01; 5.02; 9.99; 14.96; 19.98; 24.95; 29.98; 34.94; 39.97; 44.94 and 49.96 g□L^{-1}. No teste de produção de AIA, as estirpes foram cultivadas em meio DYGS líquido, na ausência e presença de triptofano (Trp). A solubilização de fósforo, foi realizada em meio GL líquido. Todas as estirpes foram capazes de crescer em todos os valores de pH analisados. Já em relação aos testes de diferentes concentrações salinas, produção de AIA e solubilização de fosfato, foi verificada alta diversidade, havendo estirpes que se destacaram. As estirpes UNIFENAS 100-51; UNIFENAS 100-52; UNIFENAS 100-60; UNIFENAS 100-63 e UNIFENAS 100-65, foram as que apresentaram maior crescimento nas três maiores concentrações salinas. Na presença de triptofano, as estirpes UNIFENAS 100-63 e UNIFENAS 100-69 foram as que apresentaram maior produção de AIA. Já em solubilização de Fosfato *in vitro,* a estirpe UNIFENAS 100-52 foi a que obteve melhor resposta. Baseado nesses resultados, observa-se a importância de estudos relacionados às características fisiológicas e metabólicas de bactérias diazotróficas, de modo a garantir maior sucesso no campo.

Palavras-chaves: Bactérias promotoras de crescimento. Microrganismos do solo. Sustentabilidade na produção animal.

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INTRODUCTION

In Brazil, about 50% of the cultivated areas are pastures (BRASIL, 2006) with a predominance of grasses of the genus *Brachiaria*. However, due to factors such as excessive stocking and lack of proper management for weed and pest control and nutrient replenishment, it has been estimated that a large proportion of pasture soils (more than 50%) are being degraded (MACEDO et al., 2014). This is negatively affecting the productive capacity and competitiveness of the livestock sector. Therefore, there is a need to develop management techniques and practices to recover these soils and to make them more productive. According to Neves Neto et al. (2013), in degraded soils, the forages present low nutritional value; therefore, soil degradation and pasture are directly related. Nitrogen fertilization is a fundamental practice, with emphasis on nitrogen fertilizers, which promotes positive changes in the structural and morphological characteristics of pasture, such as leaf area index and tiller grass density, leading to a greater dry matter accumulation of forage (LARA et al., 2012).

However, it is necessary to evaluate the impact of nitrogen fertilization, since nitrogen is one of the nutrients that suffers more biochemical transformations in the soil. One of these reactions results in the formation of nitrous oxide (N_2O) emissions, considered as greenhouse gases (NOGUEIRA et al., 2015). According to Nogueira et al. (2015), nitrogen fertilization is the major factor that influences N_2O emissions. N_2O emissions have been found to be higher in managed pastures than in unmanaged areas. Thus, low doses of nitrogen fertilizers result in lower N_2O emissions and may be an effective management technique (RODRIGUES et al., 2017).

The use of N_2-fixing bacteria, also known as diazotrophic bacteria, is one of the alternatives for a partial or total replacement of nitrogen fertilizers. Studies by Reis et al (2004) indicate that these bacteria are widely distributed in pasture soils. In addition to contributing with nitrogen to the plant, some diazotrophic bacteria can produce phytohormones such as indoleacetic acid (IAA), which accelerates plant growth (GOPALAKRISHNAN et al., 2015) and increases the availability of nutrients, such as phosphorus (P) and potassium (K), through the solubilization of nutrients present in minerals or rocks (FLORENTINO et al., 2017). Therefore, these bacteria can contribute to the development of forage grasses.

After nitrogen, P is the most limiting nutrient for plant growth. Soil microbes help in P release into the environment from which plants absorb only soluble P in its monobasic ($H_2PO_4^-$) and dibasic ($H_2PO_4^{2-}$) forms (BHATTACHARYYA; JHA, 2012).

Soil salinization is one of the most serious forms of soil degradation, and similar to plants, diazotrophic bacteria present great variation in tolerance to salinity. Finding strains that tolerate this condition is important to increase symbiotic performance and maintain plant productivity sustainably (NÓBREGA et al., 2004). However, the density and diversity of these microorganisms in the soil is influenced both by edaphoclimatic conditions and vegetation type (VITORAZI FILHO et al., 2012). Studies by Guimarães et al. (2011) and Bolsa et al. (2016) report the potential contribution of inoculation with some bacterial strains to the development of *Brachiaria* spp. Still, studies of inoculation with nitrogen-fixing bacteria in forage grasses are incipient, requiring *in vitro* research studies to identify desirable physiological properties in bacterial strains that could potentially promote grass growth and soil enrichment with a great success when applied in the field.

