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Characterization of associative diazotrophic bacteria in torch ginger

Caracterização de bactérias diazotróficas associativas em bastão-do-imperador

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Highlights:
Associative diazotrophic bacteria.
Potassium and phosphorus solubilization.
Phytohormone production.

Abstract

Associative diazotrophic bacteria perform several processes that promote increased plant development and production, allowing a reduction in the use of agricultural inputs and costs. However, for some species, such as torch ginger, there are still no reports of studies aimed at identifying diazotrophic bacteria associated with this species. On this basis, this study proposes to isolate and characterize associative diazotrophic bacteria in rhizospheric soils and roots of torch ginger as well as analyze the potential of these isolates in solubilizing phosphorus (P) and potassium (K) and producing indole-3-acetic acid (IAA). Soil and roots samples of torch ginger were inoculated into five different semi-solid and semi-selective culture media, namely, NFb, JNFb, LGI, JMV and FAM, where bacterial growth was diagnosed by the formation of a characteristic film on the surface of the media. Subsequently, the bacterial isolates were analyzed for their ability to solubilize P and K in liquid medium, using phosphate rock powder (AO-15) and potassium rock powder (phonolite) as sources of P and K, respectively. All culture media showed bacterial growth, making this the first report of isolation of diazotrophic bacterial strains in this species. Eight of the obtained strains originated from rhizospheric soils and four from roots of torch ginger. Of these, 10 solubilized P, with the UNIFENAS 100-340, UNIFENAS 100-342 and UNIFENAS 100-348 strains standing out. Six strains showed K solubilizing ability, UNIFENAS 100-346 being the most efficient. All strains were able to produce the IAA phytohormone, both in the presence and absence of tryptophan, with superior results obtained by UNIFENAS 100-344 and UNIFENAS 100-351.

Key words: Biotechnology. Ornamental plants. Plant growth-promoting bacteria.

Resumo

As bactérias diazotróficas associativas realizam diversos processos que promovem o maior desenvolvimento e produção vegetal, reduzindo o uso de insumos agrícolas e custos. No entanto, para

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algumas espécies, como o bastão-do-imperador, ainda não existem relatos de estudos visando identificar as bactérias diazotróficas associadas à esta espécie. Objetivou-se isolar e caracterizar bactérias diazotróficas associativas em solos rizosféricos e raízes de bastão-do-imperador e analisar o potencial destes isolados em solubilizar fósforo (P) e potássio (K) e produzir ácido 3-indol acético (AIA). As amostras de solos e raízes do bastão-do-imperador foram inoculadas em cinco diferentes meios de cultura semi-sólidos e semisseletivos, NFb, JNFb, LGI, JMV e FAM, em que o crescimento bacteriano foi diagnosticado pela formação de película característica na superfície dos meios. Posteriormente, os isolados bacterianos foram analisados quanto à capacidade de solubilizar P e potássio K em meio líquido, utilizando pó de rocha fosfatada, AO-15 e potássica, fonolito, como fontes de P e K, respectivamente. Todos os meios de cultivo utilizados apresentaram crescimento bacteriano, sendo este o primeiro relato de isolamento de estirpes bacterianas diazotróficas desta espécie. Das estirpes obtidas, oito foram de solos rizosféricos e quatro das raízes do bastão-do-imperador. Destas, 10 solubilizaram P, sendo as estirpes UNIFENAS 100-340 UNIFENAS 100-342 e UNIFENAS 100-348 as que se destacaram. Quanto a solubilização de K, seis estipes apresentaram a capacidade de solubilização, sendo a estirpe UNIFENAS 100-346 a mais eficiente. Em relação à produção de AIA, todas foram capazes de produzir esse fitormônio, tanto na presença quanto ausência do triptofano, com destaque para as estirpes UNIFENAS 100-344 e UNIFENAS 100-351.

Palavras-chave: Bactérias promotoras crescimento vegetal. Biotecnologia. Plantas ornamentais.

