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Bioprospecting phosphate-solubilizing bacteria in soils with different fertility levels

Bioprospecção de bactérias solubilizadoras de fosfato em solos com diferentes fertilidades

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Highlights

Phosphorus is essential for crop development but has high soil fixation.
Solubilizing bacteria can release fixed phosphorus from the soil.
Pantoea sp., *Enterobacter* sp., and *Klebsiella* sp. can solubilize phosphate.
The presence of the isolates did not interfere with rice seed germination.
Normal seedlings were obtained in the presence of the three inocula.

Abstract

Phosphate-solubilizing bacteria (PSB) have the ability to release fixed phosphorus (P) that is adsorbed in the soil, converting insoluble phosphate into soluble forms, making it readily available for plant absorption. Occurring naturally in various environments, with soil being the main reservoir, PSB are deemed beneficial and safe for agricultural applications. Their potential lies in isolation, multiplication, and reintroduction to the rhizosphere (through inoculants, biofertilizers, biopesticides, or biostimulants) to stimulate plant growth through direct and/or indirect mechanisms. However, identifying efficient isolates adapted to different crops and cropping systems remains a key challenge. This study aimed to prospect PSB from soils of different locations and select efficient strains with high potential for agricultural use via *in vitro* assays, as well as to evaluate the effects of inoculation on upland rice seeds. From soil samples collected in a conserved Amazonian biome, a productive Cerrado biome agricultural area, and a degraded with exposed subsoil, located in the Cerrado biome area, 32 P-solubilizing isolates were obtained. Among these, three isolates were selected for their early onset ability to solubilize Ca and/or Fe phosphates, and for further analysis. Identified as *Pantoea* sp., *Enterobacter* sp., and *Klebsiella*

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sp., these isolates proved to be non-harmful to rice seed health, germination, and seedling emergence, additionally promoting increased root length.
Key words: Phosphorus. Solubilization. *Pantoea* sp.. *Enterobacter* sp.. *Klebsiella* sp.

Resumo

As bactérias solubilizadoras de fosfato (BSF) possuem capacidade para liberar o fósforo (P) que está adsorvido no solo, convertendo fosfato insolúvel em formas solúveis, tornando-o disponível para a absorção pelas plantas. Estas bactérias podem ser encontradas naturalmente no ambiente, tendo o solo como maior reservatório. Além de serem consideradas benéficas e seguras, as BSF representam uma alternativa viável para uso agrícola, pois podem ser multiplicadas e devolvidas à rizosfera (inoculantes, bioestimulantes, biofertilizantes, biopesticidas) para estimular o crescimento das plantas por meio de mecanismos diretos e/ou indiretos. No entanto, encontrar isolados eficientes e adaptados às diversas culturas e sistemas de cultivo permanece um grande desafio. Esta pesquisa teve por objetivo prospectar BSF a partir do solo de diferentes áreas e selecionar, por meio de ensaios *in vitro*, cepas eficientes e com maior potencial para uso agrícola, bem como, avaliar os efeitos da inoculação em sementes de arroz de terras altas. A partir de amostras de solo coletadas em área conservada do bioma Amazônia, área agrícola produtiva do bioma Cerrado e área degradada com subsolo exposto do bioma Cerrado, foram obtidos 32 isolados solubilizadores. Destes, três foram selecionados pela capacidade de solubilização in vitro para fosfato de cálcio e/ou fosfato de ferro, e com início de solubilização precoce. Identificados como *Pantoea* sp., *Enterobacter* sp. e *Klebsiella* sp., os mesmos não apresentaram danos à sanidade das sementes de arroz, ou à germinação e emergência das plântulas, além de promoverem aumento do comprimento das raízes.
Palavras-chave: Fósforo. Solubilização. *Pantoea* sp.. *Enterobacter* sp.. *Klebsiella* sp.