Thus, the objective of this study was to evaluate the capacity of bacterial strains isolated from *Brachiaria brizantha* cv. Marandu rhizosphere to grow at different pH values and salt concentration, to produce IAA, and to solubilize phosphate.

MATERIAL AND METHODS

Identification of bacterial strains

We selected 18 strains of diazotrophic bacteria isolated from the rhizospheric soil of *Brachiaria brizantha* cv. Marandu (DIAS, 2015). Table 1 shows the main morphological characteristic of these strains when grown in FAM medium (MAGALHÃES; DÖBEREINER, 1984) with the following composition: 5 g sucrose, 0.12 g KH_2PO_4, 0.03 g K_2HPO_4, 0.2 g $MgSO_47H_2O$, 0.02 g $CaCl_2$, 0.066 g FeEDTA, 0.1 g NaCl, 0.002 g $NaMoO_4.2H_2O$, 0.00235 g $MnSO_4$, 0.0028 g H_3BO_3, 8×10-5 g $CuSO_4.5H_2O$, 0.00024 g $ZnSO_4.7H_2O$, 0.1 mg biotin, 0.2 mg pyridoxine HCl, 1.75 g agar, 1000 mL water, adjusted to a pH = 6.0. Additionally, Table 1 presents the morphological characteristics of Embrapa strains grown in culture media 79 containing the following composition: 10 g mannitol, 0.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.5 g yeast extract, 1000 mL water, adjusted to a pH = 6.8, and 15 g agar.

Table 1. Identification, medium used for bacterial isolation, and morphological characteristics of strains isolated from the rhizospheric soil of *Brachiaria brizantha* cv. Marandu in FAM medium containing bromothymol blue as pH indicator.

Identification of strains	Culture medium of origin (FAM)	Morphological characterization		
		pH[1]	Colony color[2]	EPS[3]
Strains used in the study				
UNIFENAS 100-01	JNFb	N	y	++++
UNIFENAS 100-02	JMV	N	Cr	++
UNIFENAS 100-13	JMV	N	Cr	+++
UNIFENAS 100-16	JNFb	N	Cr	++++
UNIFENAS 100-21	LGI	N	Cr	+++
UNIFENAS 100-35	JMV	N	y	++
UNIFENAS 100-39	LGI	Ac	y	++++
UNIFENAS 100-40	LGI	Ac	y	++
UNIFENAS 100-45	LGI	Ac	y	+++
UNIFENAS 100-51	JMV	Ac	y	++++
UNIFENAS 100-52	JMV	Al	Cr	+
UNIFENAS 100-60	LGI	Al	Cr	++
UNIFENAS 100-63	LGI	Al	Cr	+++
UNIFENAS 100-65	JMV	Al	y	++
UNIFENAS 100-69	LGI	Al	y	+++
UNIFENAS 100-71	LGI	N	y	+
UNIFENAS 100-78	JMV	N	y	+++
UNIFENAS 100-94	NFb	N	y	+++
Strains from Embrapa collection				
Morphological characteristics in FAM medium				
Ab-V5 (*Azospirillum brasilense*)		Al	Cr	++
Morphological characteristics in medium 79				
BR 29 (*Bradyrhizobium elkanii*)		Al	Wh	+
BR 322 (*Rhizobium tropici*)		Ac	y	+++
BR 2003 (*B. elkanii*)		Al	Wh	+
BR 8802 (*Rhizobium* sp.)		Ac	y	++

[1] pH reaction in culture medium after the growth of the strain, evaluated by the color change of the indicator (N – Neutral, Ac – Acid, Al – Alkali).
[2] Colony color (y – yellow, Cr – cream, Wh – white).
[3] Production of exopolysaccharides: (EPS; ++++ – High, +++ – Intermediate, ++ – low, + – scarce).