Introduction

Nitrogen is one of the nutrients most required by the majority of plants, and the use of nitrogen fertilizers accounts for a significant share of production costs (Hungria, Nogueira, & Araujo, 2013). Although atmospheric air consists of about 78% nitrogen gas (N_2), this form is inaccessible to most living beings and only a portion of prokaryotes N_2 fixers or diazotrophs are able to convert atmospheric nitrogen (N_2) to ammonia (NH_3), through an enzymatic complex called nitrogenase. This process, known as biological nitrogen fixation (BNF), has great agricultural and environmental importance (Reis et al., 2004).

Biological nitrogen fixation can contribute to plant development through interactions of symbiosis or association between these bacteria and plants. In associations, the bacteria are found in plant tissues, endophytically and in the rhizosphere. Studies have proven the benefits of inoculating diazotrophic bacterial strains for the development of maize (Breda, Alves, & Reis, 2016), wheat (Mumbach et al., 2017), rice (Beutler et al., 2012) and sugarcane (Donato, Andrade, Souza, França, & Maciel, 2004). In addition to BNF, these bacteria perform other processes, e.g., production of siderophores (Fedrizzi, 2006), solubilization of phosphate (Gomes, Oliveira, Silva, & Marriel, 2014) and potassium (Uroz, Dessaux, & Oger, 2009) and production of phytohormones such as indole-3-acetic acid (IAA) (Florentino, Silva, Landgraf, & Souza, 2017a), thus acting as plant growth promoters.

Despite the various benefits arising from the use of these bacteria, the adoption of this biotechnology is still restricted to some species of economic importance legumes and grasses used in human and animal nutrition, mainly. Thus, there is a need to expand the knowledge about microorganisms associated with other plants. However, for this to be possible, it is important to consider that each plant species has a unique microbial community in its tissues and rhizosphere (Costa et al., 2014).

The family Zingiberaceae has around 52 genera and 1300 species, which can be herbaceous or rhizomatous and have diverse uses, such as ornamental, food and medicinal (Yunus, Aziz, Kadir, & Rashid, 2012). Torch ginger (*Etlingera elatior*) stands out among the ornamental species due to its great commercial importance owing to its large size and lush inflorescences with colored bracts (Machado, Jasmin, & Ponciano, 2013). As stated by Chakraborty, Kundu, Mukherjee

and Ghosh (2019), endophytic microorganisms contribute to increasing productivity and tolerance to biotic and abiotic stresses in plants of the family Zingiberaceae. Nonetheless, for the specific case of torch ginger, there are no reports on the microorganisms associated with the species.

Therefore, this study was developed to isolate and characterize associative diazotrophic bacteria in rhizospheric soils and roots of torch ginger as well as analyze the potential of these isolates to solubilize phosphorus and potassium and to produce IAA.

Material and Methods

Diazotrophic bacteria were isolated from roots of torch ginger and from soil samples collected near the plants, in the germplasm bank of the floriculture section of José do Rosário Vellano University (UNIFENAS), located in Alfenas-MG (geographical coordinates: 21°25'46'' S and 45°56'50'' W, at 880 m of altitude).

Each plant was considered an experimental unit and eight composite samples were used (four root and four soil samples). The soil was classified as a Dystrophic Red Latosol (Oxisol), according to the Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA] (2013) Brazilian soil classification system. A composite sample was made from four single samples collected at 10 cm from the plant stem, at a depth of 0-20 cm. The collected material (soil and roots) was kept in cool boxes at 4 ºC and taken to the Soil Microbiology Laboratory at UNIFENAS.

Soil samples were subjected to routine chemical characterization (Silva, Lima, Teixeira, Motta, & Santana, 2011), and the following results were obtained: pH = 5.3; P = 75 mg dm^{-3}; Ca^{2+} = 1.3 cmolc dm^{-3}; Mg^{2+} = 0.6 cmolc dm^{-3}; Al^{3+} = 0.4 cmolc dm^{-3}; H + AL = 5.0 cmolc dm^{-3}; and OM = 11 g dm^{-3}.

