INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 8, No. 4, p. 124-144, 2016

RESEARCH PAPER OPEN ACCESS

In vitro selection of phosphate solubilizing strains of *Trichoderma* spp.

Dorcas Zúñiga-Silgado

Environmental Engineering Study Program, Faculty of Architecture and Engineering, Mayor College of Antioquia University Institution, Career. 78 N ° 65-46 Robledo, Medellín, Colombia

Key words: Phosphorous biosolubilization, *Trichoderma spp.*, biosolubilization, bioacidulation.

http://dx.doi.org/10.12692/ijb/8.4.124-144 Article published on April 30, 2016

Abstract

Phosphorous (P) is considered to be the macronutrient with the lowest availability for plants in the majority of tropical soils, and is thus a limiting factor for vegetal growth. The application of Phosphoric Rock (PR) has been widely studied in order to satisfy its requirement in crops, but the quantity of liberated P is too low to satisfy demand. A viable alternative for managing this problem is the use of rhizospheric microorganisms that solubilize P (PSM) in chemically unavailable forms. In the search for microorganisms that solubilize phosphates, studies report that *Trichoderma spp.*, in addition to their effects as pathogen biocontrollers, also exhibit phosphodissolvent capacities. The objective of this investigation was to evaluate in vitro strains of Trichoderma spp. isolated from rhizospheres of beans and corn with phosphate solubilizing abilities. Different strains of Trichoderma were isolated and selected from rhizospheric soil and rhizospheres. Three medium types were evaluated utilizing both solid and liquid media in order to determine their biometric characteristics, relative efficiency of solubilization of PR, bioacidulant capacity of the culture medium, and concentration of P in solution. In liquid media, these fungi were inoculated with and without PR, the quantity of soluble P was quantified, and the pH was taken. There were significant differences with respect to mycelial growth between solid media with and without the presence of PR, solubilization halos, and relative efficiency of solubilization in relation to incubation time. Liquid media with and without PR showed significant differences with respect to the quantity of soluble P. The pH of these media was inversely proportional to the quantity of soluble P, as the probable principle mechanism of dissolution of PR is the production of organic acids.

* **Corresponding Author:** Dorcas Zúñiga-Silgado ✉ dorcas.zuniga@colmayor.edu.co

Introduction

Phosphorous (P) is considered to be a macronutrient of low mobility and availability for plants in the majority of tropical soils (Lynch and Brown, 2008), being one of the most serious factors limiting plant growth. The low mobility of phosphate ions ($H_2PO_4^-$ and $HPO_4^=$) is due to their retention of constituent colloidal soil minerals (Kirkby and Johnston, 2008), which determine that only a small proportion of the phosphorous will be present as ions in the soil solution (Londoño, 2010; Lynch and Brown, 2008; Guppy *et al.,* 2005; Boul and Eswaran, 2000; Barber, 1995; Sánchez and Salinas, 1983).

En general, highly weathered tropical soils stand out for their high capacity for fixing P (Lynch and Brown, 2008) on the surface of secondary minerals (kaolinite, gibbsite, ferrihydrite, goethite, and hematite) (Boul and Eswaran, 2000; Fontes and Weed, 1996; Sparks, 1995; Dixon and Weed, 1989; Lins and Cox, 1989). Also, the precipitation of P with free ions in the soil solution, such as $Fe(OH)x^{(3-x)+}$ and $Al(OH)x^{(3-x)+}$ allow P to form highly insoluble and stable complexes, particularly at low pH values (Lynch and Brown, 2008; Sparks, 1995; Dixon andWeed, 1989). The application of phosphoric rock (PR) has been widely studied in order to satisfy cultivational phosphoric requirements (Osorio 2011; Oehl *et al.*, 2002; Entz *et al.,* 2001; Sánchez and Salinas, 1983). However, the quantity of P liberated in the soil solution through direct application of PR is too low to meet the demand of most crops (Vassilev *et al.,* 2001), which limits its extensive use.

Due to the situation described above, the idea of implementing low technological cost alternatives in order to improve the aquisition of P on the part of plants; among them, the use of rhizospheric microorganisms that promote the solubilization of P from chemically unavailable forms, including PR (Trolove *et al.,* 2003; Gyaneshwar *et al.,* 2002; Richardson, 2001), stand out. Due to this ability, these organisms have earned the generic name phosphate-solubilizing microorganisms (PSM). The association of phosphate-solubilizing microorganisms with the rhizospheres of plants allows them to meet plant phosphoric demands in ecosystems in which phosphorous is a limiting resource, and also offers an important microbial germplasm bank with potential uses in agricultural activity. These fungi exhibit various mechanisms for solubilizing inorganic compounds of P, with the production of lithic enzymes during the decomposition of organic material standing out (Marschner, 2008; Bar-Yosef *et al.,* 1999; Iyamuremye *et al.*, 1996) along with the use of organic acids that generate competition between organically produced anions and phosphate ions in absorption sites on the surfaces of clayey soil minerals (Bolan *et al.,* 1994). Some of the organic acids commonly associated with the microbial solubilization of P in fungi are: gluconic (Rodríguez *et al.*, 2006; Bar-Yosef *et al.,* 1999), oxalic, citric (Osorio, 2008; Kim *et al.,* 1997; Kucey and Leggett, 1989), lactic, tartaric, and aspartic (Venkateswardu *et al.*, 1984). These acids are products of microbial metabolism, and in some cases of oxidative respiration or of the fermentation of carbonaceous substrates (ex: glucose) (Prescott *et al.,* 2004; Mathews *et al.,* 2002; Atlas and Barta 1998).

Other proposed mechanisms are the excretion of protons due to the assimilation of NH_4^+ by microorganisms (Whitelaw, 2000; Abd-Alla, 1994; Roos and Luckner, 1984; Kucey, 1983); desorption of P ions at absorption sites (He and Zhu, 1998; 1997); and chelation of Al and Fe (Marschner, 2008; Bar-Yosef *et al.,* 1999; Lyamuremye*et al.*,1996).

The availability of using phosphate-solubilizing microorganisms within biological fertilization strategies for crops offers large prospects for agricultural applications while lowering fertilization costs. Their use can be seen as a tool for ecological management that only permits small disturbances in soil quality. However, despite many reports on the beneficial effects of PSM's, the frequency of results are inconsistent (Chen *et al.,* 2007a; Mc Spadden Gardener, 2004; Reva *et al.,* 2004; Garbeva *et al.,* 2003). It is thought that this inconsistency in results is because the utilization of only one inoculation of

microorganisms in the soil-plant system alters the the dynamics of the rhizospheric ecosystem (de Freitas *et al.,* 1997; Habte and Osorio, 2001; Chigineva *et al.*, 2011). It is also presumed that there are different reactions depending on the vegetal species (Flach et al., 1987; Gregory, 2006), the mineral composition of the soil (Boul and Eswaran, 2000), and interactions between different microbial functional groups in the soil (Chigineva *et al.*, 2011; Vessey, 2003; Habte and Osorio, 2001). Reports done by Caipo *et al.* (2002) also show that the type and quantity of inoculum affects the cellular physiology of microorganisms and the host plant, as well as the availability of carbon substrate(s) as a(n) energy source(s) (Caldeira *et al.,* 2008; Knox *et al.,* 2000), the competitiveness of other autochthonous soil microorganisms (Collados, 2006; Silvieira *et al.,* 2003), and environmental conditions (Johnson *et al.,* 1997); all of which are indicated in the differential responses of crops to microbial inoculation.

In the search for microorganisms that solubilize phosphates, studies show that in the case of *Trichoderma* spp., besides having biocontrolling effects on pathogens, its inoculation has other benefits to plants through the decomposition of organic material, as this allows for the liberation of nutrients in forms that are chemically available for plants (Howell, 2003; Godes, 2007). It also shows phosphate solubilizing activity (Valencia *et al.*, 2007; Valero, 2007; Vera *et al.*, 2002), which is why it is frequently utilized as a biofertilizing organism in many commercial products (Moreno *et al.*, 2007). This capacity of *Trichoderma* spp. promotes the growth and development of crops by producing metabolites that stimulate processes of vegetal development (Sutton and Peng, 1993).

It is also known that *Trichoderma* spp. have the capacity to multiply in the soil and colonize the roots of plants, liberating growth factors (auxins, gibberellins, and cytokinins) that stimulate the germination and development of plants (Altomare *et al.*, 1999). The production of indole-3-acetic acid, a substance that acts like a vegetal hormone, favoring the development of root systems, has been reported among other benefits (Valencia *et al.*, 2005). These substances produced by *Trichoderma* act as catalysts or accelerators of primary meristematic tissues in the young parts of the plant, accelerating their cellular reproduction, and ensuring that they develop more rapidly than plants that have not been treated with this microorganism (Valencia *et al.* 2007). *Trichoderma* has also been reported to be a promoter of vegetal growth in cultures of eggplant, vetch, beans, coffee, tomato, potato, and forest species, among others (Zambrano, 1989; Börkman *et al.*, 1998; Dandurand and Knudsen, 1993).

