Anais da Academia Brasileira de Ciências
ISSN: 0001-3765
aabc@abc.org.br
Academia Brasileira de Ciências
Brasil

MATOS, AMANDA D.M.; GOMES, IZABELA C.P.; NIETSCHE, SILVIA; XAVIER, ADELICA A.; GOMES, WELLINGTON S.; DOS SANTOS NETO, JOSÉ A.; PEREIRA, MARLON C.T.

Phosphate solubilization by endophytic bacteria isolated from banana trees

Anais da Academia Brasileira de Ciências, vol. 89, núm. 4, octubre-diciembre, 2017, pp. 2945-2954
Academia Brasileira de Ciências
Rio de Janeiro, Brasil

Anais da Academia Brasileira de Ciências (2017) 89(4): 2945-2954
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201720160111
www.scielo.br/aabc | www.fb.com/aabcjournal

Phosphate solubilization by endophytic bacteria isolated from banana trees

AMANDA D.M. MATOS[1], IZABELA C.P. GOMES[1], SILVIA NIETSCHE[2], ADELICA A. XAVIER[1], WELLINGTON S. GOMES[1], JOSÉ A. DOS SANTOS NETO[1] and MARLON C.T. PEREIRA[1]

[1]Programa de Pós-Graduação em Produção Vegetal no Semiárido, Universidade Estadual de Montes Claros, Departamento de Ciências Agrárias, Campus de Janaúba, Av. Reinaldo Viana, 2630, 39400-000 Janaúba, MG, Brazil
[2]Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Av. Universitária, 1000, Universitário, 39404-547 Montes Claros, MG, Brazil

Manuscript received on February 29, 2016; accepted for publication on January 16, 2017

ABSTRACT

Forty isolates of endophytic bacteria isolated from banana tree roots were assessed as to their capacity to solubilize phosphate in a solid culture medium supplemented with different inorganic and one organic source of phosphorus. The amount of phosphorus (P) in each liquid medium was quantified, and an indirect assessment of acid phosphatase activity was performed. All assays had a fully randomized design, with three repetitions. Approximately 67.5% of the 40 isolates assessed in solid medium solubilized phosphorus from tricalcium phosphate and 7.5% of the isolates solubilized phosphorus from soy lecithin; no isolates exhibited P solubilization capacity in medium supplemented with iron phosphate. Acid phosphatase activity was detected in 65% of the isolates; *Aneurinibacillus* sp. and *Lysinibacillus* sp. isolates presented with the best solubilization indexes. All of the assessed isolates exhibited a capacity to reduce the potential of hydrogen in liquid medium supplemented with tricalcium phosphate. Isolate EB. 78 (*Bacillus* sp.) exhibited P solubilization capacity in solid media when $Ca_3(PO_4)_2$ and soy lecithin were used as P sources; this isolate significantly reduced the pH of the liquid medium and exhibited acid phosphatase activity. The results of the present study highlight isolates that exhibit variations in their capacity to solubilize P. These isolates should be used in future tests to assess their field performance.

Key words: acid phosphatase, *Bacillus* sp., lecithin, *Musa* spp., PGPB.

INTRODUCTION

Phosphorus (P) is considered to be an essential macronutrient and depending on its availability P might limit the growth of plants because of its structural, functional and metabolic properties (Stauffer and Sulewski 2004). It is found in soils in both organic and inorganic forms. Despite their large stores of phosphorus in agricultural lands; its availability to plants is strongly influenced by the various biogeochemical processes to which this element might be subjected (Martinazzo et al. 2007).

The adsorption of phosphorus is estimated to be approximately 75% of that applied (Lin et al. 2006). Adsorption and precipitation of phosphorus in the soil usually depends on the soil pH and type.

Correspondence to: Silvia Nietsche
E-mail: silvia.nietsche@gmail.com

In acidic soils, weak aluminum and iron oxides and oxyhydroxides are able to retain phosphorus. In contrast, precipitation occurs in alkaline soils due to the presence of calcium; this results in less efficient solubilization of phosphate fertilizers (Igual et al. 2001).

Due to the significance of phosphorus to plants and its high cost, the search for alternative methods to optimize its production is considered a priority in agricultural systems.

The use of endophytic microorganisms in agriculture has increased in recent years. Such microbes promote the growth of plants and facilitate the control of biological pests and phytopathogens, as well as the production of metabolites of pharmaceutical interest (Azevedo et al. 2000). Several soil microorganisms, including bacteria and fungi, are able to mineralize organic phosphates and solubilize inorganic phosphates. Phosphate solubilization might be achieved via several mechanisms, such as hydrolysis or processes involving enzymes like phosphatases. Phosphatases produce organic and inorganic acids through pH reduction, carbon dioxide formation and the enzymatic reduction of metals (Nautiyal 1999, Souchie et al. 2005, Barroso and Nahas 2008, Bashan et al. 2013).