Growth in media containing different pH values and salt concentrations

Two experiments were carried out to evaluate the growth of the strains in culture media containing different pH values and salt concentrations. For both the tests, strains were cultivated in liquid FAM medium for 3 days, until the strains reached the log phase of their growth, with a cell density of approximately 10^8 cells.mL^{-1}. Next, 1 mL of each strain culture was transferred to a 1.5 mL sterile microtube and centrifuged at 8,000 rpm at 4°C for 10 minutes. The supernatant was discarded and cells were resuspended in 1 mL of a sterile saline solution (0.85% NaCl) and centrifuged again. This cell washing process was repeated three times to remove residues from the culture medium that could result in false positives, and then resuspended (NÓBREGA et al., 2004).

Next, 100 µL aliquots of the cell suspensions were inoculated and streaked with a Drigalsky loop on plates containing modified solid FAM medium with different concentrations of NaCl (g.L^{-1}): 0.01 (control treatment), 5.02, 9.99, 14.96, 19.98, 24.95, 29.98, 34.94, 39.97, 44.94, and 49.96. All treatments were randomized, with three replicates. After 7 days of incubation at 28°C, the presence (+) or absence (-) of bacterial growth was evaluated, based on the ability to grow at the highest salt concentration as the maximum tolerated concentration.

To evaluate bacterial growth at different pH values, medium 79 was adjusted to the following pH values: 4.0, 5.0, 6.0, and 7.0 (FLORENTINO et al., 2012). As treatment-control, the FAM culture medium was used with a pH value of 6.8. Treatments were randomly distributed with four replicates. Aliquots were streaked and plates were incubated at 28°C for 7 days; the presence (+) or absence (-) of bacterial growth was then evaluated.

Production of indoleacetic acid

To determine IAA production, the 18 strains isolated from *B. brizantha* and a positive control, *A. brasilense* strain Ab-V5, were cultivated in DYGS medium in the absence and presence (100 µg.mL^{-1}) of tryptophan (Trp), according to the methodology described by Pedrinho et al. (2010). DYGS medium was composed of 2 g glucose, 1.5 g peptone, 2 g yeast extract, 0.5 g KH_2PO4, 0.5 g $MgSO_4.7H_2O$, 1.5 g glutamic acid, and 1000 mL water; pH was adjusted to 6.8. This experiment was performed

according to a completely randomized design with a factorial scheme of 19 × 2, which refers to the 19 bacterial strains and 2 experimental conditions (the presence or absence of Trp in the culture medium) tested.

IAA concentration was evaluated by a quantitative colorimetric method, during the log phase of bacterial growth with approximately 10^8 UFC.mL^{-1}. Final IAA concentration was estimated with the help of a standard curve previously obtained using sterilized DYGS medium and known concentrations of IAA (0, 25, 50, 75, and 100 µg.mL^{1}). Absorbance was measured with a spectrophotometer (Shimadzu UV-1800) at a wavelength of 535 nm.

Natural phosphate solubilization *in vitro*

In the present study, in addition to the 18 strains isolated from *B. brizantha*, the following five strains from the Embrapa Agrobiology collection were also used: *Azospirillum brasilense* Ab-V5, *Bradyrhizobium elkanii* BR 29 and BR 2003, *Rhizobium tropici* BR 322, and *Rhizobium* sp. BR 8802. All bacterial strains were cultivated in Petri dishes containing FAM medium for 3 days at 28 °C.

After the 7-day incubation period, supernatants were separated by centrifugation (10,000 rpm, at 4° C, for 10 min) and their final pH values were measured on a pH meter. The concentration of soluble P was analyzed by a spectrophotometer (brand TECNAL, model 1105) (TEDESCO et al., 1995).

Statistical analyses

Data obtained from the experiments described above were submitted to analysis of variance, and the means of the replicates were compared by the Scott-Knott test, at 5% probability, using the Sisvar program (FERREIRA, 2011).

For the phosphate solubilization test, a linear correlation analysis was also performed between the P concentration and pH value from each treatment, using the AgroEstat - version 1.0 program (BARBOSA; MALDONADO JÚNIOR, 2011).

RESULTS AND DISCUSSION

Growth in media containing different pH values and salt concentrations

The 18 isolated strains from the rhizosphere of *B. brizantha* cv. Marandu presented a high growth diversity at different concentrations of NaCl in FAM medium (Table 2).