Due to the relevance of this fungus, the adequate selection of strains at the laboratory level will contribute to the development of strategies for integrated management of crops through the elaboration, evaluation, and application of bioproducts with multifunctional effects occasioned by the biocontrolling activity of pathogens in concert with their phytostimulating and biofertilizing effects. Based on the above, our hypothesis suggests that success in the *in vitro* selection of strains of *Trichoderma* with phosphodissolvent capacities is a function of half the growth medium and kinetic biosolubility of PR. The central aim of this investigation was the selection at the *in vitro* level of strains of *Trichoderma* spp. with solubilizing capacities for isolated phosphates through the rhizosphere of beans and corn.

Materials and methods

The present investigation took place in the Biotechnology Laboratory of the Faculty of Sciences and Health of the Institución Universitaria Colegio Mayor of Antioquia, and in the Laboratories of Ecology and Environmental Conservation and Vegetal Health of the Faculty of Agricultural Sciences of the Medellin Campus of the Universidad Nacional de Colombia (6°15´ N y 75°34´ W, 1450 m altitude).

Composition of fungic community in soil and rhizosphere of beans and corn

Twenty samples of corn and bean crops with 30, 60,

and 90 days of maturity; along with roots and rhizospheric soil; were collected in the municipalities of Rionegro and San Pedro (Antioquia). Samples were stored in a refrigerated storage room with 250 g of soil for their transfer to the labratory. Samples were processed according to the methodology reported by Calvo (2008).

Isolation of Morphotypes of Trichoderma spp., solubilizers of PR

The isolation of morphotypes of *Trichoderma* spp. was done utilizing rhizospheric soil and root segments of beans and corn following the methodology reported by Hoyos-Carvajal (2008). Roots collected were submerged in 10mL of sterile 0.85% NaCL solution and centrifuged for 10 minutes at 200rpm (Reyes *et al.,* 2002). Subsequently, 1g of roots of each sample were transferred to 100mL Erlenmeyer flasks. These contained 20mL of 10% peptone water and the roots were agitated at 100rpm for 5 minutes. Next, the roots were separated from the supernatant and dried with sterile absorbent towels. Half the roots were then dissected in segments of 1mm and planted in petri dishes with Rose Bengal Agar (RBA) with 300 mg L^{-1} of Sulfate of Streptomycin and 100 mg L^{-1} chloramphenicol (SIGMA) in total darkness.

The other half of the root samples were transferred to a mortar for maceration. Aliquots from the maceration were planted in petri dishes with Rose Bengal Agar (RBA) medium and 300 mg L^{-1} of Sulfate of Streptomycin and 100 mg L^{-1} chloramphenicol (SIGMA) in total darkness. All petri dishes were incubated for 24 hours at 25±1°C. A total of 30 dishes were planted.

Experiment 1. Solubilization of PR in solid media

The methods of solid culture utilized were a qualitative indicator of the solubilizing action of PR for strains of *Trichoderma* spp. This capacity could be observed by the formation of clear halos of solubilization around the mycelia (Hernández *et al.,* 2011; Sundara and Sinnha, 1963). In order to determine the efficiency of biosolubilization of PR, three different solid culture media were tested:

(i) MMHA with hydroxyapatite (Ca_{10} (PO_4) $_6(OH)_2$) as a sources of P: (MM-HA; Ca10 (PO_4) $6(OH)_2$) per liter of media, which contained: 0.1g NaCl; 0.4g NH_4Cl; 0.78g KNO_3; 0.50g $MgSO_4 \cdot 7H_2O$; 0.1g $CaCl_2 \cdot 2H_2O$; 0.5mg $FeSO_4 \cdot 7H_20$; 1.56mg $MnSO_4 \cdot H_2O$; 1.40mg $ZnSO_4 \cdot 7H_20$; 10g glucose; and 2.7g MMHA (0.05% P) all strained to 2µm; and 20g of agar (Döbereiner *et al.,* 1999; Reyes *et al.,* 1999). The initial pH was adjusted to 7.0. Additionally, for the isolation of phosphodissolvent fungi, 30mg of streptomycin sulfate and 100mg of chloramphenicol were added.

(ii) Pikovskaya (PVKM) growth medium modified-Agar was used with 0.5 g ferric phosphate ($FePO_4 \cdot H_2O$) per liter instead of tricalcium phosphate (Nopparat *et al.*, 2009), the composition of which was: 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.004 g $MnSO_4 \cdot H_2O$, 0.2 g NaCl, D10 g -Glucose, 0.002 g $FeSO_4 \cdot 7H_2O$ (Sigma-Aldrich), 0.5 g yeast extract (Bioxon), 18 g agar (Bioxon) and distilled water (900 mL). For the isolation of phosphodisolvent fungi, 0.1 g chloramphenicol (Pfizer) was added. A solution of phosphate was added to this mixture: 0.5 g gum arabic (Kremer), 0.5 g ferric phosphate ($FePO_4 \cdot H_2O$) (Química-Barquim), and 100 mL sterile distilled water. In this medium, disolution can be seen when a clear halo forms around the colony or mycelia (Sundara and Sinha, 1963).

(iii) Osorio and Habte medium for solubilizers of PR (MSPOH) (Osorio and Habte, 2001), the composition of which was: 1.0 g NaCl; 0.2 g $CaCl_2 \cdot 2H_2O$; 0.4 g $MgSO_4 \cdot 7H_2O$; 1.0 g NH_4NO_3; 10 g glucose L^{-1}; and 3.5 g unacidulated PR per liter of medium.

Additionally, the PDA culture medium was used as a reference. The pH of the different media were adjusted to 7.0 with 1.0M NaOH. The three different media were sterilized in an autoclave for 15 minutes at 121°C. Ten petri dishes of each growth medium were used for the seeding of each morphotype of

Trichoderma.

Biometric determination of strains of Trichoderma spp

In order to measure the effects of the media on the development of strains, diameter of colonies (DC) was measured by function of time (3, 6, 9, and 12 days after inoculation). Additionally, diameter of halo of solubilization (DHS) and relative efficiency of solubilization of PR (RES) in MSPOH media were measure (in order to increase appropriateness). The pH values of the media were measured using pH indicator strips on the surface of the media. RES was calculated using the equation proposed by Vera *et al.* (2002):

$$RES = 100 \times (DHS \div DC)$$

This value indicates the radius of action of the fungus on the substrate it solubilizes and is calculated in relation to the size of the mycelium extended from the fungus. Data indicates the average of the 4 petri dishes inoculated per strain.

Quantification of sporulation of Trichoderma spp

In order to see the effects of the media on fungal sporulation, 12 days after inoculation a circle 5mm in diameter was cut in the center of the colony. Each disk was then placed into a 1.5 mL capacity vial with 1mL of sterile distilled water and Tween 80 and agitated in a vortex for 1 minute. One mL of solution was taken from each of these solutions, which was then added to test tubes that contained 9mL of sterile 0.85% NaCL solution. Eight successive decimal dilutions from 10^{-1} to 10^{-8} were made from each suspension, from which four spore counts by dilution were done with a Neubauer chamber. Results were expressed in number of spores per mL^{-1}.

Quantification Colony-Forming Units (CFU) ofTrichoderma spp

The viability of spores after being inoculated with solubilizing broths of PR was tested with fluorescein following the method reported by Calich *et al.* (1979). Simultaneously, in order to compare counting methods, (Neubauer chamber and CFU count in Petri dishes), one mL of each dilution was seeded in four petri dishes of Standard Plate Count (SPC) (SIGMA) media for a total of 36 inoculated petri dishes for each fungal strain. The petri dishes were incubated at 25±1°C for 12 hours. Later, a count of germinated spores was done by dilution. Each axenic culture was obtained monosporically following the methodology reported by Zúñiga *et al.* (2010) with the purpose of evaluating cytogenically homogenous strains. These were stored at 4°C until their evaluation.

Quantification of Biomass of Trichoderma spp

On day 15, dry biomass (g) of the fungi was measured through the separation of cellular mass from the medium and the PR. To this end, fragmented media were transferred to a 100 mL beaker to be agitated with a magnet and progressively heated from 27 to 31°C over the course of 1 hour. Later, samples were maintained at 31°C on a hot plate and hot liquid media was transferred to plastic tubes for its centrifugation at 100rpm for 5 minutes at 31°C. Later, it was strained through a paper filter in a Buschner funnel using a vacuum for its forced filtration. The remaining biomass on the filter paper was washed several times and the washings were collected in a Petri dish. Finally, this was heated on a hot plate (60°C for 24 hours) in order to determine fungal dry mass.