Among the bacteria able to solubilize phosphate, the genera *Rhizobium* (Sridevi and Mallaiah 2009), *Agrobacterium*, *Pseudomonas*, *Burkholderia*, *Erwinia* (Verma et al. 2001, Garg et al. 2001), *Paenibacillus* (Wang et al. 2012), *Bacillus* (Silva Filho and Vidor 2000) and *Lysinibacillus* sp. (Andrade et al. 2014) are of interest. These bacteria are present in significant numbers in environments of ecological relevance.

Studies aimed at selecting bacteria and fungi able to solubilize and mineralize soil phosphorus have been performed to promote the development of sustainable agriculture. This is a global trend that seeks to reduce the use of chemical fertilizers and favors the development of ecologically balanced agricultural environments. The selection of candidate microorganisms as phosphorus solubilizers depends on both the soil of origin and the soil intended for use. Our biological and chemical understanding of this nutrient in the soil indicates what phosphate sources should be tested concomitantly to identify the potentiality of microorganisms for making phosphorus available in the soil (Bashan et al. 2013).

Considering the amount of data available in the literature and the large demand for new scientific information, the aims of the present study were to assess the capacity of endophytic bacteria isolated from banana tree roots to solubilize *in vitro* organic and inorganic phosphate and select the isolates with the highest solubilization potential.

MATERIALS AND METHODS

SELECTION OF ISOLATES AND PREPARATION OF BACTERIAL SUSPENSIONS

A total of 40 endophytic bacteria isolates from banana tree (*Musa* sp.) roots characterized by Souza et al. (2013) were assessed. Partial sequences of the bacterial 16S rRNA region were deposited in the EMBL (European Molecular Biology Laboratory)/ GenBank database (Table I).

The isolates were individually grown in Petri dishes containing TSA (*Tryptic Soy Agar*) medium at 28 ºC for 24 hours. Isolates were then transferred to TSB (*Tryptic Soy Broth*) liquid medium and kept in an automatic agitator at 100 rpm and 28 ºC for 48 hours. The suspension was centrifuged for 10 minutes at 10,000 rpm to pellet the bacterial cells. Cells were resuspended in 0.85% saline solution under aseptic conditions in a laminar flow chamber. The concentration of bacterial cells in the suspension was adjusted based on spectrophotometer absorbance readings at a wavelength (λ) of 540 nm to achieve an optical density (OD) equal to 1.0 Abs.

SOLID MEDIUM SCREENING FOR *in vitro* SOLUBILIZATION OF INORGANIC ($CA_3(PO_4)_2$ AND FE_3PO_4) AND ORGANIC (SOY LECITHIN) PHOSPHATES

Three preliminary experiments were performed to assess the bacterial potential for phosphorus solubilization in solid medium containing the following inorganic and organic sources of phosphorus: $Ca_3(PO_4)_2$ and Fe_3PO_4 as inorganic sources and soy lecithin as an organic source (Katznelson and Bose 1959). The culture medium used was NBRIP (National Botanical Research Institute Phosphate) (Nautiyal 1999) supplemented with various sources of phosphorus as follows: $Ca_3(PO_4)_2$ (5.0 g L^{-1}), Fe_3PO_4 (2.0 g L^{-1}) and soy lecithin (15.0 g L^{-1}). The culture media were placed in an autoclave at 121 ºC for 20 minutes. Then, 25 mL aliquots were added to each Petri dish. A 10 µL volume of bacterial suspension was added to the center of each Petri dish. Plates were incubated in an (BOD-like) incubator at 28 ºC for 15 days; the solubilization zone was then assessed.

Solubilization zones and bacterial colony diameters were measured using a DIGIMESS digital caliper. The results were used to calculate the solubilization index (SI) (Berraqueiro et al. 1976), which was determined as the ratio of the solubilization zone diameter to the colony diameter. The solubilization efficiency was assessed based on the scale formulated by Silva Filho and Vidor (2000), where values under 1.0 were classified as very low solubilizers, values from 1.0 to 2.0 were classified as low solubilizers, values from 2.0 to 3.0 were classified as medium solubilizers, and values above 3.0 were classified as high solubilizers.

The three phosphorus solubilization assays were independently performed in solid media following a fully randomized design, with 40 treatments and three repetitions. Each experimental unit comprised three Petri dishes.

QUANTIFICATION OF ORGANIC AND INORGANIC PHOSPHATE SOLUBILIZATION IN LIQUID MEDIUM

The isolates that tested positive in the solid-medium solubilization assays were selected for testing in NBRIP liquid medium at pH 7.0; three sources of phosphorus were added to the liquid media. Twenty-seven isolates tested positive in the assay using culture medium containing 0.25 g L^{-1} $Ca_3(PO_4)_2$. Three isolates tested positive in the assay using culture medium containing 0.75 g L^{-1} soy lecithin. One-milliliter aliquots of bacterial suspensions were transferred to Erlenmeyer flasks containing 50 mL of culture medium. The control treatment was performed in Erlenmeyer flasks containing 50 mL of liquid medium without bacterial inoculum.