Introduction

Nutrient availability in the soil is one of the factors that define crop growth and productivity. Phosphorus (P) is one of the nutrients applied in the largest quantities for plant development, however, it can be adsorbed to the iron oxides present in the soil, decreasing its availability for absorption. Thus, the total P amount in the soil may be high, and excessive in many cases, but it is not available in sufficient quantity to meet the demand of crops (Shahzad et al., 2017). This low P availability is one of the major problems of current agriculture (Withers et al., 2018).

Soil and rhizosphere microorganisms play a key role in nutrient acquisition by plants and are involved in many biological processes involved directly and indirectly in crop development (Elhaissoufi et al., 2020). Among these, some microorganisms are capable of mineralizing soil organic matter, promoting root growth by inducing the production of phytohormones, acting in biological N_2 fixation, and P solubilization (Timofeeva et al., 2022). Phosphorus solubilization is complex and derives from numerous changes occurring within the rhizosphere, such as soil acidification by the release of organic acids, exudation of

hydrolytic enzymes, and modulation of rhizosphere microbial activity (Elhaissoufi et al., 2020). Phosphate-solubilizing bacteria (PSB) have been considered an efficient, safe, economically viable, and environmentally friendly alternative to enable plants to absorb P that is fixed in the soil.

Studies on the selection and inoculation of these bacteria have brought positive results (Panda et al., 2016; Shahzad et al., 2017), but finding efficient isolates for distinct crops and cultivation systems remains a major challenge. Soil represents the main source of microorganisms on the planet, and the diversity of PSB in a given location depends on its physicochemical properties, organic matter and P contents, plant species growing in the area, environmental conditions, and land use system (Kour et al., 2021). Soils from preserved environments generally present a higher diversity of PSB, while those obtained from soils under extreme environmental conditions, such as nutrient-deficient soils or high-temperature environments, tend to solubilize more phosphate than those obtained from soils under temperate conditions (Timofeeva et al., 2022). The search for new PSB isolates should therefore cover different soil collection sites to improve the diversity and efficiency of isolates.

Rice (*Oryza sativa* L.) is a cereal of foremost importance because it serves as a basis for the diet of about one-third of the world's population (Irfan et al., 2020). This crop requires P for its growth and development, thus being a limiting factor for tillering and, hence yielding. Of all the P absorbed by rice plants, 65% is exported by grains, which makes this crop an important source of nutrients in human nutrition (Crusciol et al., 2007).

Based on the hypothesis that establishing sustainable mechanisms for P supply in upland rice can constitute a valuable tool for global agricultural production, our goal was to prospect PSB from the soil of different areas and select, through *in vitro* assays, efficient strains with greater potential for agricultural use, as well as evaluating the effects of inoculation on upland rice seeds.

Materials and Methods

Isolation and selection of strains

To isolate BSF, 4 composite soil samples were collected from three areas with the potential to contain this type of microorganism: 1- undisturbed soil area, belonging to the Amazon biome, located in the Teles Pires River basin, in the municipality of Paranaíta-MT (9°32'44.9"S 56°18'30.3"W); 2- agricultural area that has not received phosphate fertilization in the last five years, without a decrease in productivity, located in the Cerrado biome, municipality of Selvíria-MS (20°20'45.8"S 51°24'27.9"W); 3- degraded area with an exposed subsoil that, in previous recovery experiments, showed microbiological activity related to increases in organic matter content and hence P availability (A. A. Santos et al., 2018; Boni et al., 2022), located in the Cerrado biome, Selvíria-MS (20º23'02"S 51º24'24"W).

From the soil samples, serial dilutions were performed in sterile saline solution (NaCl 0.85%), followed by plating on GL solid culture medium (glucose 10 g L^{-1}, yeast extract 2 g L^{-1}, and agar 15 g L^{-1}).