To isolate the diazotrophic bacteria, the soil and root samples were inoculated in flasks containing semi-solid and semi-selective culture media, namely, NFb (*Azospirillum* spp.), JNFb (*Herbaspirillum* spp.), LGI (*A. amazonense*) (Döbereiner, Baldani, V. L. D., & Baldani, J. I, 1995), JMV (*Burkholderia* spp.) (Reis et al., 2004) and FAM (*A. amazonense*) (Magalhães & Döbereiner, 1984). The root samples, around 2 mm, were superficially disinfected in sodium hypochlorite (1% v/v) for 2 min and then washed successively in sterile distilled water, following the methodology proposed by Diniz et al. (2012) and Santos, Santos, Bakke and Bakke (2013). The soil samples, weighing about 100 mg, were inoculated directly into the media.

The flasks containing the media were incubated for 12 days (following the methodology of Diniz et al. (2012) and then diazotrophic bacterial growth was evaluated, which was characterized by the appearance of a whitish film on the surface of the medium.

For the isolation and purification of the bacterial strains, colonies of the semi-solid media were transferred to the same solid media until isolated colonies were obtained. The isolated colonies were inoculated into the original semi-solid media to confirm N_2-fixing ability, which was evidenced by the formation of the film. Isolates that showed N_2-fixing ability were characterized morphologically as suggested by Pires et al. (2018) and received the UNIFENAS code followed by numbers for their identification and conservation in the collection of diazotrophic bacteria.

Bacteria that showed a proven N_2-fixing ability were evaluated for their ability to solubilize phosphate and potassium. The isolates were sub-cultured into FAM culture medium, incubated at 28ºC for three days and then transferred to liquid FAM medium for three days, until the log growth phase.

The phosphorus and potassium solubilization experiments were laid out in a completely randomized design in which the treatments consisted of the bacterial strains and a negative control (without bacterial inoculation). Four replicates were used.

For the solubilization of phosphorus, the methodology of Parmar and Sindhu (2013) was followed, in which 15 µL of the bacterial suspension grown in liquid FAM medium were transferred to 30 mL of liquid GL medium with the addition of 10 g of AO-15 as the phosphorus source. The flasks were kept under agitation (120 rpm, 25ºC) for seven days. Afterwards, the flasks were centrifuged (1000 rpm, 4ºC, 10 min) and the supernatant was used to measure the pH value and the concentration of soluble phosphorus in accordance with the methodology described in Tedesco, Gianello, Bissani, Bohnen and Volkweiss (1995).

For the solubilization of potassium, the method proposed by Florentino et al. (2017b) was employed, in which 500 µL of the bacterial suspension grown in liquid FAM medium was transferred to 50 mL of Aleksandrov medium (Parmar & Sindhu, 2013) containing 10 g of phonolite as the potassium source. The flasks were incubated with shaking (120 rpm, 25ºC) for seven days. Subsequently, the flasks were centrifuged (1000 rpm, 4ºC, 10 min) and the supernatant was used to determine the pH value and the potassium concentration following the methodology described in Lopes-Assad, Rosa, Erler and Ceccato-Antonini (2006).

To determine IAA production, the diazotrophic bacteria isolated from the rhizosphere and roots of the torch ginger were grown in DYGS medium in the absence and presence (100 µg mL^{-1}) of tryptophan, according to the methodology described by Pedrinho, Galdiano, Campanharo, Alves and Lemos (2010). The DYGS medium had its pH value adjusted to 6.8. The experiment was laid out in a completely randomized design in a factorial arrangement (with or without tryptophan) with four replicates in which the treatments consisted of diazotrophic bacteria isolated from rhizospheric soils and roots of torch ginger.