Table 2. Maximum tolerated concentrations (MCT) of NaCl (g.L^{-1}) in FAM medium by strains of diazotrophic bacteria isolated from rhizospheric soils under *Brachiaria brizantha* cv. Marandu.

Isolates from *B. brizantha* rhizosphere	Maximum tolerated NaCl concentration (g.L^{-1})
UNIFENAS 100-52	49.96
UNIFENAS 100-51; UNIFENAS 100-60	44.94
UNIFENAS 100-63; UNIFENAS 100-65	39.97
UNIFENAS 100-01; UNIFENAS 100-13; UNIFENAS 100-16	34.94
UNIFENAS 100-02; UNIFENAS 100-94	29.97
UNIFENAS 100-21; UNIFENAS 100-39	24.95
UNIFENAS 100-35; UNIFENAS 100-45	19.98
UNIFENAS 100-40	14.96
UNIFENAS 100-78	9.99
UNIFENAS 100-71	5.02
UNIFENAS 100-69	0.01

Strains UNIFENAS 100-51, UNIFENAS 100 -52, UNIFENAS 100-60, UNIFENAS 100-63, and UNIFENAS 100-65 grew in the 3 highest salt concentrations, while strain UNIFENAS 100-69 presented the lowest capacity of growth when compared to the others, growing only at the control condition of 0.01 g.L^{-1} NaCl

Production of exopolysaccharides (EPS) by the bacterial cell is one of the mechanisms that confers the strains a greater tolerance to stress conditions, since EPS provides cellular protection and avoids water loss (XAVIER et al., 2007). After analyzing the EPS production of the strains under study (Table 1), it was not possible to determine a relationship between these characteristics. Moreover, the same phenomenon was observed in studies carried out by Nóbrega et al. (2004), where no tolerance differences were observed between strains that produced EPS (rapid growth) and those with slow growth.

Most living cells respond to extracellular osmolality changes by modulating their own cytoplasmic osmolality (MILLER; WOOD, 1996). According to Santos et al. (2012), microorganisms use flexible osmoadaptation strategies that allow

them to respond to salinity fluctuations in the external environment. Similar to plants, rhizobia produce several groups of metabolites such as solutes [e.g., trehalose, N-acetylglutaminylglutamine amide (NAGGN), and glutamate], osmoprotectants [e.g., betaine, glycine betaine, proline betaine, glucans, trehalose, sucrose, ectoine, 3-dimethylsulfoniopropionate (DMSP), and 2-dimethylsulfonioacetate (DMSA)], and pipecolic acid and cations (calcium, potassium) as components of their tolerance mechanisms. (STREETER, 2003; SUGAWARA; CYTRYN; SADOWSKY, 2010).

The high diversity of diazotrophic bacteria tolerant to different salt concentrations has also been observed in previous studies (NÓBREGA et al., 2004; THRALL et al., 2009). Furthermore, the use of bacterial strains tolerant to salinity could guarantee a greater efficiency in the N_2-fixing process under these conditions (SUBBARAO et al., 1990), justifying the importance of these studies.

In relation to pH values, it was observed that all the strains were able to grow at all the pH values tested. This was consistent with the results obtained by Florentino et al. (2012) for strains of N_2-fixing bacteria of the genus *Cupriavidus*, indicating a high adaptability of these bacteria to soil conditions. Considering the importance of the biological nitrogen fixation process carried out by bacteria that can establish ecological interactions with plants through association or symbiosis, tests to evaluate bacterial tolerance to different pH values are relevant since agricultural soils have a wide range of pH variation.

Production of indoleacetic acid

Production of IAA among tested strains was highly variable, as displayed in Table 2. Strains UNIFENAS 100-63 and UNIFENAS 100-69 produced high amounts of IAA both in the presence and absence of Trp. Moreover, Ab-V5 was one of the strains that produced the highest amounts of IAA, but only in the presence of Trp. Similar results for strain Ab-V5 have been obtainedpreviously (PEDRINHO et al., 2010; FLORENTINO et al., 2017).

Among the benefits of the interaction of bacteria with forage grasses, the synthesis of IAA stands out (FIGUEREDO et al., 2016). IAA is a phytohormone that contributes to plant growth, directly acting on root development. In the present study, a higher production of this substance was mostly observed when bacteria were cultivated in a tryptophan-rich medium (Table 3), which can be explained by the fact that this amino acid acts as an important precursor for IAA synthesis.