To test the solubilizing capacity of bacterial isolates, culture media supplemented with calcium phosphate ($CaHPO_4$) or iron phosphate ($FePO_4$), equivalent to 0.89 g P L^{-1}, were prepared (Sylvester-Bradley et al., 1982; Silva & Vidor, 2000). Calcium phosphate was obtained by adding 1 mL of 5% K_2HPO_4 solution and 1 mL of 10% $CaCl_2$ solution per 10 mL of medium, while iron phosphate was used in the ferric form (iron phosphate III). After plating, the plates were kept at room temperature at 28 ºC for 7 days, with the growth and formation of a transparent halo around colonies being checked daily to identify phosphate solubilization in the medium (Souchie & Abboud, 2007). The colonies containing transparent halos were individually re-plated for the purification of isolates.

Next, a colony of each isolate was transferred to an Erlenmeyer containing GL liquid culture medium (glucose 10 g L^{-1} and yeast extract 2 g L^{-1}) for incubation at 30 ºC, under 150 rpm stirring for nearly 24 h, when the optical density of all inoculants was adjusted to 1 at 600 nm (O.D.600) (Estrada et al., 2013).

To measure diameters (Ø) of colonies and solubilization halos, 10 µL of the culture of each isolate was transferred to Petri plates containing GL solid culture medium, supplemented with the P sources. In each plate, 3 aliquots were added, at equidistant points, with 3 plates for each isolate and each P source. The plates were kept at 28 ºC for 15 days, with measurements being taken every other day. The solubilization index (SI) was calculated for each isolate as the ratio between halo and colony diameters (SI = Ø halo [mm]/ Ø colony [mm]) (Berraquero et al., 1976). Accordingly, the isolates were classified as low (SI < 2), medium (2 > SI < 4), and high (SI > 4) solubilization capacity. Furthermore, according to the onset of solubilization, the isolates were classified as early, whose activity began up to the third day; late, with the onset from the third day onwards; or non-solubilizing, those that did not show solubilization until the fifteenth day of evaluation (Hara & Oliveira, 2004). Isolates showing high solubilization capacity, early onset, and ability to solubilize both P sources were considered the best ones.

Seed germination and seedling emergence tests

To evaluate the influence of the selected isolates on seedling health, germination, and emergence tests were carried out with inoculation in seeds of upland rice cultivar BRS Esmeralda. The inoculants were prepared by individual multiplication of each BSF in GL liquid culture medium with incubation at 30 ºC and stirring at 150 rpm for about 18 h, reaching a minimum concentration of 1.0 x 10^9 CFU mL^{-1}. Inoculation was performed using the dose often recommended for commercial inoculants (100 mL per 50 kg seeds).

Germination tests were set up by distributing 50 inoculated seeds on two sheets of paper towels (Germitest™) moistened with deionized water at 2.5 times the weight of the dry paper, covered with another sheet, and rolled up. Four replicates were performed for each isolate plus a control treatment (without inoculation). The rolls were placed in transparent polyethylene bags and stored at 25 °C. Seed germination

and seedling quality were evaluated daily until germination stabilized at 14 days. At this point, the percentages of normal seedlings (those with intact essential structures and defects in less than 50% of the tissue), abnormal seedlings (those with damaged, deformed, fungal-infected or albino essential structures), and dead seeds (soft seeds with pathogen presence at the end of the test) were counted (Ministério da Agricultura Pecuária e Abastecimento [MAPA], 2009).

For seedling emergence and health (normal and abnormal) determination, plastic boxes (40 x 15 x 8 cm) containing a substrate composed of sand, peat, vermiculite, and organic residue were prepared, with four replicates of 50 seeds per isolate plus the control treatment (without inoculation). Seeds were sown 2 cm deep, and the boxes were kept at room temperature, with daily irrigation to maintain substrate moisture. Seedling emergence was assessed daily until stabilization at 14 days, at which point the percentage of normal and abnormal seedling emergence, shoot and root length, and fresh and dry weight of shoots and roots were determined (MAPA, 2009). Moreover, the emergence speed index (ESI) was calculated for each treatment by summing the number of seedlings that emerged each day, divided by the number of days since sowing, as proposed by Maguire (1962).