The samples were incubated at 24 ºC under constant agitation in an automatic agitator at 120 rpm for 72 hours. The phosphorus concentration was measured using the ascorbic acid method (Braga and Defelipo 1974). The amount of solubilized phosphorus was calculated based on the difference in soluble P between the inoculated and non-inoculated cultures. The pH of the filtrate was measured using a digital pH meter (Hanna Instruments). The inorganic and organic phosphate solubilization assays in liquid medium were performed independently using a fully randomized design and with three repetitions.

DETECTION OF ACID PHOSPHATASE ACTIVITY

This experiment was performed using Petri dishes containing 25 mL of TSA medium and 0.5% phenolphthalein diphosphate (sterilized and filtered) at a ratio of 2%. A 10 µL aliquot of bacterial suspension was added to the center of each dish. The dishes were incubated in a BOD incubator at 28 ºC for 48 hours. The presence of acid phosphatase activity was detected through the addition of three or four drops of (8.4%) ammonium hydroxide. Fifteen minutes later, acid phosphatase activity was assessed based on the formation of a

pink zone around the bacterial colonies (Romeiro 2007). The zone diameter was measured using a digital caliper, and the results were classified as follows: (+) diameter up to 1 cm or (++) diameter greater than 1 cm.

The phosphatase activity assay had a fully randomized design, with 40 treatments and three repetitions; each experimental unit consisted of one Petri dish.

STATISTICAL ANALYSIS

The experimental results were subjected to analysis of variance and compared using the Scott-Knott test at a significance level of 5% (Scott and Knott 1974). The statistical software Sisvar (Ferreira 2011) was used for these analyses. The qualitative data were subjected to frequency distribution analysis.

RESULTS

SOLID MEDIUM SCREENING FOR *in vitro* SOLUBILIZATION OF INORGANIC ($CA_3(PO_4)_2$ AND FE_3PO_4) AND ORGANIC (SOY LECITHIN) PHOSPHATES

Approximately 67.5% of the 40 isolates assessed were able to solubilize tricalcium phosphate *in vitro* in the solid medium. Four distinct groups were formed. Six isolates were classified as high solubilizers, two were classified as medium solubilizers, and 19 were classified as low solubilizers. No isolates were classified as very low solubilizers (Table I).

Significant differences were found in the average colony and solubilization zone diameters. Based on these results, the isolates were divided into seven groups. Isolate EB. 12 (*Bacillus pumilus*) exhibited the largest colony diameter at an average of 29.3 mm. Isolate EB. 18 (*Bacillus* sp.) exhibited the largest solubilization zone diameter (45.43 mm) (Figure 1).

Only three isolates exhibited *in vitro* phosphate solubilization capacity when soy lecithin was used as a P source. Two such isolates belonged to the genus *Bacillus*; one isolate was *Bacillus pumilus* (Table I, Figure 2). Significant differences were found in the diameters of the solubilization zone; isolate EB. 135 (*Bacillus pumilus*) had the largest average solubilization zone (18.0 mm). All three of the above isolates exhibited low solubilization indexes (Table I, Figure 2).

None of the 40 isolates assessed exhibited *in vitro* phosphate solubilization capacity when Fe_3PO_4 was used as a P source (Table I).

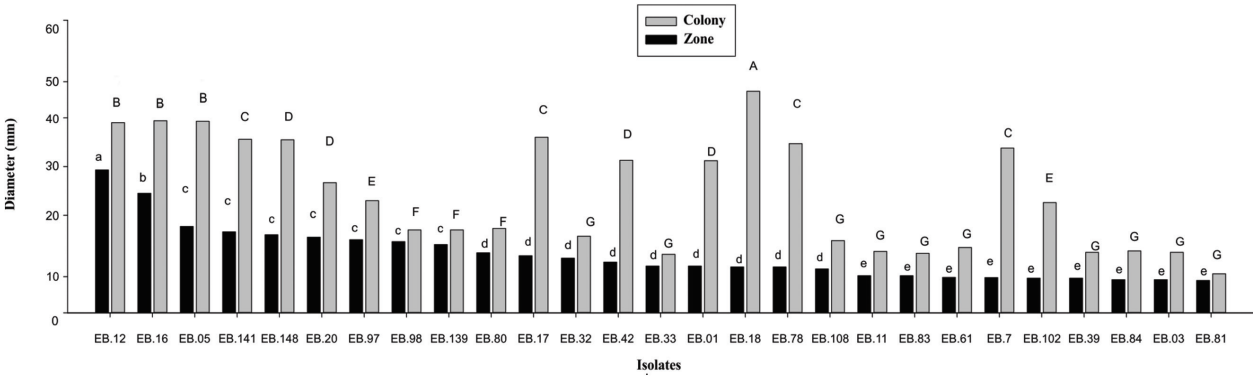

Figure 1 - Average colony (colony Ø) and phosphate solubilization zone (zone Ø) diameters in NBRIP medium supplemented with tricalcium phosphate [$Ca_3(PO_4)_2$] for endophytic bacteria isolated from "Prata Anã" banana tree roots. Data are in mm. (Vertical bars represent the standard error). The means followed by the same upper and lower case letters indicate they belong to the same group through the Scott-Knott test at a significance level at $P < 0.05$.

