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ROCK PHOSPHATE SOLUBILIZING MICROORGANISMS ISOLATED FROM MAIZE RHIZOSPHERE SOIL

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ABSTRACT - The selection of microorganisms capable of solubilizing phosphorus (P) from rock phosphates (RP) may contribute to reduce the dependence of imported fertilizers in grain crops, reducing the costs of agricultural production, and also the environmental impacts. This study tested 59 microorganisms (46 bacteria and 13 fungi) isolated from maize rhizosphere for solubilization of two RP, Araxá and Itafós phosphate *in vitro* (PA and PI, respectively). Among the 59 microorganisms solubilizing PA, 51% of the bacteria and 8% of fungi were classified as efficient. For PI, among 18 isolates, 50% of the bacteria and no fungi were efficient. There were significant differences in the availability of P among strains for both phosphates and most isolates evaluated for both types of rocks released more soluble P from PI than PA. Bacterial isolates CMSB58, CMSB32, CMSB20 and CMSB46 solubilized almost 20% of the P total in the PA and CMSB58, CMSB82, CMSB91 and CMSB48 solubilized more than 25% of the PI. The solubilizing activity of both phosphates was associated with a reduction of pH which suggests that the acidification of the culture medium can be one of the mechanisms involved in the solubilization of P. There was a dominance of the genera *Burkholderia* and *Bacillus* in the group of the most efficient bacteria and *Talaromyces* and *Penicillium* in the fungi group. The contribution of these strains to increasing the phosphorus nutrition of grain crops should be investigated further by in vivo experiments.

Key words: Biosolubilization; Phosphorus; *Zea mays*; Araxá phosphate; Itafós phosphate.

MICRORGANISMOS SOLUBILIZADORES DE FOSFATO DE ROCHA ISOLADOS DA RIZOSFERA DE MILHO

RESUMO - A seleção de microrganismos capazes de solubilizarem fósforo (P) a partir de fosfatos de rocha (FR) pode contribuir para reduzir a dependência de fertilizantes importados em culturas de grãos, reduzindo os custos da produção agrícola e também os impactos ambientais. Este estudo avaliou 59 microrganismos (46 bactérias e 13 fungos), isolados da rizosfera de milho, quanto à solubilização de dois FR, Araxá e Itafós *in vitro* (FA e FI, respectivamente). Entre os 59 microrganismos solubilizadores de PA, 51% das bactérias e 8% dos fungos foram classificados como eficientes. Para FI, entre 18 isolados, 50% das bactérias e nenhum fungo foram eficientes. Houve diferença significativa na disponibilidade de P entre as cepas em ambos os fosfatos e a maioria dos isolados avaliados em ambos os tipos de rocha liberaram mais P solúvel de FI em comparação com FA. As bactérias CMSB58, CMSB32, CMSB20 e CMSB46 solubilizaram quase 20% do P total em FA e CMSB58, CMSB82, CMSB91 e CMSB48 solubilizaram mais que 25% de FI. A atividade de solubilização para ambos os fosfatos foi associada com a redução de pH, sugerindo que a acidificação do meio de cultura pode ser um dos mecanismos envolvidos na solubilização de P. Houve predominância dos gêneros *Burkholderia* e *Bacillus* no grupo de bactérias mais eficientes e *Talaromyces* e *Penicillium* no grupo dos fungos. A contribuição destes isolados na melhoria da nutrição de P em milho precisa ser investigada futuramente em experimentos in vivo.

Palavras-chave: Biossolubilização; Fósforo; *Zea mays*; fosfato de Araxá; fosfato de Itafós.

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One of the limiting factors in tropical agriculture soils such as the Oxisol of the Brazilian acid savannas (Cerrado), is the low pH and the high phosphorus (P) fixation capacity of the soil, resulting in low availability of this nutrient to plants (Novais & Smyth, 1999). Phosphorus is one of the most limiting macronutrients for agricultural production in many soils of the world as the overall efficiency of applied fertilizer can be less than 10% (Baligar et al., 2001). P deficiency is generally alleviated through application of P fertilizers. However only a small portion of these is used by plants and the most P fertilizer readily form insoluble complexes with the constituents of the soil, becoming unavailable to plants, which leads to the need for frequent applications of this nutrient (Novais & Smyth, 1999). Furthermore, the production of chemical fertilizers requires fossil energy for processing, transportation and distribution, which increases the production costs and environmental risks (Schröder et al., 2010).