According to Naveed et al. (2015), L-tryptophan can promote an increase in bacterial productivity of IAA, since its presence in culture medium causes a stimulatory effect in IAA biosynthesis pathways. Thus, the production of IAA may be dependent on the concentration of Trp in the culture medium (CHAGAS-JUNIOR et al. 2009).

Table 3. IAA production by bacterial strains isolated from *Brachiaria brizantha* cv. Marandu, grown in DYGS medium with or without tryptophan.

Bacterial strain	IAA (μgmL^{-1})	
	Tryptophan	
	With	Without
UNIFENAS 100-01	7.30 F a	6.14 D a
UNIFENAS 100-02	37.64 B a	12.90 C b
UNIFENAS 100-13	8.32 F a	8.91 C a
UNIFENAS 100-16	22.19 D a	10.03 C b
UNIFENAS 100-21	12.89 E a	11.89 C a
UNIFENAS 100-35	12.35 E a	6.90 D b
UNIFENAS 100-39	13.86 E a	5.66 D b
UNIFENAS 100-40	14.46 E a	7.94 C b
UNIFENAS 100-45	6.19 F a	7.05 C a
UNIFENAS 100-51	7.60 F a	7.02 C a
UNIFENAS 100-52	13.06 E a	12.04 C a
UNIFENAS 100-60	16.27 D a	3.66 D b
UNIFENAS 100-63	45.16 A a	33.29 A b
UNIFENAS 100-65	35.36 B a	22.79 B b
UNIFENAS 100-69	49.67 A a	37.04 A b
UNIFENAS 100-71	15.23 E a	3.99 D b
UNIFENAS 100-78	5.85 F a	2.39 D b
UNIFENAS 100-94	27.43 C a	3.88 D b
Ab-V5 – *Azospirillum brasilense*	49.03 A a	22.05 B b

Means followed by distinct letters, uppercase in the column and lowercase in the row, differ by Scott Knott's test at a 5% probability.

Our results on the concentration of IAA produced by bacteria were similar to those obtained by other authors (TSAVKELOVA et al., 2006). Machado et al. (2013), investigating the biosynthesis of IAA in culture medium by diazotrophic plant growth-promoting bacteria, observed that 90% of the bacteria that produced IAA in the presence of Trp were also capable of producing indoleacetic acid in its absence, but at lower concentrations. The same can be observed in Table 2, where IAA concentrations were generally higher in media containing Trp. However, further studies are needed to evaluate the contribution of these strains in providing IAA for plants.

Natural Phosphate Solubilization *in vitro*

Solubilization of reactive natural phosphate in the culture medium assay revealed that the levels of P ($g.L^{-1}$) present in the GL medium after inoculation were higher than in the control treatment, except for the treatment inoculated with *Azospirillum brasilense* strain Ab-V5. It was also observed that strains differed in their ability to solubilize phosphate (Table 4). Similar results were obtained by Filho, Narloch, and Scharf (2002) who found that the solubilization potential varied between strains. According to Pineda (2014), this is due to the ability of the strains to reduce pH through the release of organic acids.

It can also be observed that strains that solubilized significant amounts of P showed higher acidification of the medium (Table 4), which was confirmed by the correlation analysis between pH and P content as shown in Figure 1. Several factors may have affected this relationship, such as the amount of P immobilized by microorganisms during growth and the phosphate source.

Table 4. Phosphate solubilization capacity, performed under *in vitro* conditions ($g.L^{-1}$), by diazotrophic bacterial strains isolated from *Brachiaria brizantha* cv. Marandu rhizosphere.