The IAA concentration was evaluated by the quantitative colorimetric method (Gordon & Weber, 1951) after three days of culture, by reading the absorbance in a spectrophotometer at a wavelength of 535 nm.

Phosphorus and potassium solubilization and IAA production data were subjected to analysis of variance, and the means of the four replicates were compared by the Scott-Knott test at 5% probability, using Sisvar Ferreira (2014) software.

Results and Discussion

Twelve diazotrophic bacteria were obtained. Of these, eight were isolated from rhizospheric soils, from the LGI, NFb, JNFb and JMV media; and four from roots of torch ginger, isolated from the FAM and JMV media, the latter being the only medium in which bacteria were isolated from rhizospheric soils and roots. None of these culture media had N in their composition, which favored the growth of diazotrophic bacteria. In addition, they were constituted by different sources of carbon and other supplements, making them specific to each genus/species.

Diazotrophic bacterial strains were obtained from all the media used in this study, indicating greater diversity and, consequently, a greater probability of contribution to plant growth (Hara & Oliveira, 2004). These results also reveal the diversity of bacteria associated with torch ginger, making this the first report of isolation of diazotrophic bacteria for this plant. According to Chakraborty et al., (2019), there is a complex and specific interaction between the host and endophytic microorganisms, warranting the need to identify the microorganisms associated with different plant species.

Morphological data of the 12 bacterial strains were used to construct the similarity dendrogram, as shown in Figure 1.

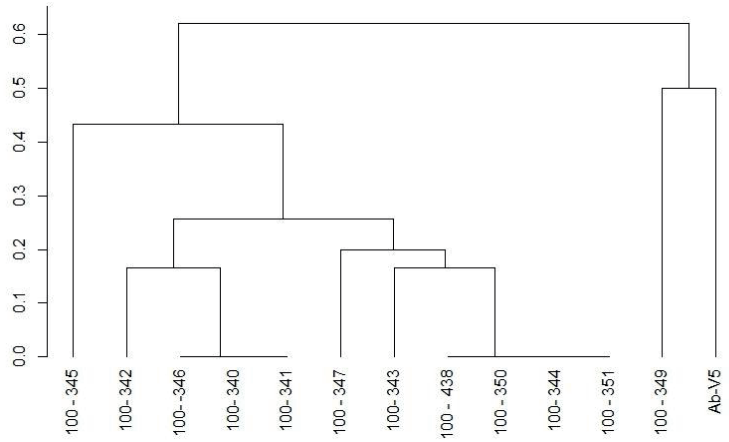

Figure 1. Bacterial-strain clustering dendrogram based on morphological traits of diazotrophic bacteria isolated from rhizospheric soils and roots of torch ginger plants, Cluster Analysis method.

Four distinct groups were obtained at 80% similarity. The first group contained the UNIFENAS 100-345 strain, isolated from the root. The second cluster, which was the largest, was formed by 10 bacterial strains, whose bacterial colonies had highly similar traits regardless of their origin (soil/root). The third group was formed only by the UNIFENAS 100-349 bacterium, isolated from rhizospheric soil in JMV medium. Lastly, the fourth group contained bacterial strain Ab-V5, of the species *Azospirillum brasilense*.

Overall, most strains were able to solubilize phosphorus and potassium from the phosphate and silicate rocks, respectively (Table 1). This table also shows the pH values of the media after bacterial culture.