TABLE I

Mean solubilization index (SI) values corresponding to 40 endophytic bacteria isolates obtained from a "Prata Anã" banana tree grown in solid medium supplemented with $Ca_3(PO_4)_2$ or soy lecithin. Isolates are classified according to solubilization level and the presence of indirect acid phosphatase activity.

Isolate	Most frequent genus/species	Solubilization index ($Ca_3(PO_4)_2$)	Classification	Solubilization index (Lecithin)	Classification	Acid phosphatase
EB. 01	*Bacillus pumilus*	3.29 B	HS	*	*	+
EB. 03	*Bacillus pumilus*	1.82 C	LS	*	*	+
EB. 05	*Bacillus pumilus*	2.33 C	MS	*	*	+
EB. 06	*Bacillus subtilis*	*	*	*	*	*
EB. 07	*Agrobacterium tumefaciens*	4.82 A	HS	*	*	+
EB. 11	*Bacillus* sp.	1.87 C	LS	*	*	+
EB. 12	*Bacillus pumilus*	1.34 D	LS	*	*	+
EB. 14	*Bacillus pumilus*	*	*	*	*	*
EB. 16	*Bacillus* sp.	1.61 C	LS	*	*	+
EB. 17	*Bacillus* sp.	3.13 B	HS	*	*	+
EB. 18	*Bacillus* sp.	4.79 A	HS	*	*	+
EB. 20	*Bacillus* sp.	1.71 C	LS	*	*	+
EB. 32	*Bacillus* sp.	1.43 D	LS	1.37 A	LS	*
EB. 33	*Bacillus* sp.	1.26 D	LS	*	*	*
EB. 39	*Streptomyces* sp.	1.77 C	LS	*	*	+
EB. 42	*Bacillus* sp.	3.00 B	MS	*	*	+
EB. 45	*Lysinibacillus sphaericus*	*	*	*	*	*
EB. 61	*Bacillus* sp.	1.84 C	LS	*	*	+
EB. 69	*Bacillus* sp.	*	*	*	*	*
EB. 78	*Bacillus* sp.	3.75 B	HS	1.16 B	LS	++
EB. 80	*Bacillus* sp.	1.40 D	LS	*	*	+
EB. 81	*Bacillus* sp.	1.34 D	LS	*	*	+
EB. 83	*Bacillus* sp.	1.62 C	LS	*	*	+
EB. 84	*Bacillus subtilis*	1.86 C	LS	*	*	+
EB. 86	*Artrobacter* sp.	*	*	*	*	*
EB. 97	*Bacillus amyloliquefaciens*	1.53 D	LS	*	*	+
EB. 98	*Micrococcus luteus*	1.16 D	LS	*	*	+
EB. 102	*Bacillus pumilus*	3.16 B	HS	*	*	+
EB. 106	*Rhizobium* sp.	*	*	*	*	*
EB. 107	*Bacillus thuringiensis*	*	*	*	*	*
EB. 108	*Rhizobium* sp.	1.70 C	LS	*	*	++
EB. 132	*Bacillus subtilis*	*	*	*	*	*
EB. 135	*Bacillus pumilus*	*	*	1.50 A	LS	+
EB. 139	*Acetobacter* sp.	1.22 D	LS	*	*	+
EB. 141	*Lysinibacillus* sp.	2.28 C	MS	*	*	++
EB. 148	*Aneurinebacillus* sp.	1.90 C	LS	*	*	+
EB. 154	*Bacillus pumilus*	*	*	*	*	*
EB. 162	*Bacillus pumilus*	*	*	*	*	*
EB. 181	*Paenibacillus* sp.	*	*	*	*	*
EB. 200	*Bacillus pumilus*	*	*	*	*	*

*Indicates isolates with negative results.

The means followed by the same upper case letters indicate they belong to the same group through the Scott-Knott test at a significance level at $P < 0.05$.

HS – high solubilization / MS – medium solubilization / LS – low solubilization. Classification formulated by Silva Filho and Vidor (2000).

(+) Zones of up to 1.0 cm in diameter and (++) zones with diameters over 1.0 cm (Romeiro 2007).

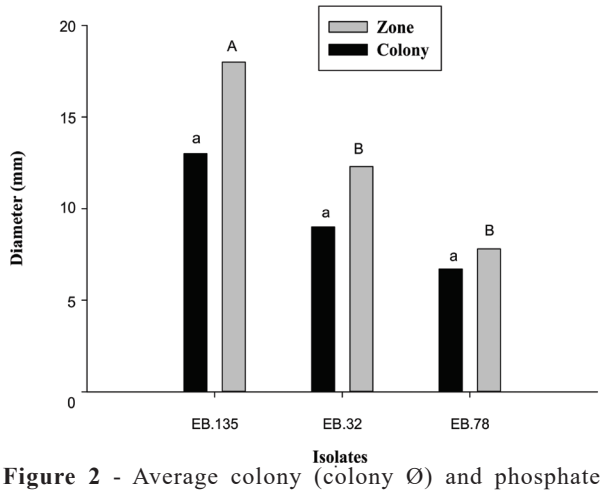

Figure 2 - Average colony (colony Ø) and phosphate solubilization zone (zone Ø) diameters in NBRIP medium supplemented with soy lecithin for endophytic bacteria isolated from "Prata Anã" banana tree roots. Data are in mm. (Vertical bars represent the standard error). The means followed by the same upper and lower case letters indicate they belong to the same group through the Scott-Knott test at a significance level at $P < 0.05$.

