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Original Article

Solubility curve of rock powder inoculated with microorganisms in the production of biofertilizers

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ABSTRACT

The study was conducted at the Biochemistry Engineering Laboratory of the Federal University of the Rio Grande do Norte to verify the efficacy of microorganisms as solvents of apatite and biotite rock powder to enable the availability and rapid production of biofertilizers. Bacteria *Paenibacillus polymyxa*, *Ralstonia solanacearum*, *Cromobacterium violaceum* and *Acidithiobacillus thiooxidans* and fungi *Penicillium fellutanum* and *Tricoderma humatum* were inoculated into biotite rock powder and apatite rock powder originating from the States of Paraíba and Paraná, respectively, in Brazil. Rock powder samples were taken on Petri plates, 10% sulfur was added to each, and were subsequently inoculated and co-inoculated for a period of 72 days. Every 12th day, the samples were withdrawn and their mineral release curve was studied. From our results, the co-inoculations with *Paenibacillus polymyxa* + *Ralstonia solanacearum* and *Paenibacillus polymyxa* + *Cromobacterium violaceum* rendered higher solubility of K and P, respectively, at 36 days.

Introduction

The growing concern for environmental conservation has stressed on the need for effective agricultural systems emphasizing ecology, profitability and social equality. The ecological aspect of agricultural undertaking strives for the responsible use of natural resources such as soil, water, fauna, flora, energy and minerals (Oliveira et al., 2003). One of the main overheads in crop production is fertilizer, mainly distinguished by its macronutrient content of nitrogen, phosphorus, and potassium (Alves et al., 2010). Among the three main nutrients, plants require K and P in greater quantities for their metabolic functions (Rheinheimer et al., 2008; Chotchutima et al., 2016).

Geological characteristics have replaced vital quantities of elements from the soils of Brazil by intemperate factors over millions of years. Brazil is a substantial consumer and importer of potassium fertilizer, and the local source of raw mineral potassium chloride would support the demand up to 2017. However, the trend in the supply of apatite rock in Brazil is more encouraging, for the national companies supply 80% of this mineral asset (Rodrigues, 2009).

Since the high dependence on import of fertilizers entails heavy national expenditures, it becomes urgent to find ecologically safe, sustainable and economical means to fertilize the soil using ground rocks whose main characteristic is gradual solubility of its nutrients in highly appropriate and favorable conditions for mineral lixiviation (Beneduzi et al., 2013).

But according to Alves et al. (2010), the release of rock powder nutrient solution into the soil to be absorbed by plants may be slow in eroded tropical soil. One of the most promising alternative mechanisms for increasing crop yields is the use of biotechnology, and many microorganisms have been recognized for their ability to promote bioquimical transformations of the nutrients, mainly N, P and K, to a form absorbable by plants, through nutrient decomposition and cycling of nutrients. Within these capabilities of the microorganisms are involved the mechanisms of production of organic and inorganic acids, chelation, ionic exchanges, enzymes and others (Guimarães et al., 2006; Aria et al., 2010).

The biological processes that arise in the soil/plant, performed by microorganisms, form the basis upon which agriculture

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agroecology is sustained. The use of microorganisms for improvement of agricultural productivity is likely to be one of the most important tools for agriculture in the current world, mainly due to emerging demand for reducing dependence on chemical fertilizers and the need for sustainable agriculture development (Moreira and Siqueira, 2006; Dastager et al., 2011).

Experiments in solubility involving microorganisms have shown promising results (Aria et al., 2010; Chagas Junior et al., 2010). The search for strains, isolated or combined, that deliver high solubility is ongoing. The discovery of the knowledge of the mechanisms of microbial interactions with the soil, as well as the invention of technology to maximize the benefit to sustainable agriculture, is a great challenge. Hence, it has become important to investigate the time needed for the biosolubility activity of P and K to reach its peak in order to be made available to plants. Thus, the objectives of this study were to verify the effect of inoculation and co-inoculation with bacteria and fungi on the solubility of P and K contained in the rock powder and analyze the effective time by which biosolubility of apatite rock and biotite rock could be achieved for effective production of biofertilizers.

Materials and methods

First bioassay

The bioassay was run at the Biochemistry Engineering Laboratory of the Chemical Engineering Department of the Federal University of the State of Rio Grande do Norte (UFRN). The efficiency of the microorganisms, alone as well as in combination, was tested. The solubility of K and P in ground rock due to the action of the microorganisms was also evaluated.