Table 1
Soluble phosphorus (P), potassium (K) and pH values of culture media supplemented with phosphate and silicate rocks, respectively, and incubated for seven days

Treatment	Phosphorus		Potassium	
	P (mg dm^{-3})	pH	K (mg dm^{-3})	pH
Control	20.33 e	4.41 a	27.50 e	6.93 a
100-340	28.33 e	4.07 a	86.00 c	3.98 c
100-341	195.00 c	4.76 a	6.00 e	4.71 b
100-342	286.00 a	3.51 b	44.75 d	4.40 c
100-343	180.00 c	3.91 b	11.00 e	3.95 c
100-344	176.00 c	4.54 a	26.50 e	3.73 c
100-345	186.00 c	3.81 b	39.33 d	3.95 c
100-346	132.50 d	3.41 b	365.33 a	4.00 c
100-347	243.50 b	3.21 b	6.00 e	3.93 c
100-348	285.50 a	4.02 a	24.50 e	4.67 b
100-349	178.00 c	4.52 a	22.00 e	4.36 c
100-350	52.00 e	4.32 a	58.500 d	5.06 b
100-351	17.00 e	4.08 a	161.50 b	5.11 b

Means followed by the same letters do not differ by the Scott-Knott test at 5%.

For the solubilization of phosphorus, the UNIFENAS 100-342 and UNIFENAS 100-348 strains stood out when compared to with control treatment. It was not possible to establish a relationship between the pH values and the soluble phosphorus content of the different treatments. According to Richardson (2001), the solubilization of phosphorus by microorganisms is the result of acidification, which is caused by the release of organic acids from the culture medium. In the present study, the culture medium containing the UNIFENAS 100-342 and UNIFENAS 100-348 strains showed a soluble phosphorus content approximately 15 times higher than that of control treatment. Between these two strains, UNIFENAS 100-342 showed a pH value below that of control treatment. These results suggest that these bacterial strains have other mechanisms to solubilize phosphorus, corroborating the data obtained by Barroso and Nahas (2008), who described that there was no correlation between the pH value and the solubilized phosphorus content.

The following bacterial strains promoted a decrease in the pH of the medium, when compared with control treatment: UNIFENAS 100-342, UNIFENAS 100-343, UNIFENAS 100-345, UNIFENAS 100-346 and UNIFENAS 100-347.

According to Barroso and Nahas (2008), the release of organic substances from microbial metabolism can reduce the adsorption of phosphorus to soil particles, increasing the availability of this element, which makes it a process of great importance for plant nutrition. Additionally, Garcia, Knaak and Fiuza (2015) reported that the production of siderophores by different groups of microorganisms can increase the release of phosphorus. This occurs due to the affinity of these substances with iron (Fe) that breaks the bonds between iron and phosphorus and consequently increases the availability of phosphorus.

In the solubilization of potassium, the UNIFENAS 100-346 strain was superior to the others and to control treatment, showing the highest soluble potassium value and pH decline in the medium (Table 1). Overall, all culture media containing bacterial strains exhibited lower pH values than control treatment, which can be attributed to the production of organic acids (Meena, Maurya, & Verma, 2014).

Results described by Silva Girio et al. (2015) revealed a correlation between pH decline and the increase in potassium solubilization. The pH of the medium was acidified by the B-30-*Burkhoderia* strain, which increased the solubilization of K by 70% from phonolite rocks. Florentino et al. (2017b) analyzed the capacity of strains of associative diazotrophic bacteria to solubilize K from phonolite and obtained similar results.

All bacterial strains were able to produce IAA, both in the presence and absence of tryptophan (TRP) (Table 2). The UNIFENAS 100-344 and UNIFENAS 100-351 strains produced more IAA in the presence of TRP, with the latter standing out with greater production of this phytohormone in the absence of TRP.

Of the analyzed strains, UNIFENAS 100-341, UNIFENAS 100-344, UNIFENAS 100-348 and UNIFENAS 100-351 produced more IAA in the presence of TRP, and only the UNIFENAS 100-346 strain showed the opposite behavior, producing more IAA in the absence of TRP.

Tryptophan is the precursor amino acid for the synthesis of IAA in the plant metabolism (Baldotto, Baldotto, Olivares, Viana, & Bressan-Smith, 2010), which is directly related to the growth of the apical and root regions of plants (Taiz & Geizer, 2013).