Experiment 2. Kinetics of dissolution of PR in liquid media

The efficiency of biological dissolution of PR of fungi was evaluated following the method developed by Osorio (2008). For this purpose, a solution consisting of 1.0 g NaCL, 0.2 g $CaCl_2 \cdot 2H_2O$, 0.4 g $MgSO_4 \cdot 7H_2O$, 1.0 g NH_4NO_3, 10 g glucose L^{-1}, and 3.5 g unacidulated PR per liter of media was added to a 250 mL Erlenmeyer flask. The pH was adjusted to 7.0 with 0.1 M NaOH. The Erlenmeyer flasks and their contents were sterilized in an autoclave at 120°C and 1.2kg cm^{-2} for 20 minutes. Suspensions of spores with 1 mL de $CaCl_2 \cdot 2H_2O$ 0.01*M* at a concentration of $7X10^9$ spores per mL (Capuchino

and Sherman, 1998). One mL of these suspensions inoculated the Erlenmeyer flasks. As a control, the uninoculated Erlenmeyer flasks received 1mL of a solution of $CaCl_2 \cdot 2H_2O$ 0.01*M*.

The broths were incubated at 25 ± 1°C in an orbital agitator at 100 rpm for 12 days. Each treatment had 4 replicas. Each 3 days, 1mL of broth was collected from each Erlenmeyer flask in order to measure the pH (Conductronic PC45, México) and the concentration of soluble P using the blue molybdate spectrophotometer method at 890 nm (Genesys 20, ThermoSpectronic, EUA (Murphy and Riley, 1962), and thus establish the kinetics of dissolution of PR. For the above, it was necessary to pass the sample through filter paper and later centrifuge the filtrate at 4000 rpm for 10 minutes, after which they were filtered in a vaccuum by duplicate through Millipore 0.2 µm Ø and 0.45 µm Ø, filter paper, respectively. The procedure above was done with the goal of separating fungal cells from particulate matter.

Experimental design and statistical analysis

The experimental design was completely random, the treatments had a 4X4 factorial arrangement (4 incubation times, 4 culture methods). The dependent variables were the RES (%), the final pH of the medium, the number of spores mL^{-1}, the UFC, the biomass, and the concentration of P in solution (mgL^{-1}). Each treatment had 4 replications.

Results were analyzed using an analysis of variance (ANOVA), from which assumptions were determined in the residuals. A Duncan test was used for the separation of media. Analysis was done with a (P) ≤ 0.05 level of significance. All analyses were completed with Statgraphics Centurion XVI software.

Results

Composition of fungal community in soil and rhizospheres of beans and corn

Table 1 shows fungal populations present in two microhabitats: rhizospheric soil and rhizospheres. The results demonstrated that significant differences of (P) ≤ 0.05 existed in the populations studied. This may be explained by the fact that each environment presents favorable conditions for the growth of microorganisms (Hernandez, 2003). In this investigation, the median values of total fungi per gram of soil are expressed at an order of 5.0 X 10^4 UFC g^{-1}. Highly significant differences were found in the number of fungal genera in the two microhabitats and the two cultures studied ((P) ≤ 0.01).

Table 1. Composition of fungal communities in soil and rhizospheres of beans and corn.

Substrate	Total microbiota UFC g^{-1}	Log UFC g^{-1}	Growth media	Fungal genera present in soil and bean rhizosphere (UFC g^{-1})					Fungal genera present in soil and rhizosphere of corn (UFC g^{-1})		
				Trichoderma	*Aspergillus*	*Penicillum*	*Paecillomices*	*Rhizopus*	*Trichoderma*	*Aspergillus*	*Penicillum*
Soil	1,6 x 10^4	4,01	PDA	6,5 x 10^4	5,4x 10^4	3,9 x 10^4	3,3x 10^4	2,4x10^4	2,5 x 10^4	1,4x 10^4	1,9 x 10^4
			MM-HA	7,8 x 10^4	6,0 x 10^4	3,4 x 10^4	3,5 x 10^4	2,0x 10^4	2,5 x 10^4	1,5 x 10^4	1,4 x 10^4
			PVKM	4,5 x 10^4	4,5 x 10^4	5,0 x 10^4	4,0 x 10^3	2,5x 10^4	6,0 x 10^4	1,5 x 10^4	1,5 x 10^4
			MSPOH	8,5 x 10^4	7,3 x 10^4	7,5 x 10^4	4,2 x 10^4	2,3x 10^4	5,5 x 10^4	1,3 x 10^4	1,5 x 10^4
Roots	1,3 x 10^4	2,5	PDA	1,8x 10^4	2,5 x 10^4	2,5 x 10^4	1,7 x 10^4	1,5x 10^4	2,8x 10^4	4,5 x 10^4	4,5 x 10^4
			MM-HA	1,0 x 10^4	2,0 x 10^4	2,9 x 10^4	1,3 x 10^4	1,0x 10^4	5,5 x 10^4	5,0 x 10^4	4,9 x 10^4
			PVKM	1,5 x 10^4	2,5 x 10^4	1,2 x 10^4	1,0 x 10^3	1,5x 10^4	5,0 x 10^4	4,5 x 10^4	4,0x 10^4
			MSPOH	1,8 x 10^4	1,5 x 10^4	1,5 x 10^4	1,7 x 10^3	1,5x 10^4	5,0 x 10^4	5,0 x 10^4	4,0 x 10^4

All values shown are the average of 4 replicates per treatment.

Isolation of morphotypes of phosphodissolvent Trichoderma spp

The processing of soil samples and roots permitted the recovery of 68 primary isolates which were grouped into 18 morphotypes. Through micromorphological, physiological, biochemical, and metabolic tests, 9 strains of *Trichoderma spp*. with phosphodissolvent and AIA producing capacities were selected.

Experiment 1. Solubilization of PR in solid media. Biometric determination of strains of Trichoderma spp

In MSPOH media on the sixth (6) day of incubation, the diameter of the TRIC7 strain showed the following characteristics: DC 42.6 cm Ø, DHS 7.2 cm Ø, RES 276%, pH of media 3.5 (Table 2) and concentration of soluble P ($H_2PO_4^-$) near 64.3 mg L^{-1} (Table 3).

Similar results were obtained with the TRIC11 strain in the same culture medium and incubation time. This strain showed a DC de 3.4 cm Ø, a DHS of 7.5 cm Ø, an RES of 221%, a pH of 3.5 (Table 2), and a concentration of soluble P ($H_2PO_4^-$) of 75.0 mg L^{-1} (Table 3). Consonantly, the strains TRIC13 and TRIC48 showed a DC of 3.3 and 3.5 cm Ø, a DHS of 8.5 and 7.9 cm Ø, a maximum RES of 257 and 226%, a pH of 3.5 (Table 2) and a concentration of soluble P ($H_2PO_4^-$) of 89.8 and 86.2 mg L^{-1} in the same culture medium and incubation, respectively (Table 3).

On the sixth day of incubation, the RES of TRIC7, TRIC11, TRIC13, and TRIC48 oscillated between 221 y 276%. These results show a solubilization halo for these strains that is three (3) times larger than the diameter of their mycelia (Table 2). In contrast, in the TRIC33, TRIC36, TRIC53, TRIC57, and TRIC61 strains, the halo was smaller, indicating low RES values.

With respect to diametral growth of the fungal strains, this was larger in control treatments than in phosphate media ($p \leq 0.05$) (Table 2).

Table 2. Relative solubilization efficiency (RSE) of fungal strains evaluated on three different solid media. Myceliar diameter and solubilization halo.