Use of substrate

The substrate used was biotite rock (containing 17.7% potassium (K_2O)) from the State of Paraíba (Brazil) and apatite rock (containing 12% phosphorus (P_2O_2)) from the State of Paraná (Brazil). The powdered rock was mixed with 10% elementary sulfur (S) or 12 g of sulfur per 120 g of rock powder on Petri plates (Stamford et al., 2008). The chemical analysis followed the Embrapa (2009) methodology, with P and K extracted by the Mehlich-1 (HCl 0.05 M + H_2SO_4 0.0125 M). Potassium was determined by flame photometry and P by colorimetric determination.

Bacterium and fungi used in the bioassay

The genera and origin of different strains used in the present study are presented in Table 1.

Multiplication of microorganisms

The genus *Paenibacillus polymyxa* was cultivated in TSB (tryptic soya broth) medium at pH 7.0, 32 °C/150 rpm for 2 days (Silva et al., 2007). The bacterium *A. thiooxidans* was cultivated in TK (Tuovinen and Kell) medium (Tuovinen and Kelly, 1973) at pH 2.8, 30 °C/170 rpm for 15–20 days. For *Ralstonia solanacearum*, Kelman's (Kelman, 1954) methodology was applied, at pH 7.0 and 33 °C/150 rpm for 3 days. *C. violaceum* was purified in Luria-Bertani medium at pH 7.0, 30 °C/170 rpm for 2 days (Sambrook and Russel, 2001). For *P. fellutanum*, malt extract was used (2% malt extract, 0.1% peptone and 2% glucose), and for *T. humatum* was used 2% malt extract at pH 5.0 and 30 °C/150 rpm for 3–5 days (Rifai, 1969). When necessary, 1.5–2% agar was used to store the microorganisms. All microorganisms were multiplied until reach stationary phase.

Inoculation and incubation period

Treatments by separate inoculations (with only one microorganism) and co-inoculations (with two organisms) using all possible combinations were carried out. These treatments were incubated in Petri plates containing apatite and biotite rocks separately, with addition of sulfur. Inoculations and co-inoculations were carried out by placing 6.0 mL of inoculum of each microorganism per Petri dish. The treatments were kept with water content of 80% of field capacity during the incubation period. The bioassay lasted 72 days. At 12-day intervals, samples were taken and the biosolubility reaction of K and P observed. Immediately after collection, the samples were incubated at 70 °C for 3–4 days for later analysis.

Statistical analysis

The experiment was carried out in a completely randomized design with split plots in time and three replications. The data were analyzed statistically with the analysis of variance, and when a significant F value was detected means were compared using the Tukey test at the 0.05 level of significance, using 21 treatments with three replicates using the software SISVAR 5.3 (Ferreira, 2010).

Results and discussion

A variance analysis demonstrated that the variables of the treatment, time and treatment × time, were significant at a level of 5% probability by the Tukey test.

This study provides valuable information as to the peak solubility time brought about by inoculation and co-inoculation of apatite and biotite rock powder samples. The K and P release curves of rock powder during an incubation period of 72 days (Figs. 1 and 2) suggest the four phases of the microbial growth curve in an enclosed environment. Extracting solution of Mehich I solubilized 39.54 mg/dm^3 of available K in the potassic rock and 602.81 mg dm^{-3} of available P in the phosphate rock.

From our results, the bacteria produced higher solubility levels as compared to fungi. Notes that this capability differs among genus and family of microorganisms, and although a larger number of bacteria are known to cause higher solubility, several of the fungi too could demonstrate this ability (Moreira and Siqueira, 2006).

Table 1
Genera and origin of bacteria and fungi used in the bioassay.

Genera of bacteria and fungi	Batch code	Origin
Paenibacillus polymyxa	421	Bacterial Physiology Laboratory Osvaldo Cruz Institute/FIOCRUZ; Dr. Leon Rabinovitch.
Acidithiobacillus thiooxidans	–	Microbiology Laboratory (UFRPE); Dr. Newton Pereira Stamford.
Ralstonia solanacearum	381	EMBRAPA vegetable garden (CNPH); Dr. Carlos Lopes.
Cromobacterium violaceum	–	Molecular and Genotype Biology Laboratory (UFRN); Dr. Silvia Regina Batistuzzo.
Penicillium fellutanum	4229	Osvaldo Cruz Institute/FIOCRUZ Fungi Culture Collection; Dr. Maria Inez de M. Sarquis.
Trichoderma humatum	3861	Osvaldo Cruz Institute/FIOCRUZ Fungi Culture Collection; Dr. Maria Inez de M. Sarquis.