QUANTIFICATION OF ORGANIC AND INORGANIC PHOSPHATE SOLUBILIZATION IN LIQUID MEDIUM

All of the 27 isolates that were positive in the phosphate solubilization assay in solid medium using tricalcium phosphate as a P source also exhibited phosphate solubilization capacity in liquid medium with the same P source (Figure 3). Significant variation in the results was observed, which resulted in the definition of three groups of isolates. Isolates EB. 102 (*Bacillus pumilus*), EB. 12 (*Bacillus pumilus*), EB. 42 (*Bacillus* sp.*)*, EB. 17 (*Bacillus* sp.), EB. 81 (*Bacillus* sp.) and EB. 05 (*Bacillus pumilus)* exhibited the highest average amounts of solubilized P. The greatest amount of P solubilization, 15.12 mg L^{-1}, was exhibited by isolate EB. 102 (Figure 3).

Three isolates were assessed in liquid medium containing soy lecithin as a P source; *in vitro* P solubilization capacity was confirmed only for EB. 32 (*Bacillus* sp.*)* and EB. 135 (*Bacillus pumilus*). Significant differences were observed in P solubilization capacity; isolate EB. 135 solubilized an average of 2.32 mg L^{-1} of P (Figure 4).

pH analysis revealed that all of the 27 isolates were able to reduce the potential of hydrogen in liquid medium containing tribasic calcium phosphate as a P source. The pH of the culture medium was adjusted to 7.0 initially; at the end of the assay, the pH value decreased to 4.34 (Figure 3). Significant differences were found in the results of the pH analysis, resulting in the definition of eight groups of isolates. Isolates EB. 07, EB. 17, EB. 18, EB. 78 and EB. 141 induced, on average, the greatest reductions in pH in the culture medium supplemented with $Ca_3(PO_4)_2$ (Figure 3).

Significant reductions in pH were also induced by isolates grown in the medium supplemented with

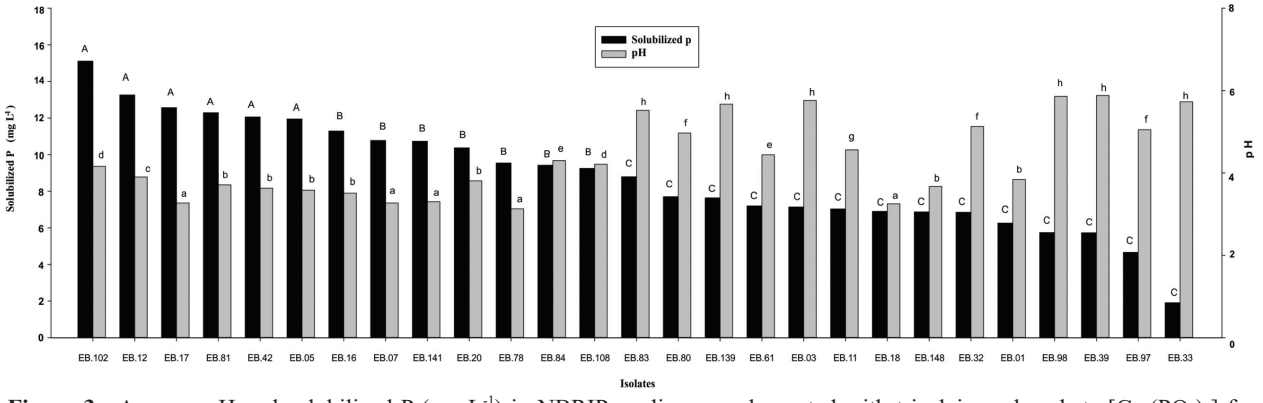

Figure 3 - Average pH and solubilized P (mg L^{-1}) in NBRIP medium supplemented with tricalcium phosphate [$Ca_3(PO_4)_2$] for endophytic bacteria isolated from "Prata Anã" banana tree roots. (Vertical bars represent the standard error). The means followed by the same upper and lower case letters indicate they belong to the same group through the Scott-Knott test at a significance level at $P < 0.05$.