Data were analyzed using analysis of variance (ANOVA), with a 5% probability F test to detect differences between treatments. When differences were found, means were compared using the Tukey test at 5% probability. Statistical analyses were performed using SISVAR software (Ferreira, 2019).

Molecular analysis

Isolates with no negative effects on seed germination and seedling health were sent for molecular identification. A colony of each isolate was transferred to an Erlenmeyer flask containing GL liquid medium, where they were incubated at 30 °C and 150 rpm for 24 hours. An aliquot of 2 mL suspension was then centrifuged at 10,000 rpm for 5 minutes, and the supernatant was discarded. The resulting bacterial mass from each isolate was subjected to DNA extraction using the "GenElute™ Bacterial Genomic DNA" kits (Sigma Aldrich, USA), following the manufacturer's instructions. After extraction, the DNA was quantified using a NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, USA) and diluted to a final concentration of 25 ng μL^{-1}. PCR reactions were performed in a total volume of 15 µL containing 2.0 µL of template DNA, 3.0 mM of $MgCl_2$, 0.6 mM of dNTPs, 0.6 µM of each primer, and 0.3 U of Taq polymerase. A negative control without template DNA was also used.

The amplifications were performed in a Proflex thermal cycler (Applied Biosystems, USA) under the following cycling conditions: initial denaturation at 95 °C for 5 minutes followed by 35 cycles of 95 °C for 30 seconds, annealing at 65 °C for 1 minute, extension at 72 °C for 1 minute, and final extension at 72 °C for 7 minutes. The amplification of the 16s rDNA was performed using the universal primers P027F (5'-GAGAGTTTGATCCTGGCTCAG-3')and1378R (5'-CGGTGTGTACAAGGCCCGGAACG-3'). After amplification, 5 µL of the PCR reaction was evaluated by agarose gel electrophoresis (1.0%) in 1x TBE buffer (Tris HCl, boric acid, and EDTA) and stained with bromophenol blue.

The PCR products were sent to the Biological Resources and Genomics Center (CREBIO) in Jaboticabal-SP, for sequencing in an ABI 3730xl Automated DNA Analyzer (Applied Biosystems, USA). The obtained sequences were analyzed and aligned in the Geneious R software version 9.0.5 (Biomatters, New Zealand). Finally, the isolates were identified by similarity analysis with sequences available in the databases of the National Center for Biotechnology Information (NCBI).

Results and Discussion

Isolation and selection of strains

Thirty-two bacterial isolates with potential for phosphate solubilization were selected, ten from a conserved area in Paranaíta-MT (ACA), eighteen from an agricultural area in Selvíria-MS (AAC), and four from a degraded area in Selvíria-MS (ADC). All isolates could solubilize calcium phosphate, but only eight (25%) solubilized iron phosphate (Figure 1). The majority (72%) of calcium phosphate-solubilizing isolates displayed low capacity (SI < 2), while a smaller portion (22%) demonstrated medium capacity (2 > SI < 4), and only two isolates (6%) stood out with high capacity (SI > 4). Conversely, all iron phosphate-solubilizing bacteria had low solubilization capacity, i.e., SI < 2 (Figure 1).

This high prevalence aligns with previously documented findings, such as Hara and Oliveira (2004) reporting 39% of calcium phosphate-solubilizing isolates and only one with high solubilization capacity. Additionally, using the same method employed in our study, Silva & Vidor (2000) evaluated the solubilizing potential of bacteria and fungi and found that 98% of the isolates solubilized calcium phosphate. These authors also noted that, although none of the isolates solubilized iron phosphate, 11% showed better growth in the presence of this P source.