In this context, the use of natural rock phosphate (RP) as P source for crops has been evaluated. The application of RP as fertilizer in tropical environments has numerous advantages, especially in the rate of dissolution of these rocks, and the reaction between mineral surfaces and soil solution, which are intensified with temperature and humidity present in these soils (Van Straaten, 2006). However, depending on the properties of the RP, the soil, climatic conditions, crop and on management practices (Sale & Mokwunye, 1993), it could take up to 4 yr of annual application before RP treatments become as effective as super-phosphate (Ghani et al., 1994). The direct use of natural sources of P as fertilizer, mainly for annual crops is not economically viable, particularly in soils with high adsorption and low ion exchange capacity (Simpson et al., 1997), as the Brazilian Cerrado soils.

For these reasons, various strategies have sought the use of microorganisms with potential for RP solubilization (Rajapaksha et al., 2011) to increase the availability of this nutrient from different types of phosphates of low solubility (Oliveira et al., 2009; Singh & Reddy, 2011), reducing the cost and energy loss for the agronomic use of these sources of P (Mohammadi, 2012). The great advantage of this combined use, in addition to the exploration of alternative sources for P fertilization (Khan et al., 2007), is the use of rocks that have low levels of P, which are inadequate for the fertilizer industry because they contain a high degree of impurities, such as marginal rocks and wastes from industry.

The key mechanism associated with solubilization of mineral phosphates is the reduction of the pH of the medium by the release of low molecular weight organic acids by microorganisms (Chung et al. 2005; Barroso & Nahas, 2008; Gulati et al. 2010). These organic acids act removing inorganic P from soil particles of clay either by direct exchange as chelation of metal ions in complex P-cations (Rodríguez & Fraga, 1999). The release of anions also results in the rhizosphere acidification, directly increasing the solubility of inorganic precipitated salts of P. However, the soil microorganisms show wide variation in their ability to secrete organic acids and thus solubilize mineral phosphate (Richardson et al., 2009).

The recommendation of strains as inoculants able to solubilize P depends on the type of RP. Oliveira et al. (2009) isolated microorganisms capable of solubilizing organic and inorganic insoluble sources of P from the rhizosphere of maize genotypes efficient in the use of P. However, in that

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work, the focus was the solubilization of inorganic synthetic phosphate, such as tricalcium phosphate and aluminum phosphate, and the solubilizing potential of different natural RP from Brazilian natural mines by these microorganisms has not been evaluated.

In this paper, the main goal is to evaluate, among the microorganisms assessed by Oliveira et al. (2009), those isolates with potential to solubilize Brazilian natural RP to use as a source of P for grain crops.

Materials and Methods

Isolates of bacteria and fungi, belonging to the collection of multifunctional microorganisms of Embrapa Maize and Sorghum, were evaluated for their ability to solubilize two natural phosphates, named Araxá (PA) and Itafós (PI), extracted from RP mines in Brazil. Most microbial isolates were obtained from samples of rhizosphere soil of maize genotypes contrasting for P use efficiency under different soil management systems in the Brazilian Cerrado soil (Oliveira et al., 2009). The microorganisms were conserved in mineral oil and reactivated growing on PDA plates (200 g l^{-1} of potato, 20 g l^{-1} of dextrose and 15 g l^{-1} of agar), using the method of streaking for obtaining pure colonies.

In order to evaluate the potential of P solubilization by the selected microorganisms, the NBRIP liquid culture medium was used (10 g l^{-1} glucose, 0.15 g l^{-1} of $(NH_4)_2$ SO_4, 0.2 g l^{-1} of KCl, 5 g l^{-1} of $MgCl_2$. 6 H_2O and 0.25 g l^{-1} of $MgSO_4.7H_2O$) (Nautiyal, 1999) plus 5 g l^{-1} PA or PI, both containing approximately 24% of P_2O_5.

The experiment to evaluate the bioavailability of P from PA in vitro was a completely randomized design with three replications. Each plot consisted of a 250 ml Erlenmeyer flask containing 50 ml of culture medium plus RP. The treatments were 59 microorganisms individually inoculated into the culture medium (46 bacteria and 13 fungi). Also, one control containing only the culture medium and the other one with the culture medium and PA were included. In order to assess the potential of PI solubilization, 18 microorganisms were randomly selected using the same experimental model.