Strain	Time (d)	Diameter of the fungus colony DC (cm)				Halo diameter solubilization HDS (cm)				Relative solubilization P Efficiency RES (%)				Final pH of the medium			
		PDA	MMHA	PVKM	MSPOH	PDA	MMHA	PVKM	MSPOH	PDA	MMHA	PVKM	MSPOH	PDA	MMHA	PVKM	MSPOH
Trichoderma sp. (TRIC7)	3	1.4	1.6	3.5	2.4	0.0	1.6	4.4	4.6	0.0	100	125	191	6.8	5.5	5.5	4.5
	6	3.8	2.8	3.6	2.6	0.0	4.8	5.4	7.2	0.0	171	150	276	6.8	4.5	5.0	3.5
	9	6.7	4.3	4.8	3.8	0.0	6.0	5.5	8.5	0.0	139	114	223	6.5	4.5	5.0	3.0
	12	10.0	5.1	5.8	4.2	0.0	6.6	6.6	8.8	0.0	129	113	209	6.0	4.0	5.1	3.0
Trichoderma sp. (TRIC11)	3	2.4	2.9	2.2	3.3	0.0	2.9	2.6	4.9	0.0	100	118	148	6.9	5.5	5.0	5.0
	6	4.3	3.3	2.6	3.4	0.0	6.5	3.5	7.5	0.0	196	134	221	6.5	4.5	5.0	3.5
	9	8.5	3.5	3.4	4.1	0.0	6.6	3.6	8.0	0.0	188	105	195	6.5	4.0	5.1	4.0
	12	10.0	4.2	4.6	5.8	0.0	6.7	4.6	8.2	0.0	159	100	141	6.0	4.0	5.0	4.5
Trichoderma sp. (TRIC13)	3	2.1	1.6	2.0	2.3	0.0	1.6	2.0	4.2	0.0	100	100	182	6.5	5.0	5.2	4.5
	6	4.6	2.1	2.3	3.3	0.0	4.6	3.6	8.5	0.0	219	156	257	6.5	4.5	5.0	3.5
	9	8.7	3.8	4.6	7.4	0.0	4.9	5.6	9.0	0.0	128	121	121	6.0	3.5	4.5	4.0
	12	10.0	4.4	4.9	8.5	0.0	4.9	5.9	9.5	0.0	111	120	111	6.0	3.5	4.5	4.5
Trichoderma sp. (TRIC33)	3	2.8	2.5	3.4	2.5	0.0	3.6	3.4	2.8	0.0	144	100	112	6.8	5.8	6.5	6.0
	6	4.9	3.4	3.5	2.7	0.0	5.4	4.5	4.3	0.0	158	129	159	6.5	5.5	6.5	5.5
	9	7.1	4.6	4.1	4.4	0.0	5.5	4.6	4.5	0.0	119	112	102	6.5	5.5	6.5	5.0
	12	10.0	4.9	4.3	4.5	0.0	5.9	4.7	4.6	0.0	120	109	102	6.5	5.5	6.5	5.0
Trichoderma sp. (TRIC 36)	3	2.3	2.4	1.7	3.3	0.0	2.8	1.8	3.9	0.0	116	105	118	6.9	5.9	5.5	6.5
	6	4.7	2.9	2.7	4.1	0.0	4.5	3.4	6.5	0.0	155	125	158	6.5	5.5	4.5	6.5
	9	8.4	3.5	3.9	5.5	0.0	4.7	3.9	7.5	0.0	134	100	136	6.5	4.5	4.5	5.5
	12	10.0	4.8	5.3	6.8	0.0	4.9	5.3	8.0	0.0	102	100	117	6.5	4.0	4.0	4.0
Trichoderma sp. (TRIC48)	3	2.2	1.5	1.5	3.9	0.0	2.8	1.8	6.9	0.0	186	120	176	6.5	5.0	4.5	5.5
	6	5.9	3.5	2.5	3.5	0.0	6.8	3.9	7.9	0.0	194	156	226	6.5	4.5	4.0	3.5
	9	9.1	5.5	4.5	6.5	0.0	7.1	5.5	8.0	0.0	129	122	123	6.5	4.5	4.0	4.0
	12	10.0	6.5	4.5	7.2	0.0	7.5	5.6	8.6	0.0	115	124	119	6.5	4.0	4.0	4.5
Trichoderma sp. (TRIC53)	3	2.3	2.1	2.1	2.1	0.0	2.6	2.1	2.2	0.0	123	100	104	6.9	5.5	6.5	5.5
	6	4.0	2.2	2.3	2.8	0.0	3.4	2.7	4.2	0.0	154	117	150	6.5	4.5	5.0	5.5
	9	8.3	3.5	2.7	4.1	0.0	3.6	2.8	4.5	0.0	102	106	109	6.5	5.0	5.5	6.0
	12	10.0	3.7	3.3	6.3	0.0	3.7	3.4	6.9	0.0	100	103	109	6.0	5.0	6.5	6.0
Trichoderma sp. (TRI57)	3	3,5	4,8	3,5	4,2	0.0	4.8	4,2	4,4	0.0	100	120	105	6.5	602	6,2	6,8
	6	3,5	4,5	3,6	4,3	0.0	5.7	4,4	5,5	0.0	126	122	128	6.4	5,7	5,2	6,6
	9	3,9	5,2	3,9	4,5	0.0	5,8	4.5	5,6	0.0	111	115	124	6.0	5,0	4,1	6,5
	12	10.0	5.6	4,2	4,7	0.0	5,8	4,6	5,7	0.0	103	109	121	5.8	5,2	5,2	6,4
Trichoderma sp. (TRIC61)	3	2,0	3,3	2,0	1,9	0.0	3,3	2.4	2.4	0.0	100	120	126	6.8	5.8	6.5	6.8
	6	2,0	3,5	2,3	2,5	0.0	5,5	3.2	4.5	0.0	157	139	180	6.6	5.5	6.3	5.5
	9	3,0	7,5	3,0	3,7	0.0	7,8	3.3	4.9	0.0	104	111	132	6.7	5.3	6.1	5.3
	12	10.0	8,0	3,3	5,0	0.0	8.0	3.5	5.2	0.0	100	106	104	6.9	5.1	6.0	5.0

All values shown are the average of 4 replicates per treatment.

In this treatment, the average diameter of mycelia on the 12th day of incubation was 10.0 Ø and the maximum decrease in pH of the media was 5.8 (Table 2). These values (DC) disagree with the concentration of soluble P ($H_2PO_4^-$) obtained with respect to different growth media (Table 3).

Quantification of sporulation of Trichoderma spp

With respect to the influence of culture media on the concentration of spores, Table 4 shows values for each of the strains evaluated. Thus, the results for TRIC7 in MSPOH media were 75 × 10^7 spores mL^{-1}. This quantity was larger than the rest of the other culture media evaluated for this strain (PVK = 35 × 10^6 spores mL^{-1}, MM-HA = 42 × 10^6 spores mL^{-1}, Control = 71× 10^6 spores mL^{-1}).

The TRIC11 strain in MSPOH media registered 84 × 10^7 spores mL^{-1}. This quantity was higher than that found in other culture media evaluated (PVK = 33 × 10^6 spores mL^{-1}, MM-HA = 45 × 10^6 spors mL^{-1}, Control = 72× 10^6 spores mL^{-1}). For the strain TRIC13 in MSPOH medium, there were 98 × 10^7 spores mL^{-1}. This quantity was higher than the rest of the other culture media evaluated (PVK = 39 × 10^6 spores mL^{-1}, MM-HA = 45 × 10^6 spores mL^{-1}, Control = 83× 10^7 spores mL^{-1}). For the strain TRIC48 in MSPOH media, there were 98 × 10^7 spores mL^{-1}. This quantity was higher than the rest of the other culture media evaluated (PVK = 55 × 10^6 spores mL^{-1}, MM-HA = 68 × 10^6 spores mL^{-1}, Control = 85× 10^7 spores mL^{-1}).

Quantification of Colony Forming Units (CFU) of Trichoderma spp

With respect to the influence of culture media on Colony Forming Units (CFU), the results for TRIC7 in MSPOH media were 25 × 10^3 spores mL^{-1}. This quantity was higher than the rest of the other culture media evaluated (PVK = 15 × 10^3 spores mL^{-1}, MM-HA = 18 × 10^3 spores mL^{-1}, Control = 20× 10^3 spores mL^{-1}) (Table 4). For theTRIC11 strain in MSPOH media, there were 27 × 10^7 spores mL^{-1}. This was higher than the rest of the other culture media evaluated (PVK = 11 × 10^6 spores mL^{-1}, MM-HA = 15 × 10^3 spores mL^{-1}, Control = 21× 10^3 spores mL^{-1}) (Table 4). For the TRIUC13 strain, in MSPOH media, there were 25 × 10^3 spores mL^{-1}. This was higher than that of the other culture media tested (PVK = 13 × 10^2 spores mL^{-1}, MM-HA = 16× 10^3 spores mL^{-1}, Control = 23× 10^3 spores mL^{-1}) (Table 4). For the TRIC48 strain in MSPOH media, there were 25 × 10^3 spores mL^{-1}. This was higher than the rest of the culture media evaluated (PVK = 13 × 10^2 spores mL^{-1}, MM-HA = 14× 10^3 spores mL^{-1}, Control = 21× 10^3 spores mL^{-1}) (Table 4).

Quantification of Biomass of Trichoderma spp

With respect to the influence of culture media on biomass, the results for TRIC7 in MSPOH media were 5.96 mg. This was higher than the PVK = 3.33 mg and MM-HA = 3.96 mg culture media, but smaller with respect to the Control=6.64 mg.