Table 2
Indole-acetic acid (IAA) production by bacterial strains in DYGS medium in the presence and absence of tryptophan (TRP)

Strain	IAA production ($\mu g.L^{-1}$)	
	With TRP	Without TRP
100-340	53.05 Ca	66.32 Ca
100-341	65.13 Ca	40.22 Db
100-342	52.61 Ca	59.84 Ca
100-343	50.41 Ca	52.23 Da
100-344	171.04 Aa	102.92 Bb
100-345	85.88 Ba	70.22 Ca
100-346	40.03 Cb	82.17 Ca
100-347	93.80 Ba	101.79 Ba
100-348	62.55 Ca	39.40 Db
100-349	86.32 Ba	69.65 Ca
100-350	60.22 Ca	48.08 Da
100-351	169.66 Aa	132.08 Ab

Means followed by different uppercase letters in the column and lowercase letters in the row differ from each other by the Scott-Knott test at 5% probability.

Bacterial metabolism is highly versatile, and there are already reports of several bacterial species synthesizing IAA in the absence of TRP (Pedrinho et al., 2010; Florentino et al., 2017a).

Conclusions

The diazotrophic bacterial strains isolated from rhizospheric soils and roots of torch ginger exhibited different morphological traits and are able to solubilize phosphorus and potassium and produce indole-3-acetic acid. Therefore, they can contribute to the development of torch ginger.

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References

Baldotto, L. E. B., Baldotto, M. A., Olivares, F. L., Viana, A. P., & Bressan-Smith, R. (2010). Seleção de bactérias promotoras de crescimento no abacaxizeiro cultivar vitória durante a aclimatização. *Revista Brasileira de Ciência do Solo, 34*(2), 349-360. doi: 10.1590/S0100-06832010000200008.

Barroso, C. B., & Nahas, E. (2008). Solubilização do fosfato de ferro em meio de cultura. *Pesquisa Agropecuária Brasileira, 43*(4), 529-535. doi: 10.1590/S0100-204X2008000400012

Beutler, A N., Muraneto, J. D., Ramão, C. J., Galon, L., Dias, N. P., Pozzebon B. C.,... Ramos, P. V. (2012). Propriedades físicas do solo e produtividade de arroz irrigado em diferentes sistemas de manejo. *Revista Brasileira de Ciência do Solo*, *36*(5), 1601-1607. doi: 10.1590/S0100-06832012000500024

Breda, F. D. F., Alves, G. C., & Reis, V. M. (2016). Produtividade de milho na presença de doses de N e de inoculação de Herbaspirillum seropedicae. *Pesquisa Agropecuária Brasileira, 51*(1), 45-52. doi: 10. 1590/S0100-204X2016000100006.

Chakraborty, A., Kundu, S., Mukherjee, S., & Ghosh, B. (2019). *Endophytism in Zingiberaceae: elucidation of beneficial impact.* Endophytes and Secondary Metabolites, Springer Nature. doi: 10.1007/978-3-319-76900-4_31-1

Costa, D. P., Dias, A. C. F., Durrer. A., Andrade, P. A. M., Gumiere, T., & Andreote, F. D. (2014). Composição diferencial das comunidades bacterianas na rizosfera de variedas de cana-de-açúcar. *Revista Brasileira de Ciência do Solo, 38*(6), 1694-1702. doi: 10.1590/S0100-06832014000600004

Diniz, P. F. A., Oliveira, L. E. M., Lopes, N. A., Florentino, L. A., Carvalho, T. S., & Moreira, F. M. S. (2012). Bactérias diazotróficas em solos sob seringueira. *Revista Brasileira de Ciência do Solo, 36*(5), 1426-1433. doi: 10.1590/S0100-06832012000500006

Döbereiner, J., Baldani, V. L. D., & Baldani, J. I. (1995). *Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas*. Brasília: EMBRAPA-SPI; Seropédica: EMBRAPA-Cnpab, 60.