The microorganisms were inoculated separately, using $5x10^7$ cells per ml of the bacterial suspension or five disks of 8 mm of the mycelium of fungi and actinobacteria. The cultures were submitted to incubation for 10 days with constant shaking of 120 rpm and a temperature of 28 ^oC (Oliveira et al., 2009 modified).

After 10 days of incubation, the cultures were centrifuged at 5000 x g for 10 min, the supernatant was filtered using paper Whatman nº 42 and the concentration of soluble P was determined by the Murphy & Riley (1962) methodology. Additionally, the pH of the filtrate from all samples, including the controls, was determined.

In order to determine the relative efficiency of P solubilization of the isolates we determined the following expression: $(N_1-N_2) / N_3 \times 100$, in which N_1 is the concentration of P (mg l^{-1}) in the presence of microorganism, N_2 is the concentration of P (mg l^{-1}) in the absence of microorganism and N_3 is the total P concentration (mg l^{-1}) contained in the RP. Then the microorganisms were classified into three groups according to the isolates that showed the best performance in P solubilization: efficient (67-100%), moderately efficient (35.5-67%) and inefficient (0-35.5%).

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Bacterial genomic DNA was extracted from cultures incubated in LB medium at 37 °C for 24 h, by the phenol/chloroform method (Ausubel et al., 1987) and amplified using the 16S rDNA primers F968 and R1401 (Nubel et al., 1996). Fungi ITS (internal transcribed sequences) rDNA fragments were amplified by the primers ITS1 and ITS4 (White et al., 1990) from genomic DNA extracted according to the method of Raeder & Broda (1985). PCR reaction was performed in a final volume of 50 µl containing 20 ng DNA, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.125 mM dNTPs, 0.4 µM primers, 1.5 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 1% (v/v) formamide for analysis of bacteria or 0.2% (v/v) DMSO for fungi. Amplification was performed using the following conditions: 94 °C for 2 min, 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and a final extension of 72 °C for 10 min for bacteria. The amplification for the fungi was performed using the following conditions: 40 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 90 sec, and a final extension of 72 °C for 7 min. The reaction products were analyzed by electrophoresis on 1.2% (w/v) agarose gel stained with ethidium bromide (1µg ml^{-1}) and displayed in the equipment Gel Logic 200 (KODAK Company, Rochester, NY).

The amplification products were removed from the gel, purified with the kit QIAquick Gel Extraction (Qiagen, Hilden, Germany) and sequenced using the kit Big Dye Terminator (Applied Biosystems, Foster City, CA) according to the recommendations of manufactures. Samples were analyzed in the automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) and the sequences were compared with the GenBank (http://www.ncbi.nlm.nih.gov/) using the program Blast N (Altschul et al., 1997).

The data obtained for phosphate solubilizing activity and pH of microorganisms were subjected to ANOVA and means were compared by the Scott Knott test using the software SISVAR (Ferreira, 2008). The linear correlation (r) between solubilized phosphate in liquid media and pH was performed using Excel 2007 (Microsoft Corporation, Redmond, WA). The differences obtained at the level of $P \leq 0.05$ were considered significant.

Results and Discussion

Among the 59 microorganisms solubilizing PA, 51% of the bacteria were classified as efficient, 13% classified as moderately efficient and 54% classified as inefficient. Related to fungi, 8% were efficient, 23% were moderately efficient and 69% were inefficient (Figure 1). For PI, 50% of the bacteria obtained were efficient, 8% were moderately efficient and 42% were inefficient. In the group of fungi were observed only 33% of the strains classified as moderately efficient and 67% as inefficient (Figure 1).

A significant difference ($p \leq 0.05$) among the values of PA solubilized that ranged from 0.94 to 100.70 mg P l^{-1} was shown in Table 1. Among the bacteria, CMSB58 (*Burkholderia*) and CMSB32 (*Bacillus*) were the most efficient, providing 100.70 and 94.98 mg P l^{-1}, respectively. Among the fungi, the isolates that showed higher solubilizing capacity of PA were CMSF14 (*Penicillium*), CMSF102 and CMSF105 (*Talaromyces*) that solubilized 75.54, 55.70 and 54.83 mg P l^{-1}, respectively.