The TRIC11 strain in MSPOH media registered 5.13 mg. This was greater than the PVK = 2.78 mg and MM-HA = 2.46 mg culture media, but smaller than the Control = 6.74.

The TRIC13 strain in MSPOH media yielded 5.56 mg. This was greater than the PVK = 2.66 mg and MM-HA = 2.56 mg culture media, but less than the Control = 6.91. The TRIC48 strain in MSPOH media yielded 5.25 mg biomass. This was higher than the PVK = 1.97 mg and MM-HA = 2.10 mg culture media, but less than the Control = 6.95. The results above are shown in Table 4.

Quantification of soluble phosphorous in liquid media

In MSPOH on day six (6) of the incubation of the TRIC7, TRIC11, TRIC13, and TRIC48 strains, there were concentrations of soluble P ($H_2PO_4^-$) of 66.3, 75.9, 89.8, and 86.2 mg L^{-1}, respectively. In the same order, these strains exhibited a decrease in pH of from 7.0 to 3.64, 3.63, 3.64, and 3.80. Considering that the strains mentioned solubilized an average of 12.5 times more P than the control treatment and the other strains evaluated, they obtained better solubilization *in vitro* of PR ($p \leq 0.01$) (Table 3).

Table 3. Concentration of soluble P (H2P04-) and pH in uninoculated liquid media inoculated with fungal strains.

Treatment	Incubation time (d)	Variables evaluated in the middle MM-HA		Variables evaluated in the middle PVKM		Variables evaluated in the middle MSPOH	
		Medium pH	Soluble P ($H_2PO_4^-$)	Medium pH	Soluble P ($H_2PO_4^-$)	Medium pH	Soluble P ($H_2PO_4^-$)
Absolute control	3	6.55	1.5	6.71	1.3	6.69	1.3
(uninoculated)	6	4.60	1.1	6.64	1.8	6.67	1.7
	9	5.45	1.9	6.35	1.8	6.46	1.7
	12	6.50	1.9	6.13	1.7	6.41	1.6
Trichoderma sp.	3	6.00	10.8	5.98	6.3	5.65	61.0
(TRIC7)	6	5.58	37.7	5.68	6.9	3.64	66.3
	9	5.48	28.3	5.56	6.7	3.03	53.0
	12	4.33	37.7	5.30	6.6	4.41	48.5
Trichoderma sp.	3	5.69	25.8	6.53	5.8	5.75	68.9
(TRIC11)	6	5.48	39.0	5.97	6.9	3.63	75.9
	9	5.38	25.3	5.86	6.2	4.01	65.3
	12	4.21	21.3	5.71	6.5	4.52	59.5
Trichoderma sp.	3	4.98	33.7	6.02	6.4	5.67	68.9
(TRIC13)	6	4.75	68.0	5.86	6.9	3.64	89.8
	9	4.83	48.8	5.54	6.7	4.02	79.8
	12	4.38	55.7	5.53	6.5	4.91	69.8
Trichoderma sp.	3	5.73	2.7	6.96	1.2	6.00	33.2
(TRIC33)	6	5.74	2.9	6.70	2.5	5.76	34.0
	9	5.76	2.6	6.77	2.7	5.30	34.2
	12	5.70	2.5	6.47	1.9	5.19	34.2
Trichoderma sp.	3	5.96	5.8	5.91	3.2	6.79	36.0
(TRIC 36)	6	5.67	6.5	4.90	4.4	6.68	36.0
	9	5.58	5.8	4.73	3.9	5.23	36.5
	12	5.45	6.1	4.42	3.5	6.31	36.5
Trichoderma sp.	3	5.87	7.3	5.59	4.2	5.53	55.0
(TRIC48)	6	5.57	7.9	5.57	5.4	3.80	86.2
	9	5.37	6.8	5.46	4.9	3.99	66.0
	12	5.18	6.5	5.45	4.3	3.45	52.0
Trichoderma sp.	3	5.82	2.1	3.97	1.4	6.82	29.3
(TRIC53)	6	5.68	2.8	2.96	1.7	6.72	29.9
	9	5.62	2.5	3.03	1.8	6.55	25.5
	12	5.55	2.3	3.04	1.0	6.34	25.0
Trichoderma sp.	3	5.92	1.3	3.47	1.4	6.62	19.3
(TRI57)	6	5.68	1.5	2.96	1.7	6.62	19.9
	9	5.72	1.4	3.83	1.8	6.05	15.5
	12	5.55	1.0	3.74	1.0	6.44	15.0
Trichoderma sp.	3	5.83	3.6	6.86	2.2	6.02	43.2
(TRIC61)	6	5.74	4.9	6.60	2.5	5.72	44.0
	9	5.76	3.7	6.57	1.7	5.00	44.2
	12	5.60	3.3	6.47	1.7	5.12	44.2

Soluble P ($H_2PO_4^-$) ($LSD_{0.05}$= 8.0) and final pH of culture media ($LSD_{0.05}$= 0.2) are data that show the behavior of fungal colonies in MM-HA, PVKM, and MSPOH liquid media. All values shown are the average of 4 replicas per treatment.

At the end of twelve days of solubilization kinetics, the concentration of soluble P ($H_2PO_4^-$) decreased (48.5 mg L^{-1} in TRIC7, 59.5 mg L^{-1} in TRIC11, 69.8 mg L^{-1} in TRIC13, and 52.0 mg L^{-1} in TRIUN48) (Table 3). This behavior confirms that at the greatest fungal biomass (Table 4), there is the smallest concentration of P in solution, allowing the selection of six (6) days as the optimal time in which the highest amount of free P is found ($p \leq 0.05$) (Table 4).

The concentration of soluble P ($H_2PO_4^-$) was lower in the other strains evaluated (Table 3).

Discussion

The results of the present investigation confirm the proposed hypothesis, indicating that success in the *in vitro* selection of strains of *Trichoderma* with phosphodissolvent capacities are a function of the median growth and kinetic biosolubilization of PR. The results obtained under experimental conditions evaluated confirm the differential response of fungi in relation to these variables.

Composition of fungal communities in soil and rhizospheres of beans and corn

One of the most important aspects that determines the composition of the microbial community in the rhizosphere is type of vegetal species, due to the fact that for different plants, following the genotype, morphology of roots, and physical as well as structural transformations that these cause in the adjacent soil; there are variations in the structure of microbial communities (Marschner *et al.,* 2001). Beans are a plant, the foliar architecture of which favors the accumulation of necromass in the foot, an aspect that supports a larger colonization of fungi due to their decompositional activity for litter accumulated around the stem (Anacona and Sabogal, 2002). Due to the above, a great amount of organic material is obtained in the soil that favors the establishment of fungi that respond to the rhizospheric effect exercised by the exudates of the bean rhizosphere.

Table 4. Sporulation efficiency, CFU formation, and biomass of fungal strains evaluated on three different solid culture media.

Strain	N° spores x 0.1mL $^{-1}$ (Dilution 10^{-3})				UFC mL^{-1} (Dilution 10^{-8})				Biomass (mg)			
	PDA	MMHA	PVKM	MSPOH	PDA	MMHA	PVKM	MSPOH	PDA	MMHA	PVKM	MSPOH
Trichoderma sp. (TRIC7)	71x10^6	42x10^6	35x10^6	75x10^7	20x10^3	18x10^3	15x10^3	25x10^3	6.64	3.96	3.33	5.96
Trichoderma sp. (TRIC11)	72x10^7	45x10^6	33x10^6	84x10^7	21x10^3	15x10^3	11x10^3	27x10^3	6.74	2.46	2. 78	5.13
Trichoderma sp. (TRIC13)	83x10^7	45x10^6	39x10^6	98x10^7	23x10^3	16x10^3	13x10^3	25x10^3	6.91	2.56	2.66	5.56
Trichoderma sp. (TRIC33)	81x10^7	53x10^6	40x10^6	88x10^7	18x10^3	13x10^3	17x10^3	19x10^3	5.56	2.31	2.19	4.84
Trichoderma sp. (TRIC 36)	85x10^7	68x10^6	55x10^6	98x10^7	18x10^3	11x10^3	8x10^3	18x10^3	6.71	2.15	2.43	4.33
Trichoderma sp. (TRIC48)	85x10^7	48x10^6	30x10^6	87x10^7	21x10^3	14x10^3	13x10^3	25x10^3	6.95	2.10	1.97	5.25
Trichoderma sp. (TRIC53)	83x10^7	41x10^6	32x10^6	82x10^7	18x10^3	8x10^3	6x10^3	17x10^3	4.78	1.98	1.80	4.14
Trichoderma sp. (TRI57)	81x10^7	40x10^6	37x10^6	96x10^7	15x10^3	7x10^3	6x10^3	16x10^3	6.78	0.98	0.80	4.05
Trichoderma sp. (TRIC61)	80x10^7	53x10^6	42x10^6	73x10^7	15x10^3	10x10^3	7x10^3	17x10^3	5.58	2.00	2.10	4.80

All values shown are the average of 4 replicates per treatment.