Donato, V. M. T. S., Andrade, A. G. de, Souza, E. S. de, França, J. G. E., & Maciel, G. A. (2004). Atividade enzimática em variedades de cana-de-açúcar cultivadas in vitro sob diferentes níveis de nitrogênio. Pesquisa Agropecuária Brasileira, *39*(11), 1087-1093. doi: 10.1590/S0100-204X2004001100006.

Empresa Brasileira de Pesquisa Agropecuária (2013). *Sistema brasileiro de classificação de solos. Centro Nacional de Pesquisa de Solos* (3. ed., pp. 353). Brasília: EMBRAPA.

Fedrizzi, S. M. G. (2006). *Produção de Metabolitos antimicrobianos e sideróforos de isolados provenientes de Terra Preta Antropogênica da Amazônia Ocidental*. Tese de Doutorado, Universidade de São Paulo, São Paulo, SP, Brasil.

Ferreira, D. F. (2014). Sisvar: um guia dos seus procedimentos de comparações múltiplas Bootstrap. *Ciência e Agrotecnologia, 38*(2), 109-112. doi: 10.1590/S1413-70542014000200001

Florentino, L. A., Rezende, A. V., Miranda, C. C. B., Mesquita, A. C., Mantovani, J. R., & Bianchini, H. C. (2017b). Potassium solubilization in phonolite rock by diazotrophic bacteria. *Comunicata Scientiae, 8*(1), 17-23. doi: 10.14295/cs.v8i1.1292

Florentino, L. A., Silva, A. B., Landgraf, P. R. C., & Souza, F. R. C. (2017a). Inoculação de bactérias produtoras de ácido 3-indol acético em plantas de alface (Lactuca sativa L.). *Revista Colombiana de Ciencias Hortícolas, 11*(1), 89-96. doi: 10.17584/rcch.2017v11i1.5780

Garcia, T. V., Knaak, N., & Fiuza, L. M. (2015). Bactérias endofíticas como agentes de controle biológico na orizicultura. *Arquivos do Instituto Biológico, 82*, 1-9. doi: 10.1590/1808-1657001262013

Gomes, E. A., Oliveira, C. A., Lana, U. G. P., Silva, U. C., & Marriel, I. E. (2014). *Potencial de microrganismos para solubilização de fosfatos de rocha.* (Boletim de Pesquisa e Desenvolvimento, 97). EMBRAPA.

Gordon, S. A., & Weber, R. P. (1951). Colorimetric estimation of indoleacetic acid. *Plant Physioogy, 26*(1), 192-195. doi: 10.1104/pp.26.1.192

Hungria, M., Nogueira, M. A., & Araujo, R. S. (2013). Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. *Biology and Fertility of Soils, 49*(7), 791-801. doi: 10.1007/s00374-012-0771-5

Lopes-Assad, M. L., Rosa, M. M., Erler, G., & Ceccato-Antonini, S. R. (2006). Solubilização de pó-de-rocha por Aspergillus niger. *Espaço & Geografia, 9*(1), 1-17.

Machado, A. S., Neto, Jasmin, J. M., & Ponciano, N. J. (2013). Indicadores econômicos da produção de flores tropicais no Estado do Rio de Janeiro. *Revista Ceres*, *60*(2), 173-184. doi: 10.1590/S0034-737X2013000200005.

Magalhães, F. M. M., & Dobereiner, J. (1984). Occurrence of *Azospirillum amazonense* in some Amazonian (Brazil) ecosystems. *Revista de Microbiologia, 15*(4), 246-252.