Similarly, the solubilization of PI showed significant variation ($p \leq 0.05$) among the 18

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strains examined, including fungi and bacteria. The highest solubilization of PI were observed in strains of the *Burkholderia* genus CMSB58, CMSB48 and CMSB82 resulting in the release of 153.11, 135.58 and 132.88 mg P l^{-1} (Table 1), respectively. Regarding the solubilization capacity of fungi in PI, the best isolates were CMSF14 (*Penicillium*), CMSF105 (*Talaromyces*) and CMSF102 (*Talaromyces*), releasing 71.36, 66.55 and 45.24 mg P l^{-1}, respectively (Table 1).

RP solubilization values similar to our results are common. Xiao et al. (2008) investigated the RP solubilizing by *Candida krissii*, *Penicillium expansum* and *Mucor ramosissimus* isolated from phosphate mines and observed that the maximum content of soluble P was 109.3 mg l^{-1} released by *C. krissii*, followed by *P. expansum* (104.5 mg l^{-1}) and *M. ramosissimus* 99.9 mg l^{-1}. Rajapaksha et al. (2011) also studied six strains of bacteria isolates from rice rhizosphere and observed that the P solubilization varied from 50 to 150 mg P l^{-1}.

The genus *Burkholderia* has been reported in other studies as a plant growth-promoting rhizobacteria and as efficient in solubilizing P from different sources (Anandham et al., 2007; Marra et al., 2011; Peix et al., 2011; Azziz et al., 2012). It has been observed that this genus is associated in large numbers with the rhizosphere of maize, ranging from 4 to 35% of the total culturable bacteria present in the rhizosphere of this crop (Hebbar et al., 1994; Balandreau et al., 2001). Besides *Burkholderia*, the genera *Bacillus* and *Paenibacillus* along with *Aspergillus*, *Penicillium* and *Talaromyces* have been reported as P solubilizers, plant growth promotion and used as commercial P inoculants (Khan et al., 2008; Khan et al., 2010; Scervino et al., 2010; Naraghi et al., 2012; Anand et al., 2013; Junges et al., 2013; Murugappan et al., 2013).

All the strains that solubilized phosphate in liquid media reduced the pH of the media compared to the non-inoculated control, regardless of the source of phosphate. There was a significant and negative correlation between the amount of soluble phosphorus and the final pH of culture media ($r = -0.89$ for PI and $r = -0.82$ for PA; $p < 0.01$) (Table 1).

The comparison of results of both types of phosphates showed that the solubilization of PI and PA were significantly different (Figure 2). The solubilization of PI was higher than PA by the isolates CMSB58, CMSB48, CMSB82, CMSB91, CMSB119, CMSB5, CMSB2, CMSF105 and CMSF80. Most of these isolates belong to *Burkholderia* genus. On the other hand, CMSB70, CMSB32, CMSB62, CMSF40, CMSB86 and CMSB124 showed opposite results, being efficient in the PA solubilization (between 18.62% and 13.97%), whereas they solubilized less than 3% of PI (Table 1), indicating the effect of the type of rock in this biological process (Figure 2). Interesting, all of these PA efficient isolates are from *Bacillus* and *Arthrobacter* genera and none of them belongs to the *Burkholderia* (Table 1 and Figure 2). In both PI and PA, the isolate CMSB58 presented the best performance in the solubilization, around 153.11 mg P l^{-1} and 100.70 mg P l^{-1} (29.20 and 19.17% of the P total content, respectively), demonstrating potential of this isolate to be used to improve the P availability of these minerals being a good candidate for a biofertilizer to be used in maize fields.

Another explanation for this difference in the solubilization of both types of RP is the different physicochemical characteristics, depending on their source material and particle

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TABLE 1. Phosphate solubilization activity of microorganisms isolates from the maize rhizosphere.