In gramines, the metabolism generates a higher quantity of exuded radicals, of which organic acids predominate. The necromass offering of gramines is deposited in the soil near the rhizosphere, which is different from beans, where the necromass remains in the foot; therefore the highest quantity of decomposing fungi are associated with corn rhizospheres, which may explain the results found. In general terms, the numbers of fungi registered are within the same range (quantity), although there was a higher number of fungi in the rhizospheres of corn in relation to beans. In addition, there were also a predomination of phosphate solubilizing fungi found in both cultures, although there was a higher proportion in corn.

Conversely, it is possible that the presence of a large quantity of phosphate solubilizing fungi found in the

rhizosphere of corn and the high micorrhization that is characteristic of gramines (Cepeda and Gamboa, 2001; Sánchez, 1996) plays an important role in the removal of phosphorous in these plants; in contrast with that obtained in beans. According to Cepeda and Gamboa (2001), the quantity of available phosphorous in the soil determined by the Bray II method was 4.2 ppm in the soils of eastern Antioquia, which is considered to be very low.

These soils also show a great quantity of Fe and Al, and an acidic pH of 4.9. These factors are related to a high percentage of phosphoric retention (98.9%), and make it so that solubilizers of phosphates are one of the most important functional groups of microorganisms associated with the predominant plants of this ecosystem.

The presence and promotive activity of vegetal growth in phosphate solubilizing fungi is summatively important, as they offer plants the possibility of achieving better adaptation to this phosphate-limited ecosystem by simultaneously promoting forms of phosphorous which can be assimilated and favor efficiency in the uptake of nutrients, while at the same time achieving greater length and proliferation of roots. Species of fungi have been found that produce enzymes such as acid phosphatases and phytases that increment soluble phosphorous in the soil, stimulating root length and sprout proliferation in different plants (Villarroel, 2009; Ocampo *et al.,* 2012; Valero, 2003; Martínez and Martínez, 2000).

Due to the above, it is inferred that the presence of phosphate solubilizing strains of *Trichoderma* spp. associated with plants may unleash similar effects and be utilized as inoculants in cold weather agricultural systems, as with the evaluated strains (Useche, 2003; Rodríguez y Rubiano, 2002; Cepeda and Gamboa, 2001). A value added to the utilization of these microorganisms in agriculture is their potential to also act as biological controls, which has been reported in different studies (Torres-Rubio et al., 2000; Rodríguez and Fraga 1999; Altamore *et al.,* 1999; Kloepper 1993).

The results obtained in this study support the additive hypothesis (Bashan *el al.,*1993), according to which, promoter microorganisms of vegetal growth act through the summation of different mechanisms that operate simultaneously or in succession and through the sum of different activities when they are introduced under auspicious environmental conditions, resulting in the stimulation of growth in plants. The association of plants with microorganisms that act through various mechanisms in an integrated manner, such as phosphate solubilizers, may be an important adaptive mechanism for ecological success, as much for plants as for microorganisms; while, at the same time, representing a valuable germplasm bank for agroecological and biotechnological applications.

Solubilization of PR in solid media

The RES of the TRIC7, TRIC11, TRIC13, and TRIC48 fungal strains, with solubilization halos three (3) times larger than the diameter of their mycelia (Table 2), are larger than those of the *Trichoderma* spp., *Paecilomyces* spp., and *Aspergillus* spp. strains evaluated by Vera *et al.* (2002) and Hernández *et al.* (2011) in comparable culture media. However, other strains evaluated in this work (TRIC33, TRIC36, TRIC53, TRIC57, and TRIC61) show incipient halo formation; their RES values were lower. Despite this, various authors (Hernández *et al.,* 2011; Whitelaw, 1999; and Jones *et al.,* 1998) mention that the absence of solubilization halos in solid culture media do not necessarily indicate that microorganisms lack solubilizing abilities, but rather that solid media are not sensitive enough to detect the activity of some of them. This indicates the need to evaluate them in liquid media in order to obtain more appropriate results.

Nahas (1996) reports that phosphodisolvent bacteria and fungi obtained in the soils of Sao Paulo (Brazil) generate concentrations of soluble P ($H_2PO_4^-$) between 13.3 and 33.1 mg L^{-1}, respectively. Sridevi and Mallaiah (2009) also report phosphodisolvent strains of *Rhizobium* spp. that exhibited RES values between 60 and 150%, accompanied by decrease of

pH of media from 7.02 to 4.04, as well as concentrations of soluble P between 15.6 and 62.0 mg L^{-1}. Consequently, the strains of *Trichoderma* evaluated in this investigation showed RES values between 128-176%, decreases in pH from 7.0 to 3.0, and soluble P values between 15.0 and 89.8 mg L^{-1} (Tables 2 and 3).

With respect to diametral growth of fungal strains, control treatments showed greater values than media with PR ($P \leq 0.05$) (Table 2). This data suggests that the production of organic acids to dilute PR has a high energy cost and deprives the production of fungal biomass. The composition of culture media has a notable influence on the growth and solubilization of fungi. With respect to this, Nahas (2007) and Hernández *et al.* (2011) emphasize that the production of substances implicated in solubilization require the absorption of nutrients, mainly carbonaceous compounds important in the accumulation of biomass.

Data found in this study for the solubilization kinetics of PR indicate a cause-effect relationship between acidification of pH of the media and the concentration of solubilized P ($P \leq 0.05$) (Tables 2 and 3). This inverse relationship suggests that the production of organic acids is the principal mechanism for the dissolution of PR; however, the fact that, at the same pH, the same concentration of P is not obtained, suggests that there may be other mechanisms in the dissolution of PR, such as those suggested by llmer and Schinner (1992) and Thomas (1985). For example, the type of organic acid used and the formation of Ca^{2+} complexes by organic anions may each have an influence. These authors affirm that under buffered conditions, microbial metabolism changes, and this has effects on the liberation of different organic acids than those commonly liberated by microorganisms when the initial pH is not adjusted to the culture medium. Thus, the microbial solubilization process of PR may be more affected by the type of organic acid secreted than by the quantity of acid (Gyaneshwar *et al.*, 1998; Halder *et al.*, 1990). For example, in work done by Osorno and Osorio (2014) with the fungus *Mortierella* sp. (M) and *Aspergillus niger* (A); in fungi that produce oxalic acid (Osorio, 2008) and citric acid (Velásquez *et al.*, 2010), respectively, significant differences were found with respect to the concentration of P in solution. Consonantly, strains of *Trichoderma* evaluated in this investigation equally diminish the pH of the medium (presumably through organic acid production), with some strains generating values as low as those of other fungi (M, A), but do not cause similar reaction dissolution of PR. In the same way that the mechanism of bioacidulation and biosolubilization of PR is influenced by the type of organic acids that the fungus generates, it also impinges the increase in concentration of phosphorous in solution.

It follows that the solubilization efficiency of PR *in vitro* shown by the strains of *Trichoderma* evaluated can be compared with other fungi of known effectiveness in dissolving PR and other phosphorous containing compounds such as *Penicillium janthinellum* (Ñustez and Acevedo, 2005), *Paecilomyces lilacinus* (Thom), Samson (Hernández *et al.*, 2011), *Trichoderma harzianum* (Zhang *et al.*, 2013), *Mortierella* sp., and *Aspergillus* sp., (Osorno and Osorio, 2014; Osorio, 2011). It is thus comparable with the solubilization efficiency of bacterial genera such as *Bacillus, Azospirillum, Pseudomonas,* and *Rhizobium* (Ahmad *et al.*, 2011; Sridevi and Mallaiah, 2009; Rodríguez *et al.*, 2004), among others.

Effects of culture media on spore production

The higher strain sporulation capacities in the MM-HA con $Ca_{10}(PO_4)_6(OH)_2$) medium with respect to the PVK medium modified with $FePO_4 \bullet H_2O$ (PVK) may be related to the degree of solubility of each chemical compound. Calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ has a higher solubility (2.21×10^{-4} g L^{-1}) than $FePO_4 \bullet H_2O$ (1.78×10^{-9} g L^{-1}); solubilizing more readily and generating a higher concentration of soluble P, which may allow better sporulation (Hernández *et al.*, 2011; Barroso and Nahas, 2005). Reyes *et al.* (1999a) found that by using NO_3^- as a nitrogen (N) source, and saccharose as a labile carbon (C) source, they obtained higher solubilization of PR

than with NH_4^+ and glucose used in these media. Also, it may be that the highest sporulation obtained with MBSP could be due to a higher presence of free Ca^{+2} ions in the medium, which coincides with results described by Mosley *et al.* (1989), Shaw and Hoch (2007), Koslova *et al.* (2010), and Hernández *et al.* (2011).