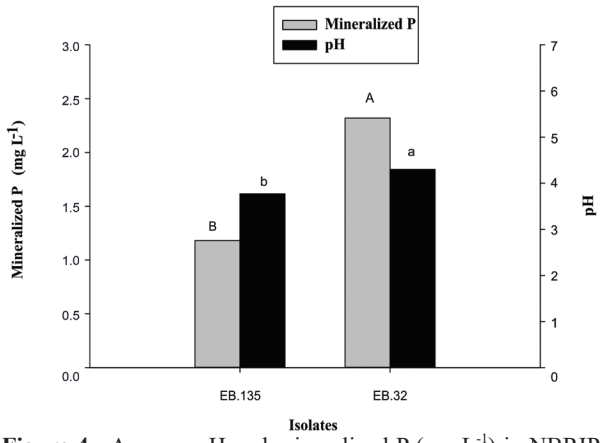

Figure 4 - Average pH and mineralized P (mg L^{-1}) in NBRIP medium supplemented with soy lecithin for endophytic bacteria isolated from "Prata Anã" banana tree roots. (Vertical bars represent the standard error). The means followed by the same upper and lower case letters indicate they belong to the same group through the Scott-Knott test at a significance level at $P < 0.05$.

soy lecithin as a P source. Isolate EB. 32 (*Bacillus* sp.) induced the greatest reduction in pH reduction, to an average value of 3.77 (Figure 4).

DETECTION OF ACID PHOSPHATASE ACTIVITY

Of the 40 isolates assessed, 26 tested positive for indirect acid phosphatase activity (Table I) (Figure 5). Activity zone diameters were over 1.0 cm for three isolates, EB. 78 (*Bacillus* sp.*)*, EB. 108 (*Rhizobium* sp.) and EB. 141 (*Lysinibacillus* sp.); these isolates were classified as having the highest acid phosphatase activity (Table I). Among the genera and species of bacteria that exhibited positive results on the phosphatase activity assay, the following were notable: *Acetobacter* sp., *Agrobacterium tumefaciens, Aneurinibacillus* sp., *Bacillus amyloliquefaciens, Bacillus pumilus, Bacillus* sp., *Bacillus subtilis, Micrococcus luteus* and *Streptomyces* sp.

DISCUSSION

Studies on the use of phosphorus solubilizing microorganisms (PSM) were first published at the beginning of the 20th century. Since then, the

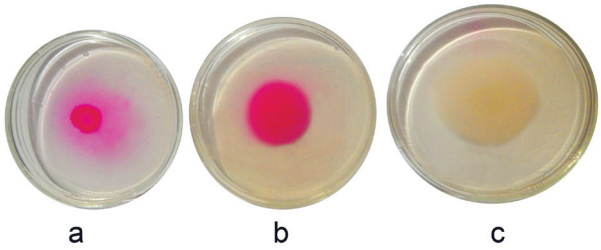

Figure 5 - Acid phosphatase detection zones. (**a**) Solubilization zone classified as ++, (**b**) solubilization zone classified as +, and (**c**) an isolate negative for acid phosphatase activity.

mechanisms of phosphate mineralization and solubilization by endophytic bacteria, rhizobacteria and fungi have been extensively researched by the scientific community.

The greatest impact of microorganisms used in agricultural systems is associated with the biological process of nitrogen (N_2) fixation. Global agricultural production is also dependent on phosphate fertilizers and is vulnerable to reductions in the availability of phosphorus, increases in the cost of phosphate fertilizer production and high levels of precipitation and immobilization of this element in the soil (Khan et al. 2007).

Within this context, several studies have investigated the capacity of microorganisms for P solubilization and mineralization in different culture media with varying P sources (Pikovskaya 1948, Gupta et al. 1994, Nautiyal 1999). The study of low-solubility inorganic P sources, such as tricalcium phosphate (Ca_3PO_4), aluminum phosphate (Al_3PO_4) and iron phosphate (Fe_3PO_4), and various organic P sources contributes to our understanding of the aforementioned mechanisms and to the selection of strains with high performance in both *in vitro* and field conditions (Bashan et al. 2013).

A large proportion of the methods used for the initial screening of PSM involve the use of solid or semi-solid culture media and tricalcium phosphate as a P source (Rodriguez and Fraga 1999, Oliveira et al. 2009). Considering the diversity of species exhibiting P solubilization capacity selected using

these techniques, the use of a single P source is not necessarily the best strategy (Bashan et al. 2013).

In the present study, P solubilization capacity of isolates was assessed using two inorganic (Ca_3PO_4 and Fe_3PO_4) P sources and one organic (soy lecithin) P source in solid and liquid media. The results suggest that the investigated species exhibited greater capacities to solubilize calcium phosphate and, less frequently, to mineralize organic phosphate. Of the 40 isolates assessed, 29 belong to the genus *Bacillus*. Of these isolates, 68.9% exhibited P solubilization capacity in solid medium supplemented with tricalcium phosphate.

The first study on endophytic bacteria isolated from banana tree roots indicated that isolates of the genus *Bacillus* sp. were the most frequent P solubilizers. The authors of that study observed wide variations in solubilization indexes and zones and characterized some of these isolates as high-efficiency solubilizers (Andrade et al. 2014).

Variations in the size of the solubilization zone were also observed in the present study. The solubilization zone occurs due to the presence of some substances, such as organic acids, that are released by microorganisms and solubilize the P in the medium. The presence of these substances generates a translucent zone around the colonies, which is indicative of solubilizing capacity (Souchie et al. 2005). This phenomenon is known as metal complexing; microorganisms release organic acids that can form metal complexes with calcium, iron and aluminum (Bashan et al. 2013).