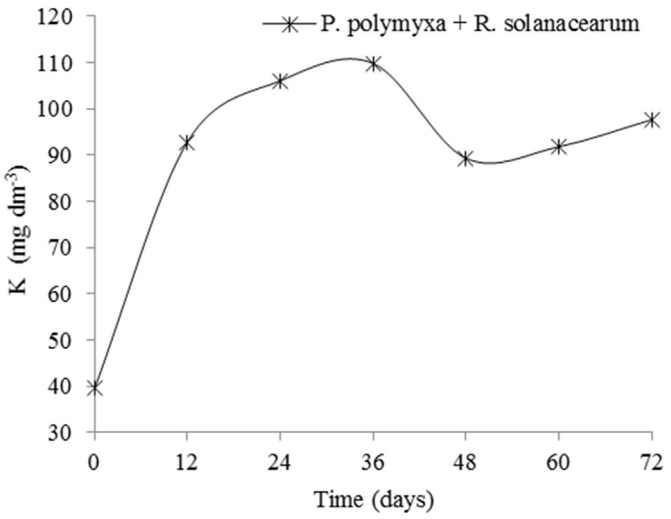

Fig. 1. Curve of K release in powdered biotite rock co-inoculated with *P. polymyxa* + *R. solanacearum* versus control (0 time, without inoculation). Standard deviations in the times: (12) = 5.24; (24) = 6.82; (36) = 3.07; (48) = 6.36; (60) = 3.24; (72) = 2.06.

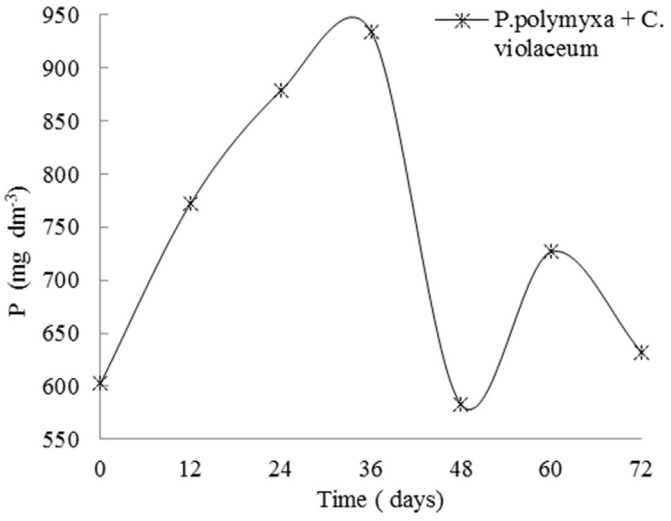

Fig. 2. Curve of P release in apatite rock powder co-inoculated with *P. polymyxa* + *C. violaceum* versus control (0 time, without inoculation). Standard deviations in the times: (12) = 31.92; (24) = 76.73; (36) = 16.36; (48) = 153.84; (60) = 63.67; (72) = 27.00.

This point was not confirmed in this study, and may be related to different nutrition factors in the medium as glucose and sources of nitrogen as yeast extract, tryptone and peptone. Silva Filho and Vidor (2000) tested the solubility of 56 isolates and found that solubility only took place in a medium having a rich source of carbon, such as xylitose, glucose, fructose or maltose, as well as of N, Fe, Ca and K, suggesting also that, nutritional deficiencies can promote the production and/or release of acids. Corroborating the present work in which the organisms presented greater solubility when the medium contained glucose and/or various sources of N.

As far as K (Tables 2–5) is concerned, the microorganisms presented a homogeneous behavior, causing an increased release of K up to 36 days ($p < 0.05$) of incubation and then a decrease later. This trend possibly occurred due to the reduction of the nutrient supply provided by the biotite rock to the growth of the studied microorganisms. Stamford et al. (2008) inoculated apatite rock and sulfur on Petri plates with a strain of the genus *Acidithiobacillus* and incubated these for a period of 30, 45, and 60 days. In that study, the peak of solubility of P and K was noted on the 60th day. In the present study, a high variability was noted in the values of K solubilized at 36 days of incubation (57.30–109.83 mg dm^{-3}) when compared to the control group (K = 39.54 mg dm^{-3}) corresponding to the biotite rock solubilized with Mehlich I extractor, without microorganisms, at time 0.

Among the inoculation treatments (Table 2), there was no significant difference in liberation values, excepting for *P. polymyxa* (101.32 mg dm^{-3}). However, a significant difference ($p < 0.05$) was noted on the Tukey test, showing a difference of 56. 54% as compared to the *P. fellutanum* treatment (57.30 mg dm^{-3}). It is known that the bacterium *P. polymyxa* is able to produce a variety of metabolites among them extracellular polysaccharides, enzymes, and organic acids such as formic acid, oxalic acid and acetic acid. These macromolecules in the presence of minerals can lead to changes in the properties of their surfaces, and these changes can be attributed to a higher affinity of minerals by these macromolecules present in the cell wall of the microorganism (Botero et al., 2008).