This study permitted the standardization of three distinct techniques for the selection of fungal morphotypes with phosphodissolvent capacities from the rhizoshpere and rhizoplane of beans and corn. Appropriate isolation permits the selection of phosphodissolvent fungi *in vitro,* and later, an evaluation that includes plant response to inoculation with these microorganisms (*e.g. in situ* and *ex situ).* The above will permit advancement in the study of these organisms as potential inoculation tools in soils with deficiencies in phosphorous and PR fertilization.

Kinetics of dissolution of PR

On the sixth day of incubation, the TRIC7, TRIC11, TRIC13, and TRIC48 strains showed higher concentrations of soluble P ($H_2PO_4^-$) and higher bioacidulation of pH. Considering this, the strains mentioned solubilized an average of 12.5 times more P than the control treatment and the other strains evaluated, obtaining a higher *in vitro* solubilization efficiency of PR ($P \leq 0.01$) (Table 2). At the end of twelve days of solubilization kinetics, the concentration of soluble P decreased in the culture media of these strains. This behavior confirms that as fungal biomass increases, concentration of P in solution decreases, allowing the selection of six (6) days as the optimal time for peak amounts of liberated P ($P \leq 0.05$) (Table 2).

In addition, Hernández *et al.* (2011) mentioned that it is probable that the quantity of solubilizing phosphorous diminishes over time because it is incorporated into the metabolism of the fungi. In their investigations with *Penicillum rugolosum* Thom, Reyes *et al.* (1999a) attributed the diminishment of liberated P to the process of immobilization of P for mycelial growth. As phosphorous is indispensable in the cellular functions of all living things, in processes such as the production of ATP, DNA, and RNA; fungi may store this element as a reserve substance in the form of polyphosphates within cellular vacuoles (Hernández *et al.,* 2011; Carlile *et al.,* 2001). Although the control treatments showed a quantity of P in solution 12.5 times less than the inoculated treatments, the results indicate that there was dissolution of PR, although these results were statistically insignificant with respect to experimentation time.

The pH of the media decreased during the time of evaluation of solubilization kinetics (Tables 2 and 3). This decrease in treatments in which TRIC7, TRIC11, TRIC13, and TRIC48 were evaluated was the effect of the phosphodissolvent activity of the fungi (Chun-Chao *et al.,* 2007; Pradham and Sukla, 2005; Gómez-Guiñan and Zabala, 2001). Hernández *et al. (*2011) also reported that one of the principal mechanisms employed by fungi to dissolve phosphatic complexes is the production of organic acids. The control treatments did not show statistically significant differences.

This investigation found an inverse relationship between quantity of soluble P and pH of media with PR($P \leq 0.01$). Control treatments did not encounter a relationship between these two variables.

The above coincides with other studies in which it was reported that when pH is decreased (e.g. From 5.5 to 2.4), the concentration of soluble P increased (0.32 to 110 mg L^{-1}) (Hernández *et al.*, 2011; Xiao *et al.,* 2009; Pandey *et al.,* 2008; Thomas *et al.,* 1985). The favorable interaction between bioacidulation versus biosolubilization depends on diverse factors, among which, the solubilizing capacity of the organism stands out, as organic acids are produced that form mineral organic complexes and reduce pH as the result of metabolic activity (Osorno and Osorio, 2014; Osorno, 2013; Scervino *et al.*, 2010; Wakelin *et al.*, 2004); as does the type of phosphatic compound used in the experiment; as well as the labile carbon source and the nitrogen source applied to the system

(Hernández *et al.,* 2011; Souchie *et al.,* 2006; Barroso and Nahas, 2005; Pradham and Sukla, 2005).

Conclusion

The results of the present investigation confirm the proposed hypothesis, indicating that success in the *in vitro* selection of strains of *Trichoderma* with phosphodissolvent capacities are a function of the median growth and kinetic biosolubilization of PR. The results obtained under experimental conditions evaluated confirm the differential response of fungi in relation to these variables.

Data found in this study for the solubilization kinetics of PR indicate a cause-effect relationship between acidification of pH of the media and the concentration of solubilized. This inverse relationship suggests that the production of organic acids is the principal mechanism for the dissolution of PR; however, the fact that, at the same pH, the same concentration of P is not obtained, suggests that there may be other mechanisms in the dissolution of PR. For example, the type of organic acid used and the formation of Ca^{2+} complexes by organic anions may each have an influence. In the same way that the mechanism of bioacidulation and biosolubilization of PR is influenced by the type of organic acids that the fungus generates, it also impinges the increase in concentration of phosphorous in solution.

Therefore, it is important to consider in subsequent investigations for a favorable interaction between bioacidulation versus biosolubilization depends on diverse factors, among which, the solubilizing capacity of the organism stands out, as organic acids are produced that form mineral organic complexes and reduce pH as the result of metabolic activity ; as does the type of phosphatic compound used in the experiment; as well as the labile carbon source and the nitrogen source applied to the system.

This study permitted the standardization of three distinct techniques for the selection of fungal morphotypes with phosphodissolvent capacities from the rhizoshpere and rhizoplane of beans and corn. Appropriate isolation permits the selection of phosphodissolvent fungi *in vitro,* and later, an evaluation that includes plant response to inoculation with these microorganisms (*e.g. in situ* and *ex situ).* The above will permit advancement in the study of these organisms as potential inoculation tools in soils with deficiencies in phosphorous and PR fertilization.

The results obtained in this study support the additive hypothesis, according to which, promoter microorganisms of vegetal growth act through the summation of different mechanisms that operate simultaneously or in succession and through the sum of different activities when they are introduced under auspicious environmental conditions, resulting in the stimulation of growth in plants.

The association of plants with microorganisms that act through various mechanisms in an integrated manner, such as phosphate solubilizers, may be an important adaptive mechanism for ecological success, as much for plants as for microorganisms; while, at the same time, representing a valuable germplasm bank for agroecological and biotechnological applications.

Referencias

Abd-Alla MH. 1994. Use of organic phosphorus by *Rhizobium leguminosarum* iovar. *Viceae* phosphatases. Biology and Fertility of Soils **18,** 216-218.
http://dx.doi.org/10.1007/BF00647669

Ahmad AA, Muhammad Z, Saima S, Muhammad N. 2011. Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for coinoculation with *Rhizobuim leguminosarum* to improven growth, nodulation, and yield of lentil. Biology and Fertility of Soils **47,** 457-465.
http://dx.doi.org/10.1007/s00374-011-0551-7

Altomare C, Norvell WA, Björkman T, Harman GE. 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and

biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Applied and Environmental Microbiology **65(7),** 2926-2933.

Anacona AC, Sabogal SR. 2002. Efecto de diferentes tamaños de claro experimental sobre el proceso de sucesión en un pastizal del embalse San Rafael (La Calera- Cundinamarca). Acta Biológica Colombiana **2(2),** 59-60.

Bar-Yosef B, Rogers RD, Wolfram JH, Richman E. 1999. *Pseudomonas cepacia* mediated rock phosphate solubilization in kaolinite and montmorillonite suspensions. Soil Science Society of America Journal **63,** 1703-1708. http://dx.doi.org/10.2136/sssaj1999.6361703x

Bolan NS, Naidu R, Mahimairaja S, Baskaran S. 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphates. Biology and Fertility of Soils **18,** 311-319.

Börkman T, Blanchard LM, Harman GE. 1998. Growth enhancement of shrunfen-2 (sh2) Sweet Corn by *Trichoderma harzianum* 1295-22: Effect of Environmental Stress. Journal of the American Society for Horticultural Science **123(1),** 35-40.

Boul SW, Eswaran H. 2000. Oxisols. Advances in Agronomy **68**, 151-195.

Caipo ML, Duffy S, Zhao L, Schaffner DW. 2002. *Bacillus megaterium* spore germination is influenced by inoculum size. Journal of Applied Microbiology **92,** 879-884. http://dx.doi.org/10.1046/j.1365-2672.2002.01597.x

Calich VL, Purchio A, Paula CR. 1979. A new fluorescent viability test for fungi cells. Mycopathologia **66,** 175-177. http://dx.doi.org/10.1007/BF00683967

Calderia AT, Feio SS, Arteiro JM, Coelho AV, Roseiro JC. 2008. Environmental dynamics of *Bacillus amyloliquefaciens* CCMI 1051 antifungal activity under different nitrogen patterns. Journal of applied microbiology **104,** 808-816.