Bacterial strain	P (gL^{-1})	pH
Control (non-inoculated)	0.36 k	6.72 b
Strains used in the study		
UNIFENAS 100-01	6.58 f	3.37 i
UNIFENAS 100-02	6.27 f	4.93 e
UNIFENAS 100-13	5.95 f	3.52 i
UNIFENAS 100-16	1.97 i	6.18 c
UNIFENAS 100-21	4.62 g	5.42 d
UNIFENAS 100-35	5.67 f	4.12 g
UNIFENAS 100-39	8.20 d	3.28 i
UNIFENAS 100-40	10.30 c	4.29 g
UNIFENAS 100-45	7.57 e	4.44 f
UNIFENAS 100-51	11.60 b	3.71 h
UNIFENAS 100-52	13.30 a	3.43 i
UNIFENAS 100-60	5.72 f	4.70 f
UNIFENAS 100-65	2.30 i	5.34 d
UNIFENAS 100-63	2.90 h	4.16 g
UNIFENAS 100-69	7.62 e	4.22 g
UNIFENAS 100-71	8.37 d	3.75 h
UNIFENAS 100-78	10.45 c	3.88 h
UNIFENAS 100-94	1.25 j	6.19 c
Strains from Embrapa collection		
Ab-V5 –*Azospirillum brasilense*	0.62 k	7.03 a
BR 322 – *Rhizobium tropici*	1.92 i	5.65 d
BR 8802 – *Rhizobium* sp.	2.95 h	4.66 f
BR 29 – *Bradyrhizobium elkanii*	1.37 j	5.98 c
BR 2003 – *Bradyrhizobium elkanii*	11.95 b	3.22 i

Means followed by the same letter do not differ statistically from each other by the Scott-Knott test at a 5% probability.

The correlation between the pH value and soluble P, shown in Figure 1, is an inversely proportional relation, in which soluble P concentrations increase as pH values decrease.

Among tested strains, the best P solubilization results were obtained with the strain UNIFENAS 100-52, followed by BR 2003 and UNIFENAS100-51 which were statistically similar to each other. The only strain that did not increase P concentration in the medium was *A. brasilense* Ab-V5, whose result was statistically equal to that from the control treatment.

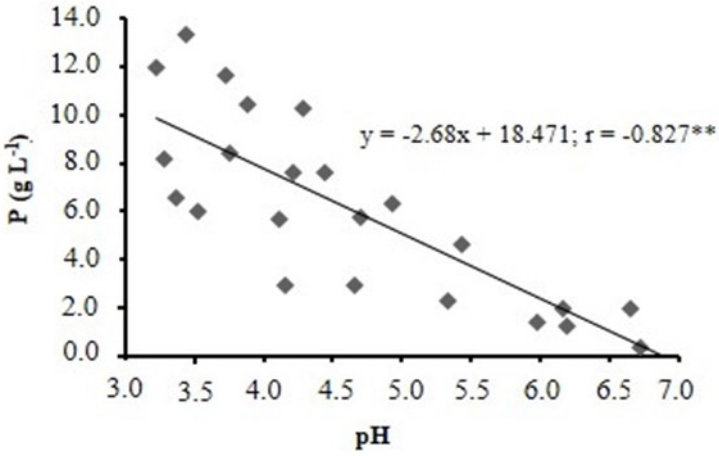

Figure 1. Correlation between pH value and phosphorus concentration in culture media containing different diazotrophic bacterial strains.

Studies have shown that the ability of the strains to solubilize phosphate is correlated with a pH decrease in the medium due to the synthesis of organic acids (HARA; OLIVEIRA, 2005). Secreted organic acids may either dissolve the mineral phosphate due to an exchange of PO_4^{3-} anions or chelation of $Fe^{2+/3+}$ and Al^{3+} ions associated with phosphate. According to Kang et al. (2008), the action of the acids is more related to the concentration of H^+ itself than to the specific type of organic acid synthesized.

All the strains, except Ab-V5, decreased the pH of the medium during culturing. The least changes occurred with strains UNIFENAS 100-94, UNIFENAS 100-16, and BR 29, which did not differ statistically. The lowest pH values were obtained by strains UNIFENAS 100-13, UNIFENAS 100-52, UNIFENAS 100-1, UNIFENAS 100-39, and BR 2003.

CONCLUSION

It was demonstrated that diazotrophic bacterial strains isolated from rhizospheric soils from *Brachiaria brizantha* cv. Marandu present different behaviors in relation to tolerance to high concentrations of salt, production of indoleacetic acid, and solubilization of phosphate. Strain UNIFENAS 100-52 presented the best response in the tests of tolerance to salinity and solubilization of phosphorus; in tests conducted to assess the production of IAA, strains UNIFENAS 100-63 and UNIFENAS 100-69 presented the highest production values. The use of these strains may increase biological nitrogen fixation and phosphorus availability in the soil solution and promote plant growth. More studies are recommended to evaluate the potential benefits of these bacteria on forage grasses, ensuring a greater success in the field.

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