With respect to co-inoculation treatments, synergism among microorganisms was observed, but without significant differences in time 36 days. According to Table 3, K solubility varied from 61.57 mg/dm^3 with *A. thiooxidans* + *P. fellutanum* to 65.84 mg/dm^3 with *R. solanasearum* + *P. fellutanum*. Similarly, the solubility of K varied from 70.85 mg/dm^3 when co-inoculated with *C. violaceum* + *P. fellutannum* and 74.96 mg/dm^3 when co-inoculated with *C. violaceum* + *T. humatum* (Table 4). Table 5 shows that K solubility presented a significant increase ($p < 0.05$) when co-inoculated with *P. polymyxa* + *R. solanacearum* (109.83 mg/dm^3), yielding an increase in rock powder solubility of 177.77% in relationship to the control group extracted with Mehlich I (39.54 mg/dm^3) (Table 5), and graphically represented in Fig. 1. It is important to note that *P. polymyxa* inoculated alone provided a K solubilization of 101.33 mg/dm^3 (Table 2). Guimarães et al. (2006) used 40 isolated fungi inoculates in a liquid medium containing fragmented rock and observed an increase of up to 80% in the contents of released K when compared to the control group and attributed this release mechanism to the genus of the fungi.

Our results suggest the formation of functional groups having similar metabolism and interacting with each other. According to Andrade and Nogueira (2005), certain microorganism populations perform complementary physiological mechanisms that are part of one or more biogeochemical cycles. In the present study, co-inoculation with *P. polymyxa* + *R. solanacearum* present a great potential for sustainable agriculture. The natural supply of K is not sufficient to sustain a high agricultural yield, as well as produce quality may be significantly affected (Taiz and Zeiger, 2009).

An instability ($p > 0.05$) was observed in the solubility and release of P with different microorganisms. Such instability in the decline phase suggests a dying process involving the majority of viable cells releasing P to the medium, while others are still dividing, mineralizing and absorbing. According to Mendes and Reis Junior, 2003, the immobilized P in the microbial biomass constitutes a readily available reserve of this nutrient.

For days 12, 24, 36 and 48 of incubation, there were no differences ($p < 0.05$), but there was a rising tendency and towards the average on the 36th day (42.8% of treatments), noticeably in the treatments (Table 6) of *C. violaceum* (817.47 mg dm^{-3}; 35.6% above the control group) and *P. polymyxa* (783.88 mg/dm^3; 30% above the control group). The simultaneous effect of the co-inoculation of *P. polymyxa* + *C. violaceum* on apatite rock reached a higher level (934.35 mg/dm^3) (Table 7), surpassing the availability of the mineral by 55% compared to the non-inoculated control group

Table 2
Effect of treatments inoculated with a microorganism, in the K solubilization of the biotite rock (mg dm^{-3}) in relation to the incubation time (days).

Treatments inoculated	Incubation time (days)					
	12	24	36	48	60	72
P. polymyxa	72.48^{aE}	86.83^{aB}	101.33^{aA}	79.34^{aCD}	85.21^{aBC}	73.19^{aDE}
C. violaceum	45.40^{bD}	56.43^{bB}	66.74^{bA}	53.06^{bBC}	50.11^{bBCD}	49.13^{bCD}
R. solanacearum	44.86^{bC}	55.33^{bcB}	62.93^{bcA}	52.81^{bB}	52.33^{bB}	49.22^{BC}
A. thiooxidans	40.96^{bC}	51.27^{bcdB}	63.71^{bcA}	50.20^{bB}	46.29^{bcBC}	46.27^{bBC}
P. fellutanum	40.92^{bC}	46.69^{dBC}	57.30^{cA}	48.08^{bB}	43.20^{cBC}	44.31^{bBC}
T. humatum	41.89^{bC}	49.02^{cdB}	62.83^{bcA}	49.46^{bB}	45.85^{bcBC}	43.33^{bBC}

Values followed by different letters are significantly different ($p = 0.05$) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. Standard deviations: treatments = 2.61; times = 2.74. CV (%) treatments = 4.68; times = 4.91. K extracted with Mehlich I, without microorganisms = 39.54 mg dm^{-3} (control group).

Table 3
Effect of treatments co-inoculated with two microorganisms in the K solubilization of the biotite rock (mg/dm^3) in relation to the incubation time (days).