In addition to genetics, variations in the diameter of the solubilization zone might be influenced by the composition of the culture media. Increased use of nitrogen sources, such as nitrates, and high concentrations of sugars, such as glucose, might affect the growth and development of the isolates. These components may also significantly influence the solubilization process.

The lack of iron phosphate solubilization capacity observed in the present study might be due to the dose that was used. Alternatively, the iron phosphate solubilization efficiency of the investigated isolates might be low. There are other mechanisms of solubilization available, and the investigated bacteria likely do not use the one involving iron phosphate (Bashan et al. 2013). Similar results were reported by Pérez et al. (2007) upon assessing 130 isolates using Fe_3PO_4 as a P source. According to the above authors, none of the isolates exhibited the capacity to solubilize Fe_3PO_4. This compound characteristically exhibits high stability and lower solubility compared to tricalcium phosphate.

The capacity to mineralize P from soy lecithin in solid and liquid media was confirmed in 7.5% and 5% of the isolates, respectively. Few studies have assessed the capacity of microorganisms to mineralize P from organic sources. Oliveira et al. (2009) investigated the microorganisms associated with the rhizosphere of corn using lecithin as a P source and found that only fungal isolates had this capability.

Organic P in the soil undergoes a mineralization process that transforms it into soluble forms of phosphorus (Rodriguez et al. 2006). Several authors have affirmed that enzymes mediate this process. Among the many enzymes involved, phosphatases are considered to be highly relevant. According to Bashan et al. (2013), phosphatases participate in a process known as enzymatically driven phosphate dissolution. In this process, enzymes are released into the extracellular compartment and catalyze the hydrolysis of organic phosphate. These inorganic forms of phosphorus are then released.

Many studies have sought to quantify the capacity of PSM to solubilize low-solubility phosphorus in liquid media (Whitelaw 2000, Souchie et al. 2005). According to the primary reports, solubilization in liquid media is chiefly due to the excretion of organic acids such as oxalic, citric and lactic acids (Khan et al. 2014). The role of such organic acids produced by PSM during the

solubilization of phosphorus is related to reductions in hydrogen potential and cation chelation (Nahas 1996).

In the present study, all 27 isolates assessed in liquid medium exhibited a capacity to reduce pH. Acidification of the medium is described as one of the most relevant processes in P solubilization (Bashan et al. 2013). In this process, protons (H^+) are released. These protons consequently reduce the pH of the medium, leading to the formation of more soluble hydrophosphates. The most remarkable and significant effects in this regard are exhibited by calcium phosphates, which served as one of the P sources in the present study.

The results described here and the various mechanisms involved in the process of phosphate solubilization and mineralization that have been described in the literature suggest that the investigated isolates exhibited significant variability. P solubilization occurred in solid and liquid media when tricalcium phosphate and soy lecithin were used as P sources. Reductions in pH and the presence of acid phosphatase activity suggest that at least the following three different solubilization processes occurred: medium acidification, metal complexing and enzymatically driven phosphate dissolution (Bashan et al. 2013).

Isolate EB. 78, a member of the genus *Bacillus*, deserves special consideration among the isolates with P solubilization capacity. This isolate exhibited P solubilization capacity in solid media containing tricalcium phosphate and soy lecithin as P sources, was able to solubilize P in the liquid medium supplemented with $Ca_3(PO_4)_2$, exhibited acid phosphatase activity and induced a significant reduction in the pH of the culture medium. Based on these results, we should consider including this isolate in future *in vivo* tests to assess its phosphorus nutritional parameters as well as its role in the parameters like estimation plant growth promoting substances like IAA, GA and antagonistic activitiy to common pathogens.

ACKNOWLEDGMENTS

The authors thank the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for their financial support and individual grants provided for the present study.

REFERENCES

ANDRADE LF, DE SOUZA GLD, NIETSCHE S, XAVIER AA, COSTA MR, CARDOSO AM, PEREIRA MCT AND PEREIRA DF. 2014. Analysis of the abilities of endophytic bacteria associated with banana tree roots to promote plant growth. J Microbiol 52: 27-34.

AZEVEDO JL, MACCHERONI JÚNIOR W, PEREIRA JO AND ARAUJO WL. 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electron J Biotechn 3: 40-65.

BARROSO CB AND NAHAS E. 2008. Solubilização do fosfato de ferro em meio de cultura. Pesq Agropec Bras 43: 529-535.

BASHAN Y, KAMNEV AA AND DE BASHAN LE. 2013. A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. Biol Fertil Soils 49: 1-2.

BERRAQUEIRO FR, BAYA AM AND CORMENZANA AR. 1976. Establecimiento de índices para el estudio de la solubilizacion de fosfatos por bacterias del suelo. ARS Pharm 17: 399-406.