The high frequency of calcium phosphate solubilization in our study (Figure 1) was likely due to the use of a culture medium containing calcium phosphate. This technique is effective in isolating bacteria that solubilize calcium phosphate, but it may not be as effective in isolating bacteria that solubilize iron phosphate. The difficulty in finding microorganisms with high or medium solubilization indices for iron phosphate has been reported, although few studies are testing this P source. Studying the solubilization potential of endophytic microorganisms in corn crops, Ribeiro et al. (2014) reported that 42% of the bacterial isolates solubilized iron phosphate but had a low SI. In a study of the solubilization potential for different P sources by microorganisms isolated from African soil, Fankem et al. (2006) found that all of the evaluated bacteria solubilized iron, but only one had a medium SI.

Regarding the onset of solubilization (Table 1), 72 and 16% of the 32 isolates studied here showed an early onset of solubilization for calcium and iron phosphates, respectively. The time of onset of solubilization is related to the ability of a microorganism to adapt to the environment in which it was inserted (Fankem et al., 2016). Therefore, this characteristic highlights the potential of using an isolate in agricultural cultivation environments.

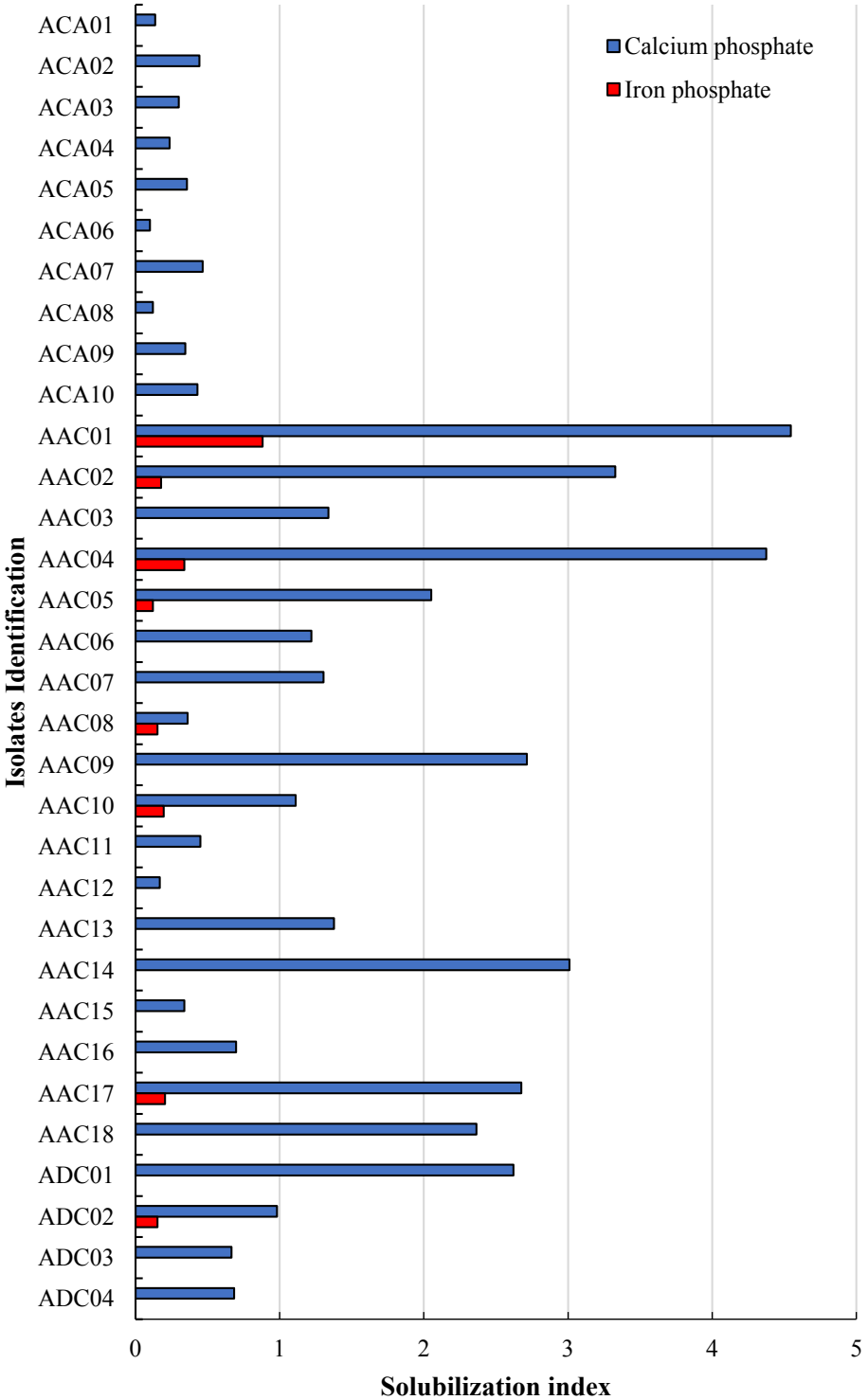

Figure 1. Solubilization index of selected isolates in GL culture medium (glucose 10 g L^{-1}, yeast extract 2 g L^{-1}, agar 15g L^{-1}) supplemented with calcium phosphate (CP) and iron phosphate (IP). Note. ACA- undisturbed soil area, located in the Amazon biome; AAC- agricultural area, located in the Cerrado biome; ADC- degraded area with an exposed subsoil, located in the Cerrado biome.

Table 1
Isolates were identified according to the location of isolation (LI) and the beginning of solubilization test using GL culture medium (glucose 10 g L^{-1}, yeast extract 2 g L^{-1}, agar 15 g L^{-1}) supplemented with calcium phosphate (CP) or iron phosphate (IP)