Treatments co-inoculated	Incubation time (days)					
	12	24	36	48	60	72
T. thiooxidans + *T. humatum*	48.28^{abB}	52.32^{aB}	64.00^{aA}	54.65^{aB}	28.70^{aC}	47.83^{abB}
T. thiooxidans + *P. fellutanum*	45.31^{bC}	54.31^{aABC}	61.57^{aA}	54.96^{aAB}	31.41^{aD}	48.18^{abBC}
R. solanacearum + *T. humatum*	54.23^{aB}	52.28^{aB}	63.89^{aA}	56.19^{aAB}	34.62^{aC}	40.79^{bC}
R. solanacearum + *P. fellutanum*	46.96^{abC}	56.40^{aB}	65.84^{aA}	52.78^{aBC}	35.84^{aD}	51.54^{aBC}
T. humatum + *P. fellutanum*	49.78^{abBC}	53.35^{aB}	63.83^{aA}	50.17^{aBC}	36.65^{aD}	42.41^{bCD}

Values followed by different letters are significantly different ($p = 0.05$) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. Standard deviations: treatments = 4.39; times = 3.73. CV (%) treatments = 8.78; times = 7.47. K extracted with Mehlich I, without microorganisms = 39.54 mg/dm^3 (control group).

Table 4
Effect of treatments co-inoculated with two microorganisms in the K solubilization of the biotite rock (mg dm^{-3}) in relation to the incubation time (days).

Treatments co-inoculated	Incubation time (days)					
	12	24	36	48	60	72
C. violaceum + *P. fellutanum*	49.86^{aB}	58.94^{aB}	70.85^{aA}	55.10^{aB}	52.13^{bB}	52.21^{aB}
C. violaceum + *R. solanacearum*	51.97^{aB}	60.19^{aB}	74.05^{aA}	61.37^{aB}	60.06^{abB}	51.52^{aB}
C. violaceum + *T. humatum*	50.58^{aC}	63.66^{aAB}	74.96^{aA}	58.64^{aBC}	64.57^{aAB}	50.66^{aC}
A. thiooxidans + *R. solanacearum*	51.22^{aC}	62.66^{aAB}	73.47^{aA}	58.43^{aBC}	58.29^{abBC}	50.83^{aC}
A. thiooxidans + *C. violaceum*	50.18^{aB}	57.76^{aB}	72.19^{aA}	59.60^{aB}	17.31^{cC}	52.02^{aB}

Values followed by different letters are significantly different ($p = 0.05$) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. Standard deviations: treatments = 5.38; times = 4.70. CV (%) treatments = 9.35; times = 8.17. K extracted with Mehlich I, without microorganisms = 39.54 mg/dm^3 (control group).

Table 5
Effect of treatments co-inoculated with two microorganisms in the K solubilization of the biotite rock (mg/dm^3) in relation to the incubation time (days).

Treatments co-inoculated	Incubation time (days)					
	12	24	36	48	60	72
P. polymyxa + *T. humatum*	90.56^{aAB}	95.73^{bA}	92.21^{cA}	89.13^{aAB}	82.76^{bB}	87.67^{bAB}
P. polymyxa + *A. thiooxidans*	88.82^{aAB}	94.99^{bA}	95.60^{cA}	86.66^{aAB}	85.67^{abB}	85.16^{bcB}
P. polymyxa + *P. fellutanum*	86.54^{aBC}	90.08^{bB}	99.91^{bcA}	82.82^{aBCD}	79.42^{bCD}	77.16^{cD}
P. polymyxa + *C. violaceum*	75.13^{bE}	98.23^{abB}	108.09^{abA}	86.52^{aCD}	94.15^{aBC}	77.73^{cDE}
P. polymyxa + *R. solanacearum*	92.82^{aC}	106.06^{aAB}	109.83^{aA}	89.32^{aC}	91.80^{aC}	97.64^{aBC}

Values followed by different letters are significantly different ($p = 0.05$) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. Standard deviations: treatments = 4.37; times = 3.80. CV (%) treatments = 4.82; times = 4.19. K extracted with Mehlich I, without microorganisms = 39.54 mg/dm^3 (control group).

(602.81 mg/dm^3; time 0), graphically represented in Fig. 2, ensuring greater effect on the solubilization. This result suggests an accumulation of enzymes for the process, among other organic products, as stated by Singh et al. (2010). According to Kiss et al. (1975), enzymes have a fundamental role in the nutrient cycle of the soil, which results in the proliferation of diverse microbial groups and in the accumulation of their enzymatic activity. This finding confirms the importance of functional groups of different organisms for plant nutrition and productivity. In the soil, microorganisms proliferate in complex communities, not in pure cultures (Dantas et al., 2009), which is of importance for the maintenance of the soil as well as of life on planet Earth (Andrade and Nogueira, 2005; Pereira et al., 2013). This consortium of the different species, according to Li et al. (2003), carries an important role in tropical and subtropical regions through their symbiotic interactions thus benefiting one another, amplifying the absorption of nutrients, especially phosphorus. *P. polymyxa* stands out in co-inoculation, revealing its significance in agriculture.

Looking at Table 8, a higher instability among the different groups of microorganisms suggests that various mechanisms may

Table 6
Effect of treatments inoculated with a microorganism in the P solubilization of the apatite rock (mg dm^{-3}) in relation to the incubation time (days).