Isolate	Taxon	Species (access number)[a] Similarity index	Araxá phosphate			Itafós phosphate		
			P (mg l^{-1})[*b]	P (%)[c]	pH[d]	P (mg l^{-1})	P (%)	pH
CMSB58	Bacteria	*Burkholderia cepacia* (AY509957) – 89%	100.70 A	19.74 A	3.05 A	153.11 A	29.20 A	3.04 A
CMSB32	Bacteria	*Bacillus pumilus* (FJ641028) – 99%	94.98 A	18.62 A	3.15 A	11.02 G	2.10 G	4.33 D
CMSB20	Bacteria	*Uncultured Paenibacillus* (EU647536) – 99%	93.45 A	18.32 A	3.15 A	-	-	-
CMSB46	Bacteria	*Burkholderia* sp. (FJ644952) – 96%	92.78 A	18.18 A	3.18 A	-	-	-
CMSB82	Bacteria	*Burkholderia* sp. (AB480713) – 98%	91.47 A	17.93 A	3.19 A	132.88 B	25.34 B	3.16 A
CMSB91	Bacteria	*B. cenocepacia* (EF602557) – 98%	90.60 A	17.76 A	3.19 A	126.21 B	24.07 B	3.14 A
CMSB48	Bacteria	*Burkholderia* sp. (FJ4334111) – 90%	89.03 A	17.45 A	3.22 A	135.58 B	25.85 B	3.15 A
CMSB5	Bactéria	*B. cepacia* (EF602558) – 98%	88.77 A	17.40 A	3.19 A	105.96 C	20.21 C	3.20 A
CMSB70	Bacteria	*B. subtillis* (FJ4835114) – 89%	86.28 A	16.91 B	3.12 A	16.84 G	3.21 G	4.08 C
CMSB62	Bacteria	*Arthrobacter* sp. (AF408967) – 98%	85.31 A	16.72 B	3.11 A	7.19 H	1.37 H	4.30 D
CMSB34	Bacteria	*Burkholderia* sp. (FJ434111) – 89%	82.62 A	16.19 B	3.10 A	-	-	-
CMSB37	Bacteria	*Burkholderia* sp. (AB480713) – 96%	79.77 B	15.63 B	3.08 A	-	-	-
CMSB43	Bacteria	*Stenotrophomonas maltophilia* (AF068009) – 96%	79.33 B	15.55 B	3.07 A	-	-	-
CMSB11	Bacteria	*Burkholderia* sp. (FJ930075) – 97%	76.74 B	15.04 C	3.09 A	-	-	-
CMSF14	Fungus	*Penicillium pinophilum* (EU360183) – 95%	75.54 B	14.81 C	3.73 A	71.36 D	13.61 D	3.51 B
CMSB44	Bacteria	*B. cepacia* (GQ359110) – 98%	75.00 B	14.70 C	3.08 A	-	-	-
CMSB17	Bacteria	*Serratia* sp. (HM045833) – 96%	71.45 B	14.00 C	3.19 A	-	-	-
CMSB86	Bacteria	*Arthrobacter* sp. (DQ985470) – 89%	71.37 B	13.99 C	3.16 A	0.00 I	0.00 I	4.25 D
CMSB124	Bacteria	*Arthrobacter* sp. (FJ685644) – 97%	71.28 B	13.97 C	3.17 A	0.00 I	0.00 I	4.46 E
CMSB2	Bacteria	*Pantoea ananatis* (FJ611812) – 94%	71.15 B	13.95 B	3.16 A	98.28 C	18.74 C	3.24 A
CMSB116	Bacteria	*B. pumilus* (FJ641028) – 99%	70.02 B	13.72 B	3.22 A	-	-	-
CMSB45	Bacteria	*Burkholderia* sp. (FJ930075) – 98%	62.29 C	12.21 D	3.43 A	-	-	-
CMSB119	Bacteria	*B. subtillis* FJ483514 – 89%	61.27 C	12.15 D	3.20 A	107.39 C	20.48 C	3.17 A
CMSF102	Fungus	*Talaromyces rotundus* (EU497950) – 95%	55.70 C	10.92 D	3.58 A	45.24 E	8.63 E	3.47 B
CMSF105	Fungus	*T. rotundus* (AF285115) – 95%	54.83 C	10.75 D	3.62 A	66.55 D	12.69 D	3.65 B
CMSB4	Bacteria	*S. maltophilia* (FJ481929) – 89%	45.78 D	8.97 E	3.44 A	-	-	-
CMSB65	Bacteria	*Uncultured Pseudomonas* (FJ542901) – 95%	42.08 D	8.25 E	3.38 A	-	-	-
CMSF94	Fungus	*T. rotundus* (AF285115) – 94%	40.67 D	7.93 E	3.61 A	-	-	-
CMSB7	Bacteria	*Citrobacter* sp. (GU056358) – 99%	35.03 D	6.87 F	3.93 B	-	-	-