Isolates	LI	CP	IP	Isolates	LI	CP	IP
*ACA01	1	Early	NS	AAC07	2	Late	NS
ACA02	1	Late	NS	AAC08	2	Early	Late
ACA03	1	Early	NS	AAC09	2	Early	NS
ACA04	1	Early	NS	AAC10	2	Early	Early
ACA05	1	Early	NS	AAC11	2	Early	NS
ACA06	1	Early	NS	AAC12	2	Late	NS
ACA07	1	Late	NS	AAC13	2	Early	NS
ACA08	1	Late	NS	AAC14	2	Early	NS
ACA09	1	Early	NS	AAC15	2	Early	NS
ACA10	1	Early	NS	AAC16	2	Early	NS
AAC01	2	Early	Early	AAC17	2	Early	Late
AAC02	2	Early	Early	AAC18	2	Early	NS
AAC03	2	Early	NS	ADC01	3	Late	NS
AAC04	2	Early	Early	ADC02	3	Late	Late
AAC05	2	Early	Late	ADC03	3	Late	NS
AAC06	2	Early	NS	ADC04	3	Late	NS

Note. *ACA- Area with undisturbed soil, located in the Amazon biome; AAC- Agricultural area, located in the Cerrado biome; ADC- Degraded area with exposed subsoil, located in the Cerrado biome. NS= non-solubilizing.

Only the isolates AAC01 and AAC04 showed high solubilization capacity for calcium phosphate, also presenting capacity for solubilization of iron phosphate (Figure 1), in addition to early onset of solubilization for both sources (Table 1). Among the isolates with medium solubilization capacity for calcium phosphate, AAC02, AAC05, and AAC17 were also able to solubilize iron phosphate. Of these, AAC02 demonstrated an early onset of solubilization for both P sources. Thus, according to the established method, AAC01, AAC02, and AAC04, obtained from Cerrado agricultural soil, were considered the isolates with the highest potential for P solubilization.

Germination and seedling emergence tests

The germination test of BRS Esmeralda rice seeds inoculated with the three selected BSF isolates showed germination rate averages above 85% for all isolates, not differing statistically from non-inoculated seeds (Figure 2). All treatments had averages above the minimum threshold of 80%, according to the national standards established for rice seed marketing (Costa et al., 2020).