BRAGA JM AND DEFELIPO BV. 1974. Determinação espectofotométrica de fósforo em extratos de solos e planta. Rev Ceres 21: 73-85.

FERREIRA DF. 2011. SISVAR: A computer statistical analysis system. Cienc Agrotec 35: 1039-1042.

GARG SK, BHATNAGAR A, KALLA A AND NARULA N. 2001. *In vitro* fixation, phosphate solubilization, survival and nutrient release by Azotobacter strains in aquatic system. Bioresour Technol 80: 101-109.

GUPTA R, SINGAL R, SANKAR A, KUHAD RC AND SAXENA RK. 1994. A modified plate assay for screening phosphate solubilizing microorganisms. J Gen Appl Microbiol 40: 255-260.

IGUAL JM, VALVERDE A, CERVANTES E AND VELAZQUEZ E. 2001. Phosphate solubilizing bacteria as inoculants for agriculture: use of update molecular techniques in their study. Agron Sustain Dev 21: 561-568.

KATZNELSON H AND BOSE B**.** 1959. Metabolism activity and phosphate-dissolving capability of bacterial isolates from wheat roots, rhizosphere, and non-rhizosphere soil. Can J MicroBiol 5: 79-85.

KHAN MS, ZAIDI A AND AHMAD E. 2014. Mechanism of Phosphate Solubilization and Physiological Functions of Phosphate-Solubilizing Microorganisms In: Phosphate Solubilizing Microbes for Crop Improvement, Springer International Publishing, Berlin Heidelberg, p. 31-62.

KHAN MS, ZAIDI A AND WANI PA. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review. Agron Sustain Dev 27: 29-43.

LIN TF, HUANG HI, SHEN FT AND YOUNG CC. 2006. The protons of gluconic acid are the major factor responsible for the dissolution of tricalcium phosphate by *Burkholderia cepacia* CC-A174. Bioresour Technol 7: 957-960.

MARTINAZZO R, SANTOS DR, GATIBONI LC, BRUNETTO G AND KAMINSKI J. 2007. Fósforo microbiano no solo sob sistema plantio direto em resposta à adição de fosfato solúvel. Rev Bras Cienc Solo 31: 563-570.

NAHAS E. 1996. Factors determining rock phosphate solubilization by microorganisms isolated from soil. World J Microbiol Biotecnol 12: 567-572.

NAUTIYAL CS. 1999. An efficient microbiological growth medium for screening phosphate soluzilizing microorganisms. FEMS Microbiol Lett 170: 265-270.

OLIVEIRA CA, ALVES VMC, MARRIEL IE, GOMES EA, MUZZI MRS, CARNEIRO NP, GUIMARÃES CT, SCHAFFERT RE AND SÁ NMH. 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol Biochem 41: 1782-1787.

PÉREZ E, SULBARÁN M, BALL MM AND YARZÁBAL LA. 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol Biochem 39: 2905-2914.

PIKOVSKAYA RI. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologiya 17: 362-370.

RODRIGUEZ H AND FRAGA R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17: 319-339.

RODRIGUEZ H, FRAGA R, GONZALEZ T AND BASHAN Y. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287: 15-21.

ROMEIRO RS. 2007. Controle biológico de doenças de plantas: procedimentos. Viçosa, MG: UFV, 172 p.

SCOTT AJ AND KNOTT M. 1974. Cluster-analysis method for grouping means in analysis of variance. Biometrics 30: 507-512.

SILVA FILHO GN AND VIDOR C. 2000. Solubilização de fosfato por microrganismos na presença de fontes de carbono. Rev Bras Cienc Solo 24: 311-319.

SOUCHIE EL, ÁZCON R, BAREA JM, SAGGIN JUNIOR OJ AND SILVA EMR. 2005. Solubilização de fosfatos em meio sólido e líquido por bactérias e fungos do solo. Pesq Agropec Bras 40: 1149-1152.

SOUZA SA, XAVIER AA, COSTA MR, CARDOSO AMS, PEREIRA MCT AND NIETSCHE S. 2013. Endophytic bacterial diversity in banana 'Prata Ana' (*Musa* spp.) roots. Genet Mol Biol 36: 252-264.

SRIDEVI M AND MALLAIAH KV. 2009. Phosphate solubilization by *Rhizobium* strains. J Microbiol Biotechnol 49: 98-102.

STAUFFER MD AND SULEWSKI G. 2004. Fósforo essencial para a vida In: Fósforo na agricultura brasileira. Piracicaba: Potafós, p. 1-12.

VERMA SC, LADHA JK AND TRIPATHI AK. 2001. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J Biotechnol 91: 127-141.

WANG Y, SHI Y, LI B, SHAN C, IBRAHIM M, JABEEN A, XIE G AND SUN G. 2012. Phosphate solubilization of *Paenibacillus polymyxa* and *Paenibacillus macerans* from mycorrhizal and non-mycorrhizal cucumber plants. Afr J Microbiol Res 6: 4567-4573.

WHITELAW MA. 2000. Growth promotion of plants inoculated with phosphate-solubilizing fungi. Adv Agron 69: 99-151.