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CMSF87	Fungus	*Acremonium strictum* (AY138846) – 93%	33.70 D	6.61 F	3.77 B	-	-	-	
CMSB15	Bacteria	*Sinomonas flava* (EU370704) – 97%	32.42 D	6.35 F	4.65 B	-	-	-	
CMSB76	Bacteria	*Pantoea* sp. (GU271945) – 96%	30.16 E	5.91 F	3.55 A	-	-	-	
CMSB3	Bacteria	*Bacillus* sp. (EU864320) – 89%	25.79 E	5.05 F	3.91 B	-	-	-	
CMSB6	Bacteria	*Acinetobacter calcoaceticus* (FJ976598) – 98%	23.74 E	4.65 F	3.89 B	-	-	-	
CMSA62	Bacteria	*Streptomyces* sp. (AB366323) – 96%	18.54 F	3.63 G	4.10 B	-	-	-	
CMSF80	Fungus	*T. rotundus* (EU497950) – 94%	18.29 F	3.58 G	3.81 B	36.25 F	6.91 F	4.83 F	
CMSB1	Bacteria	*B. megaterium* (FJ393316) – 92%	17.70 F	3.47 G	4.24 B	-	-	-	
CMSB121	Bacteria	Uncultured *Pseudomonas* (HM011904) – 98%	17.69 F	3.47 G	4.24 B	-	-	-	
CMSF93	Fungus	*Aspergillus terreus* (AY822631) – 99%	12.93 F	2.53 G	5.22 D	14.65 G	2.79 G	4.53 E	
CMSF95	Fungus	*P. citrinum* (FJ571468) – 97%	12.71 F	2.49 G	4.04 B	-	-	-	
CMSF96	Fungus	*T. rotundus* (AF408967) – 98%	10.49 G	2.06 H	4.70 D	-	-	-	
CMSB104	Bacteria	*B. cereus* (DQ884352) – 90%	10.32 G	2.02 H	5.49 D	-	-	-	
CMSF50	Fungus	*A. terreus* (FJ462767) – 95%	10.01G	1.96 H	4.65 C	-	-	-	
CMSB118	Bacteria	*B. pumilus* (FJ641028) – 96%	9.69 G	1.90 H	4.47 C	-	-	-	
CMSF39	Fungus	*A. terreus* (AJ001333) – 99%	8.57 G	1.68 H	4.38 C	-	-	-	
CMSA80	Bacteria	*Streptomyces bungoensis* (FJ486371) – 94%	7.73 G	1.52 H	4.40 C	-	-	-	
CMSB18	Bacteria	*Bacillus* sp. (EU864320) – 92%	7.42 G	1.45 H	4.66 D	-	-	-	
CMSB16	Bacteria	*B. pumilus* (EU586783) – 97%	7.24 G	1.42 H	4.70 D	-	-	-	
CMSA14	Bacteria	*Streptomyces* sp. (AB369480) – 98%	7.05 G	1.38 H	4.76 D	-	-	-	
CMSF40	Fungus	*A. terreus* (AY822630) – 99%	6.95 G	1.36 H	4.28 C	4.78 I	0.91 I	4.04 C	
CMSB52	Bacteria	*Burkholderia* sp. (AM992063) – 90%	6.70 G	1.31 H	7.33 F	-	-	-	
CMSB19	Bacteria	Uncultured *Paenibacillus* sp. (FJ481059) – 94%	6.05 G	1.19 H	4.81 D	-	-	-	
CMSA68	Bacteria	*S. spinicoumarensis* (AB184535) – 91%	5.53 G	1.08 H	4.59 D	-	-	-	
CMSA83	Bacteria	*Streptomyces* sp. (EU360176) – 94%	5.37 G	1.05 H	4.72 D	-	-	-	
CMSB31	Bacteria	*B. pumilus* (EU147190) – 97%	3.52 G	0.69 H	4.74 D	-	-	-	
CMSF79	Fungus	*A. terreus* (FJ462767) – 98%	2.35 G	0.46 H	4.65 D	-	-	-	
CMSA4	Bacteria	*S. tubercidicus* (FJ406112) – 96%	1.72 G	0.34 H	5.02 D	-	-	-	
CMSA19	Bacteria	*S. chartreusis* (EU647536) – 90%	0.94 G	0.18 H	4.67 D	-	-	-	
Control	Not inoculated	-	1.02 G	0.20 H	6.80 E	1.54 I	0.30 I	6.00 G	

*Values followed by the same letter(s) (column) indicate no significant difference (p>0.05) at 95% confidence. Values are average of three replications. [a]Molecular identification of the microorganisms. [b]Solubilized phosphorus (mg l^{-1}). [c]P solubilization efficiency (%). [d]pH after 10 days of grown at 28 °C.