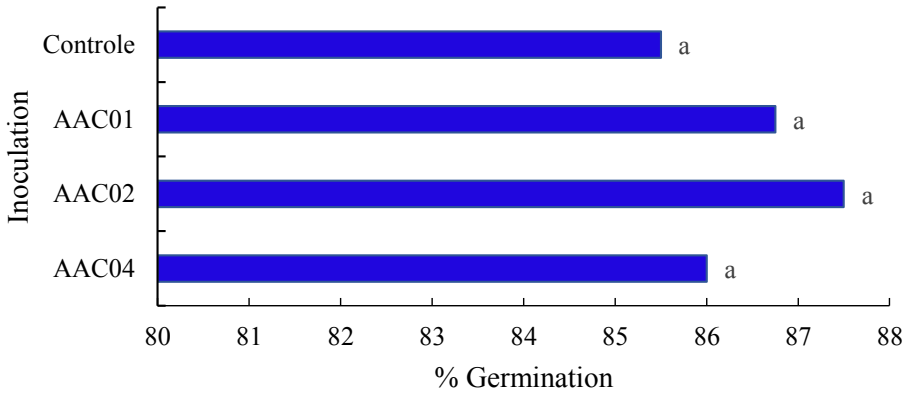

Figure 2. Average germination values of rice seeds, cultivar BRS Esmeralda, inoculated with isolates AAC01, AAC02 and AAC04, or without inoculation (control).
Note: Means followed by the same letter in the bar do not vary from each other using the Tukey test ($p > 0.05$).

The seedling emergence rate was also above 80% for all treatments (Table 2). No significant differences were observed between the control and BSF-inoculated treatments for shoot length, shoot fresh and dry masses and root dry mass of seedlings. This result, associated with the absence of abnormal seedlings or damaged seeds in all treatments, either in germination or in emergence tests, indicates that neither seed nor seedling health was affected by BSF inoculation.

Table 2
Average values for seedling germination (SG), shoot length (SL), root length (RL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM) and emergence speed index (ESI) of rice cultivar BRS Esmeralda, as a function of inoculation of phosphate-solubilizing bacteria (AAC01, AAC02 and AAC04) and without inoculation (control)

Parameters	SG	SL	RL	SFM	RFM	SDM	RDM	ESI
	- % -	------ cm -----		---------- g ----------				
Control	81.5	17.20	6.63b	0.62	0.32b	0.13	0.05	10.98
AAC01	84.5	18.09	7.10ab	0.70	0.33b	0.14	0.05	11.82
AAC02	87.0	20.74	7.38ab	0.89	0.38b	0.13	0.05	11.48
AAC04	85.0	20.22	8.30a	0.80	0.62a	0.15	0.06	12.18
F Value	0.637^{ns}	3.121^{ns}	3.223*	1.705^{ns}	3.742*	2.206^{ns}	2.279^{ns}	2.385^{ns}
Average	84.5	19.06	7.35	0.75	0.41	0.14	0.05	11.61
V.C. (%)	6.74	9.19	10.64	13.61	13.19	7.27	6.91	5.70

Note. Means followed by the same letter, in each column, do not differ according to the Tukey test ($p \leq 0.05$). V.C. = variation coefficient. ns= not significant. *significant at 1% probability using the F test.

As for the emergence speed index (ESI), values varied from 10.98 to 12.18, with the control treatment not differing from the inoculated treatments. These values are in accordance with those of Tunes et al. (2012) who, evaluating vigor in five lots of highland rice seeds, found ESI between 8.7 and 12.3. Moreover, studying the seed quality of different highland rice cultivars, H. O. Santos et al. (2015) observed high ESI for the BRS Esmeralda rice cultivar, which is considered to have a high physiological quality. Overall, the higher the ESI, the less sensitive the plant is to adverse conditions during field development (Nakagawa, 2020). Thus, this index is of foremost importance in cultivar selection.

Molecular analysis

Based on the phylogenetic tree constructed from 16S rRNA sequences, the selected strains belong to the genera *Pantoea* sp., *Enterobacter* sp., and *Klebsiella* sp., with 97, 99, and 99% similarity, respectively (Table 3). The ability of P solubilization by bacteria of these genera has already been reported. Solanki et al. (2020) associated the *Pantoea* sp. strain D2Ss73 with increasing contents of total P, nitrogen, and potassium in a soil grown with sugarcane in association with peanuts and soybeans.