Treatments inoculated	Incubation time (days)					
	12	24	36	48	60	72
C. violaceum	707.48^{aAB}	701.09^{aAB}	817.47^{aA}	717.71^{aAB}	629.87^{aB}	641.94^{aB}
P. polymyxa	720.01^{aAB}	757.21^{aAB}	783.88^{aA}	662.19^{aAB}	622.11^{aB}	636.50^{aB}
R. solanacearum	642.76^{aAB}	715.47^{aA}	563.22^{bBC}	708.76^{aA}	628.21^{aAB}	472.89^{bC}
A. thiooxidans	665.20^{aABC}	664.21^{aABC}	573.90^{bBC}	747.04^{aA}	698.63^{aAB}	544.74^{abC}
P. fellutanum	711.22^{aA}	694.00^{aAB}	697.35^{abAB}	710.10^{aA}	730.78^{aA}	564.06^{abB}
T. humatum	684.65^{aAB}	697.64^{aAB}	704.25^{abAB}	763.16^{aA}	605.85^{aB}	625.84^{aAB}

Values followed by different letters are significantly different ($p = 0.05$) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. Standard deviations: treatments = 62.01; times = 59.39. CV (%) treatments = 9.22; times = 8.83. P extracted with Mehlich I. without microorganisms = 602.81 mg/dm^3 (control group).

Table 7
Effect of treatments co-inoculated with two microorganisms in the P solubilization of the apatite rock (mg/dm^3) in relation to the incubation time (days).

Treatments co-inoculated	Incubation time (days)					
	12	24	36	48	60	72
P. polymyxa + *C. violaceum*	$772,57^{aBC}$	878.32^{aAB}	934.35^{aA}	582.86^{bcD}	726.74^{aBCD}	632.22^{abCD}
P. polymyxa + *A. thiooxidans*	688.40^{abB}	704.73^{bAB}	843.05^{abA}	733.83^{abAB}	662.22^{abB}	706.53^{aAB}
P. polymyxa + *R. solanacearum*	713.46^{abAB}	718.51^{bAB}	814.35^{abA}	736.07^{abAB}	742.61^{aAB}	660.25^{abB}
A. thiooxidans + *T. humatum*	638.27^{abB}	641.12^{bB}	832.15^{abA}	739.43^{aAB}	727.64^{aAB}	617.91^{abB}
A. thiooxidans + *C. violaceum*	669.88^{abAB}	659.09^{bAB}	736.43^{bA}	505.02^{cC}	638.95^{abABC}	549.79^{bBC}
C. violaceum + *P. fellutanum*	614.70^{bAB}	605.55^{bAB}	736.43^{bA}	631.33^{abcAB}	514.13^{bB}	628.35^{abAB}

Values followed by different letters are significantly different ($p = 0.05$) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. Standard deviations: treatments = 69.89; times = 63.31. CV (%) treatments = 10.09; times = 9.14. P extracted with Mehlich I, without microorganisms = 602.81 mg/dm^3 (control group).

Table 8
Effect of treatments co-inoculated with two microorganisms in the P solubilization of the apatite rock (mg/dm^3) in relation to the incubation time (days).

Treatments co-inoculated	Incubation time (days)					
	12	24	36	48	60	72
P. polymyxa + *T. humatum*	727.49^{abABC}	826.50^{aA}	782.32^{aAB}	837.04^{aA}	680.51^{aBC}	629.06^{aC}
P. polymyxa + *P. fellutanum*	716.83^{abcAB}	837.87^{aA}	627.02^{bB}	666.21^{bB}	697.17^{aB}	624.10^{abB}
R. solanacearum + *P. fellutanum*	786.78^{aA}	586.14^{dBC}	469.13^{cC}	609.86^{bB}	446.98^{cC}	634.55^{aB}
R. solanacearum + *A. thiooxidans*	719.07^{abcAB}	768.35^{abA}	682.62^{abAB}	455.36^{cC}	646.39^{bAB}	619.45^{abB}
R. solanacearum + *T. humatum*	744.51^{abA}	753.31^{abcA}	716.86^{abA}	653.60^{bAB}	410.90^{cC}	568.18^{abB}
R. solanacearum + *C. violaceum*	656.41^{abcA}	631.74^{bcdA}	696.85^{abA}	729.66^{abA}	685.82^{aA}	610.94^{abA}
C. violaceum + *T. humatum*	602.17^{bcCD}	795.36^{aA}	776.68^{abAB}	685.72^{bABC}	515.06^{bcD}	654.09^{aBCD}
A. thiooxidans + *P. fellutanum*	656.60^{abcA}	612.21^{cdA}	661.05^{abA}	692.22^{abA}	720.98^{aA}	606.68^{abA}
T. humatum + *P. fellutanum*	572.05^{cBC}	598.65^{dABC}	667.49^{abAB}	733.41^{abA}	604.53^{abABC}	476.07^{bC}