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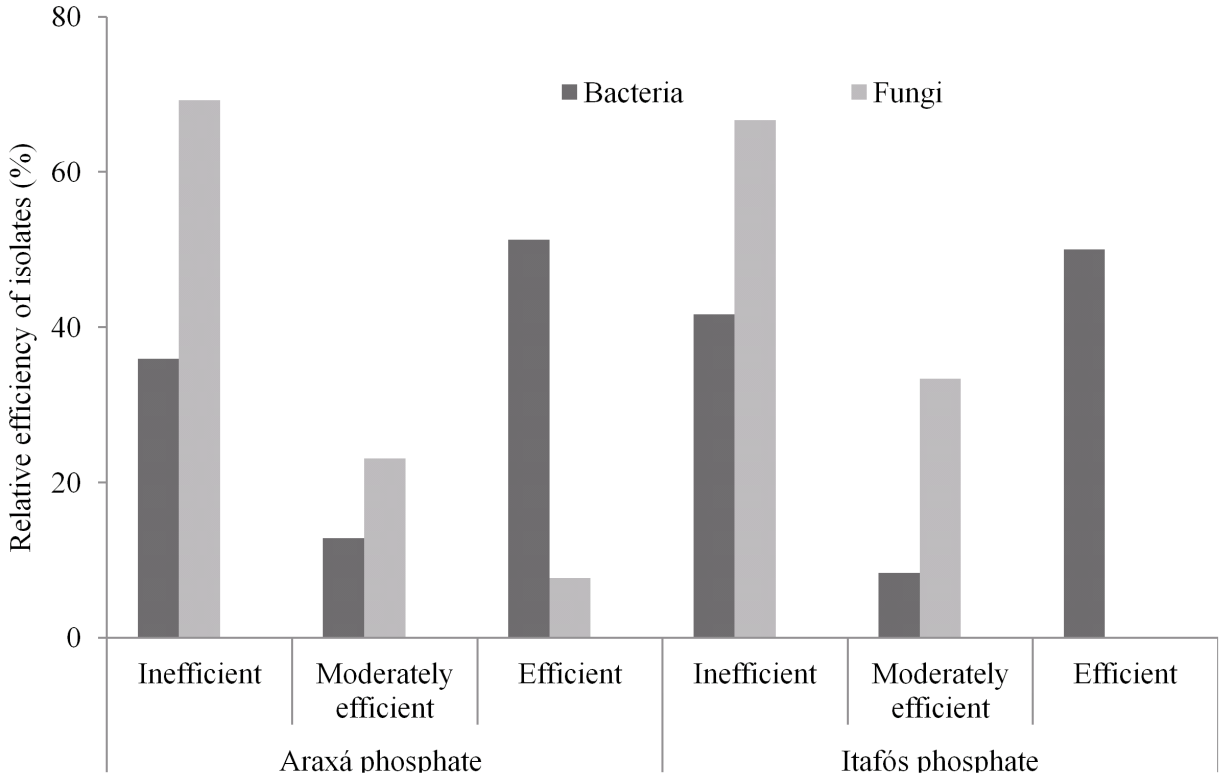

FIGURE 1. Relative efficiency of the isolates in the P solubilization (%) of the Araxá and Itafós phosphates.

size, that influence their rate of solubilization (Loureiro et al., 2008). Mendes et al. (2013) showed that fluoride limited the solubilization of Araxá RP by *A. niger* by negatively affecting metabolic processes involved in phosphate solubilization, such as decreasing fungal growth, citric acid production and medium acidification. In the case of phosphates of sedimentary origin, such as PI, generally, there are higher contents of available P in relation to the ones of igneous or metamorphic origin, such as the PA that exhibits high crystallization level and low solubility in citric acid (Kliemann & Lima, 2001). Due to these characteristics, there are significant differences compared to the natural bioavailability of nutrients, which can be altered in the presence of microorganisms (Richardson & Simpson, 2011). The differences observed in the present work according both type of RP can contribute to the process of selection of microorganisms to be used with natural phosphates in tropical agriculture. It can be suggested that for each type of rock to be used, there are different microorganisms that have potential for solubilizing P.