Table 3
Isolates were identified through similarity analysis with sequences obtained from The National Center for Biotechnology Information (NCBI)

Isolates	Strain	Similarity	Reference
AAC01	*Pantoea* sp.	97%	Solanki et al. (2020)
AAC02	*Enterobacter* sp.	99%	Khan et al. (2015)
AAC04	*Klebsiella* sp.	99%	Lin et al. (2012)

In *in vitro* experiments, Khan et al. (2015) concluded that the *Enterobacter* sp. strain HU38 can solubilize P, produce siderophores, and the plant hormone indoleacetic acid (IAA). The strain also exhibited tolerance to heavy metals (arsenic, lead, cadmium, and zinc) and promoted ryegrass growth. Evaluating the potential of bacterial isolates for sugarcane growth, Lin et al. (2012) reported that the *Klebsiella* sp. strain LC55S can solubilize P, as well as produce siderophores and IAA.

Borham et al. (2017) evaluated the inoculation of an *Enterobacter* sp. P-solubilizing isolate in wheat plants and observed increased plant height and mass, leaf area, chlorophyll index, and grain number and weight. Likewise, Roslan et al. (2020) investigated the P solubilization potential of 18 *Enterobacter* genus isolates in okra plants and observed enhanced plant growth and increased P levels in both soil and plants; they concluded that the agricultural use of *Enterobacter* sp. showed promise.

In upland rice, Li et al. (2020) found that *Klebsiella* sp. inoculation significantly increased concentrations of available P in the soil and hormone production, while also enhancing plant photosynthetic parameters. Similarly, Eke et al. (2019) observed that bacteria of this genus solubilized phosphate in the soil and stimulated the growth of tomato plants.

The search for microorganisms that benefit agriculture has intensified in recent years. Phosphate-solubilizing microorganisms, capable of releasing fixed phosphorus in the soil, hold special importance for Brazilian agriculture, as nearly half of all phosphorus applied over the past 50 years remains fixed in the soil (Withers et al., 2018). Much of the research has focused on phosphate-solubilizing fungi, and when it comes to bacteria, the genus *Bacillus* sp. has been the most extensively studied. Studies involving other bacterial genera are scarce and lack research information under Brazilian conditions. In this sense, this study demonstrates significant potential in prospecting different bacterial genera with phosphate-solubilizing capabilities, providing valuable insights for the expansion of this research field. Moreover, efficiently exploring existing diversity significantly enhances the prospects of finding more efficient strains better adapted to various crops and cultivation systems, a goal yet to be fully achieved in the realm of phosphate solubilization.

In the context of rice cultivation, securing stable phosphorus supplies is crucial to prevent potential crises in phosphorus supply and food security. This is because rice is among the most important staple crops globally, providing sustenance to approximately 50% of the world's population (Tan & Norhaizan, 2020). Moreover, it holds significant economic relevance for Brazil, with the country having produced over 10 million tons in 2022 (Food and Agriculture Organization of The United States [FAO], 2023).

Research on the agricultural use of the three bacterial genera identified in our study is still in its preliminary stages, it is limited to a handful of crops and specific environments. As emerging players in the agricultural context, these microorganisms should undergo pot and field trials for a comprehensive evaluation before recommending them for agricultural use.

Conclusions

Prospecting for phosphate-solubilizing bacteria yielded three isolates for agricultural use. The isolates *Pantoea* sp., *Enterobacter* sp., and *Klebsiella* sp. boasted significantly higher solubilization capacity for both calcium and iron phosphates, posing no harm to rice seeds nor to seed germination and seedling emergence, and significantly enhanced root length and fresh root mass in upland rice seedlings.

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design, data collection and analysis, decision to publish, or manuscript preparation.

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