Values followed by different letters are significantly different ($p = 0.05$) using the Tukey test. Upper case letters compare data in rows and lower case letters compare data in columns. Standard deviations: treatments = 54.40; times = 58.82. CV (%) treatments = 8.26; times = 8.93. P extracted with Mehlich I, without microorganisms = 602.81 mg/dm^3 (control group).

have influenced the solubility capacity (410.90 mg/dm^3), treatment co-inoculated with *R. solanacearum* + *T. humatum*, to 837.87 mg/dm^3 (treatment co-inoculated with *P. polymyxa* + *P. fellutanum*) and time 60 and 24 days, respectively. According to Hariprasad and Niranjana (2009), the capacity for biosolubility depends on the family of organisms, the type of phosphate to be solubilized and the nature of the organic material produced. Song et al. (2008) reported that the solubilization of insoluble inorganic phosphate by the bacterium *Burkholderia cepacia* species is related to the decrease of pH.

We show here that in general, biosolubility of biotite and apatite rock by inoculation and co-inoculation was best on the 36th day of incubation. Among the treatments with simple inoculation, *P. polymyxa* and *C. violaceum* exhibited higher content of K and P yield by biotite and apatite rock, respectively. In relation to treatments with co-inoculation, *P. polymyxa* + *R. solanacearum* in biotite rock presented greater availability of K, while co-inoculation with *P. polymyxa* + *C. violaceum* promoted the highest solubility of P in the apatite rock. The synergy within these lineages optimized the process of biosolubility of P and K from rocks for the efficient production of biofertilizer.

Conflict of interest

None.

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References

Alves, L., Oliveira, V.L., Silva Filho, G.N., 2010. Utilization of rocks and ectomycorrhizal fungi to promote growth of eucalypt. Braz. J. Microbiol. 41, 676–684.
Andrade, G., Nogueira, M.A., 2005. Bioindicadores para uma análise de risco ambiental: organismos geneticamente modificados e grupos funcionais de microrganismo do solo. Biotecnol. Cienc. Desenvolv. 34, 11–19.

Aria, M.M., Lakzian, A., Haghnia, G.H., Berenji, A.R., Besharati, H., Fotovat, A., 2010. Effect of *Thiobacillus*, sulfur, and vermicompost on the water-soluble phosphorus of hard rock phosphate. Bioresour. Technol. 101, 551–554.

Beneduzi, A., Moreira, F., Costa, P.B., Vargas, L.K., Lisboa, B.B., Favreto, R., Baldani, J.I., Passaglia, L.M.P., 2013. Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the South of Brazil. Appl. Soil Ecol. 4, 94–104.

Botero, A.E.C., Torem, M.L., Mesquita, L.M.S., 2008. Perspectiva e potential aplicação de biorreagentes na flotação de minerais. REM. 61, 57–61.

Chagas Junior, A.F., Oliveira, L.A., Oliveira, A.N., Willerding, A.L., 2010. Capacidade de solubilização de fosfatos e eficiência simbiótica de rizóbios isolados de solos da Amazônia. Acta Sci. Agron. 32, 359–366.

Chotchutima, S., Tudsri, S., Kangvansaichol, K., Sripichitta, P., 2016. Effects of sulfur and phosphorus application on the growth, biomass yield and fuel properties of leucaena (*Leucaena leucocephala* (Lam.) de Wit.) as bioenergy crop on sandy infertile soil. Agri. Nat. Resour. 50, 54–59.

Dantas, J.S., Souza, A.P., Farias, M.F., Nogueira, V.F.B., 2009. Las interaciones entre los grupos de microorganismos em la rizosfera. Pesqui. Apl. Agrotecnologia 2, 118–213.

Dastager, S.G., Deepa, C.K., Pandey, A., 2011. Plant growth promoting potential of *Pontibacter niistensis* in cowpea (*Vigna unguiculata* (L.) Walp.). Appl. Soil Ecol. 49, 250–255.

Embrapa-Empresa Brasileira de Pesquisa Agropecuária, 2009. Manual de Análises Químicas de Solo, Plantas e Fertilizantes, second ed. Embrapa Informação Tecnológica, Brasília.

Ferreira, D.F., 2010. Sisvar-programa Estatístico, Versão 5.3 (Build 75). Universidade Federal de Lavras, Lavras.