Other authors also observed that solubilization capacity of microorganisms depends on the type of phosphate assessed. Xiao et al. (2008) observed that solubilization capacity of three types of RP from China by the fungi *Candida krissii*, *Penicillium expansum* and *Mucor ramosissimus* was directly proportional to the P content in the rock. In other words, it might be concluded that the capability of RP solubilization was positively correlated with the grade of RP.

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This paper presents a considerable improvement in the characterization of microorganisms that have potential for use for the production of inoculants to maize, since most microorganisms selected solubilize both types of RP from Brazilian natural mines and other inorganic sources, such as aluminum phosphate. Other isolates also have the ability to mineralize organic sources of P, such as soy lecithin and phytate (Oliveira et al., 2009).

Together, these microorganisms should be evaluated in experiments in vivo in the future, using different RP and vehicles aiming the development of inoculants for P supply in agriculture applied in seeds or directly on soil to be offered to farmers as technological products for reduction of fertilizer costs and environmental impacts.

Conclusions

There were significant differences in the availability of P among strains and most isolates released more soluble P from PI than PA. The majority of the bacteria were efficient in the P solubilization of both RP. There was a negative correlation between the final pH of the culture medium and the concentration of soluble phosphate suggesting that the acidification of the culture medium can be one of the mechanisms involved in the solubilization of P by these microorganisms. There was a dominance of the genera *Burkholderia* and *Bacillus* in the group of the most efficient microorganisms and the contribution of these strains, isolated or in combination, to increase the P nutrition of maize crops should be investigated further, by *in plant* experiments in tropical soils.

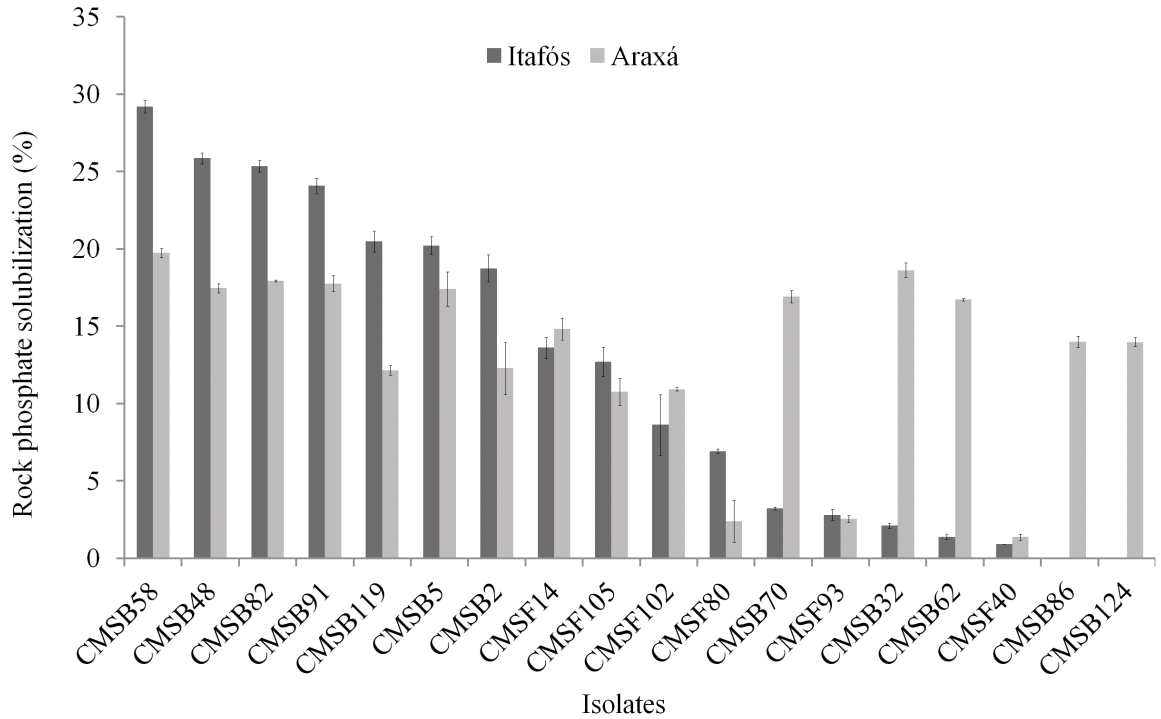

FIGURE 2. Percentage of Araxá and Itafós phosphate solubilization of 18 isolates of fungi and bacteria. Isolates identification is shown in Table 1.

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