Meena, V. S., Maurya, B. R., & Verma, J. P. (2014). Does a rhizospheric microorganism enhance K + availability in agricultural soils? *Microbiological Research*, *169*(5), 337-347. doi: 10.1016/j.micres.2013.09.003Get

Mumbach, G. L., Kotowski, I. E., Schneider, F. J. A., Mallmann, M. S., Bonfada, E. B., Portela, V. O., & Kaiser, D. R. (2017). Resposta da inoculação com *Azospirillum brasilense* nas culturas de trigo e de milho safrinha. *Scientia Agraria, 18*(2), 97-103. doi: 10.5380/rsa.v18i2.51475

Hara, F. A. S. & Oliveira, L. A. S., (2004). Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos e álicos de Presidente Figueiredo, *Amazonas. 34*(3), 343-357. doi: 10.1590/S0044-59672004000300002

Parmar, P., & Sindhu, S. S. (2013). Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. *Journal of Microbiology Research, 3*(1), 25-31. doi: 10.5923/j.microbiology.20130301.04

Pedrinho, E. A. N., Galdiano, Jr. R. F., Campanharo, J. C., Alves L. M. C., & Lemos, E. G. M. (2010). Identificação e avaliação de rizobactérias isoladas de raízes de milho. *Bragantia*, *69*(4), 905-911. doi: 10. 1590/S0006-87052010000400017

Pires, I. C., Freire, N. B., Fernandes, A. W. C., Souza, R. F. S., Silva, F. A. G., Jr., Oliveira, H. P., & Costa, M. M. (2018). Influência do polipirrol e dos níveis de salinidade na formação de biofilme em Aeromonas spp.. *Pesquisa Veterinária Brasileira, 38*(8), 1528-1536. doi: 10.1590/1678-5150-PVB-5374

Richardson, A. E. (2001). Prospects for using soil microrganism to improve the acquisition of phosphorus by plants. *Australian Journal of Plant Physiology*, 28(9), 897- 906. doi: 10.1071/PP01093

Reis, V. M., Estrada-de Los Santos, P., Tenorio-Salgado, S., Vogel, J., Stoffels, M., Guyon, S., & Balandreau, J. (2004). Burkholderia tropica sp. nov., a novel nitrogen-fixing, plant-associated bacterium. *International Journal of Systematic and Evolutionary Microbiology, 54*(6), 2155-2162. doi: 10.1099/ijs. 0.02879-0

Santos, M. C. M., Santos, D. R., Bakke, O. A., & Bakke, I. A. (2013). Ocorrência e atividade de bactérias diazotróficas em forrageiras cultivadas na região semiárida no Brasil. *Revista Caatinga, 26*(1), 27-34.

Silva Girio, L., Ferreira Dias, F. L., Reis, V. M., Urquiaga, S., Schultz, N., Bolonhezi, D., & Mutton, M. A. (2015). Bactérias promotoras de crescimento e adubação nitrogenada no crescimento inicial de cana-de-açúcar proveniente de mudas pré-brotadas. *Pesquisa Agropecuaria Brasileira, 50*(1), 33-43. doi: 10. 1590/S0100-204X2015000100004

Silva, F. W. R., Lima, H. N., Teixeira, W. G., Motta, M. B., & Santana, R. M. (2011). Caracterização química e mineralogia de solos antrópicos (terras pretas de índio) na amazônia central. *Revista Brasileira de Ciência do Solo, 35*(3), 673-681. doi: 10.1590/S0100-06832011000300002

Taiz, L., & Zeiger, E. (2013). *Fisiologia vegetal* (4a ed.). Porto Alegre: Artmed.

Tedesco, M. J., Gianello, C., Bissani, C. A., Bohnen, H., & Volkweiss, S. J. (1995). *Análises de solo, plantas e outros materiais*. (Boletim Técnico). Porto Alegre: UFRGS.

Uroz, S., Dessaux, Y., & Oger, P. (2009). Quorum sensing and quorum quenching: the yin and yang of bacterial communication. *ChemBioChem, 10*(2), 205-216. doi: 10.1002/cbic.200800521

Yunus, M. F., Aziz, M. A., Kadir, M. A., & Rashid, A. A. (2012). In vitro propagation of Etlingera elatior (Jack) (Torch ginger). *Scientia Horticulturae, 135*(24), 145-150. doi: 10.1016/j.scienta.2011.12.016