Guimarães, P.S., Lúcio, C.H., Soares, E.M., Nonato, L.V., Coelho, A.M., Alves, V.M.C., Marriel, I.E., 2006. Liberação de Potássio de Rocha Silicática Brecha Influenciada Pelo Genótipo de Fungo, in Vitro. May 2012. http://www.alice.cnptia.embrapa.brhandledoc490147./16.

Hariprasad, P., Niranjana, S.R., 2009. Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant Soil 316, 13–24.

Kelman, A., 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium,. Phytopathology 44, 693–695.

Kiss, S., Dragan-Bularda, M., Radulescu, D., 1975. Biological significance of enzymes in soil. Adv. Agron. 27, 25–91.

Li, L., Tang, C., Rengel, Z., Zhang, F., 2003. Chickpea facilitates phosphorus uptake by intercropped wheat from an organic phosphorus source. Plant Soil 248, 297–303.

Mendes, I.C., Reis Junior, F.B., 2003. Microrganismos e Disponibilidade de Fósforo (P) no Solo: Uma Análise Crítica. Embrapa Cerrados, Planaltina, p. 26 (Documentos, 85).

Moreira, F.M.S.E., Siqueira, J.O., 2006. Microbiologia e Bioquímica do Solo, third ed. Lavras, Minas Gerais.

Oliveira, A.L.M., Urquiaga, S., Baldani, J.I., 2003. Processos e Mecanismos Envolvidos na Influência de Microrganismos Sobre o Crescimento Vegetal. Embrapa Agrobiologia, Seropédica, p. 40 (Documentos 161).

Pereira, M.G., Santos, C.E.R.S., Freitas, A.D.S., Stamford, N.P., Rocha, G.S.D.C., Barbosa, A.T., 2013. Interações entre fungos micorrízicos arbusculares, rizóbio e actinomicetos na rizosfera de soja. Rev. Bras. Eng. Agríc. Ambient 17, 1249–1256.

Rheinheimer, D.S., Martinazzo, R., Gatiboni, L.C., Kaminski, J., Silva, L.S., 2008. Amplitude no fósforo microbiano em um Argissolo em pastagem nativa submetida à roçada e à introdução de espécies forrageiras com fertilização fosfatada em diferentes épocas. Acta Sci. Agron. 30, 561–567.

Rifai, M.A., 1969. A Revision of the Genus Trichoderma. Mycology Papers, Series 116. Imperial Mycological Institute, Kew Surrey, pp. 1–56.

Rodrigues, A.F.S., 2009. Mineração para o Agronegócio. In: Rodrigues, A.F.S. (Ed.), Economia mineral do Brasil. DNPM, 7. June 2016. http://www.dnpm.gov.br/conteudo.asp./16.

Sambrook, J., Russel, D.W., 2001. Molecular Cloning: a Laboratory Manual, third ed. Cold Spring Harbor Laboratory Press, New York.

Silva Filho, G.N., Vidor, C., 2000. Solubilização de fosfatos por microrganismos na presença de fontes de carbono. R. Bras. Ci. Solo. 24, 311–319.

Silva, V.N., Silva, L.E.S.F., Martínez, C.R., Seldin, L., Burity, H.A., Figueiredo, M.V.B., 2007. Estirpes de *Paenibacillus* promotoras de nodulação específica na simbiose *Bradyrhizobium*-caupi. Acta Sci. Agron. 29, 331–338.

Singh, N., Kumar, S., Bajpai, V.K., Dubey, R.C., Maheshwuari, D.K., Kang, S.C., 2010. Biological control of *Macrophomina phaseolina* by chemotactic fluorescent *Pseudomonas aeruginosa* PN1 and its plant growth promotory activity in chirpine. Crop. Prot. 29, 1142–1147.

Song, O.R., Lee, S.J., Lee, Y.S., Lee, S.C., Kim, K.K., Choi, Y.L., 2008. Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA23 isolate from cultivated soil. Braz. J. Microbiol. 39, 151–156.

Stamford, N.P., Izquierdo, C.G., Fernández, M.T.H., Moreno, M.C.M., 2008. Biofertilizantes de rochas fosfatadas e potássicas com enxofre e acidithiobacillus. In: Figueiredo, M.V.B., Burity, H.A., Stamford, N.P., Santos, C.E.R.S. (Eds.), Microrganismos e Agrobiodiversidade: o novo desafio para a agricultura, Agrolivros, Rio Grande do Sul, pp. 401–421.

Taiz, L., Zeiger, E., 2009. Fisiologia Vegetal, fourth ed. Artmed, Porto Alegre.

Tuovinen, O.H., Kelly, D.P., 1973. Studies on the growth of *Thiobacillus ferrooxidans*: use of membrane filters and ferrous iron agar to determine viable number and comparison $14CO_2$-fixation and iron oxidation as measures of growth. Arch. Microbiol. 88, 285–298.