Calvo AM. 2008. The VeA regulatory system and its role in morphological and chemical development in fungi. Fungal Genetics Biology **45,** 689-701.

Cappuccino G, Sherman N. 1998. Microbiology: A laboratory Manual. CA: Benjamin/Cumming Science Publishing.

Carlile MJ, Watkinson SC, Gooday GW. 2001. The Fungi. Academic Press, Great Britain. 588 p.

Cepeda ML, Gamboa AM. 2001. Hongos solubilizadores de fosfato aislados de rizósfera de *Espeletia grandiflora* Humb. Y Bonpl. (Páramo El Granizo-Monserrate) y su efecto sobre la disponibilidad de fósforo en el suelo. Trabajo de Grado. Universidad Nacional de Colombia. Facultad de Ciencias. Departamento de Biologia. Bogotá.

Chen XH, Koumoutsi A, Scholz R, Eisenreich A, Schneider K, Heinemeyer I, Morgenstern B, Voss B, Hess W, Reva O, Junge H, Voigt B, Jungblut PR, Vater J, Sussmuth R, Liesegang H, Strittmatter A, Gottschalk G, Borriss R. 2007. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. Nature Biotechonlogy 25. http://dx.doi.org/1007-1014. 10.1038/nbt1325

Chigineva NI, Aleksandrova AV, Marshan S, Kandeler E, Tiunov AV. 2011. The importance of mycelial connection at the soil-litter interface for nutrient translocation, enzyme activity and litter decomposition. Applied Soil Ecology **51,** 35–41. http://dx.doi.org/10.1016/j.apsoil.2011.08.009

Chun-Chao C, Yu-Lin K, Chen-Ching C, Wei-Liang C. 2007. Solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger*. Biology and Fertility of Soils **43,** 575-584.

http://dx.doi.org/10.1007/s00374-006-0140-3

Collados CC. 2006. Impacto de inoculantes basados en *Azospirillum* modificados genéticamente sobre la diversidad y actividad de los hongos de la micorriza arbuscular en rizosfera de trigo y maíz. Tesis Doctoral. Universidad de Granda. Facultad de Ciencias. Departamento de Microbiología. España.

Dandurand L, Knudsen G. 1993. Influence of *Pseudomonas fluorescent* on hyphal growth and biocontrol activity of *Trichoderma harzianum* in the spermosphere and rhizosphere of pea. Phytopathology **83(3),** 265-270.
http://dx.doi.org/10.1094/Phyto-83-265

de Freitas JR, Banerjee MR, Germida JJ. 1997. Phosphate solubilizing rhizobacteria enhance the growth and yeild but not phosphorus uptake of canola (*Brassica napus*), Biology and Fertility of Soils **24,** 358-364.

Dixon JB, Weed SB. 1989. Minerals in soil environments, Second edition. Soil Science Society of America, Madison, Wisconsin, USA. 1244 P.

Entz MH, Guilford R, Gulden R. 2001. Crop yield and soil nutrient status on 14 organic farms in the eastern portion of the northern Great Plains. Canadian Journal of Plant Science **81,** 351– 354.
http://dx.doi.org/10.2134/agronj2001.933495x

Flach EN, Quak W, Van Diest A. 1987. A comparison of the rock phosphate-mobilizing capacities of various crop species. Trop Agric (Trinidad) **64,** 347– 352.

Fontes MP, Weed SB.1996. Phosphate adsortion by clays from Brazilian Oxisols relationships with specific surface area and mineralogy. Geoderma **72,** 37-51.
http://dx.doi.org/10.1016/0016-7061(96)00010-9

Garbera P, Van Veen JA, Van Elsas JD. 2003. Predominant Bacillus spp. In agricultural soil under different management regimes detect via PCR-DGGE. Microbial Ecology **45,** 302-316.

Godes A. 2007. Perspectivas de los inoculantes fúngicos en Argentina. pp. 11-14. En: Izaguirre-Mayoral, M.L., C. Labandera y J. Sanjuán (eds.). Biofertilizantes en Iberoamérica: una visión técnica, científica y empresarial. Imprenta Denad Internacional, Montevideo.

Gómez-Guiñán Y, Zabala M. 2001. Determinación de la capacidad solubilizadora del P en hongos aislados de la rizósfera del maní (*Arachis hypogea* L.). Saber **13,** 8-13.

Gregory PJ. 2006. Roots, rhizosphere and soil: the route to a better understanding of soil science? European Journal of Soil Science **57,** 2-12.
http://dx.doi.org/10.1111/j.1365-2389.2005.00778.x

Guppy CN, Menzies NW, Blamey FPC, Moody P. 2005. Do decomposing organic matter residues reduce phosphorus sorption in highly wathered soil?. Soil Science Society of America Journal **69,** 1405-1411
http://dx.doi.org/10.2136/sssaj2004.0266

Gyaneshwar P, Naresh KG, Parekh LJ. 1998. Effect of buffering on the phosphate-solubilizing ability of microorganisms. World Journal of Microbiology and Biotechnology **14,** 669-673.
http://dx.doi.org/10.1023/A:1008852718733

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil **245,** 83–93.
http://dx.doi.org/ 10.1023/A:102066391625

Habte M, Osorio NW. 2001. Arbuscular Mycorrhizas: Producing and applying Arbuscular Mycorrhizal Inoculum. Honolulu: University of Hawaii. 47.

Halder AK, Mishra AK, Bhattacharyya P, Chakrabartty PK. 1990. Solubilization of rock phosphate by rhizobium and bradyrhizobium. The

Journal of General and Applied Microbiology **36,** 81-92.

He ZL, Zhu J. 1997. Transformation and bioavailability of specifically sorbed phosphate on varaiable-carge mineral soils. Biology and Fertility of Soils **25,** 175-181. http://dx.doi.org/ 10.1007/s003740050300

He ZL, Zhu J. 1998. Microbial utilization and transformation of phosphate adsorbed by variable charge minerals. Soil Biology and Biochemistry **30,** 917-923.

Hernández JL, Sánchez MI, García JG, Mayek N, González JM, Quiroz JDC. 2011. Caracterización molecular y agronómica de aislados de *Trichoderma* spp., nativos del noreste de México. Revista Colombiana de Biotecnología **12(2),** 176-185.

Howell CR. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Disease **87,** 4-10. http://dx.doi.org/10.1094/PDIS.2003.87.1.4

Hoyos-Carvajal LM. 2008. Evaluación de aislamientos de *Trichoderma* spp. contra *Rhizoctonia solani* y *Sclerotium rolfsii* bajo condiciones *in vitro* y de invernadero. Agronomia Colombiana.

Ilmer P, Schinner F. 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. Soil Biology and Biochemistry **24,** 389-395.

Iyamuremye F, Dick RP. 1996. Organic amendments and phosphorus sorption by soils. Advances in Agronomy **56,** 139-185.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist **135,** 575-585. http://dx.doi.org/10.1046/j.1469-8137.1997.00729.x

Kamal S, Ajay KM, Raj SM. 2009. Morphological, Biochemical and molecular characterization of *Trichoderma harzianum* isolates for their efficacy as biocontrol agents. Journal of Phytopathology **157,** 51-56. http://dx.doi.org/10.1111/j.1439-0434.2008.01451.x

Kim KY, McDonald GA, Jordan D. 1997. Solubilization of hydroxyapatite by *Enterobacter agglomerans* and cloned *Escherichia coli* in culture medium. Biology and Fertility of Soils **24,** 347-352. http://dx.doi.org/10.1007/s003740050256

Kirkby EA, Johnston E. 2008. Soil and fertilizer phosphorus in relation to crop nutrition. En: The ecophysiology of plant-phosphorus interactions. 177-223. P.J. White and J.P. Hammond (Eds.) © Springer Science. 296 p. http://dx.doi.org/10.1007/978-1-4020-8435-5_9

Kloepper JW. 1993. Plant Growth Promoting Rhizobacteria as Biological Control Agents. En: F. B. Metting (Ed), Soil Microbial Ecology: Applications in Agricultural and Environmental Management. Marcel Dekker Inc., New York, USA.

Knox OGG, Killham K, Leifert C. 2000. Effects of increased nitrate availability on the control of plantpathogenic fungi by the soil bacterium *Bacillus subtilis*. Applied Soil Ecology **15,** 227-231. http://dx.doi.org/10.1016/S0929-1393(00)00098-6

Kozlova OV, Egorov SY, Kupriyanova-Ashina FG. 2010. The Relationship between cellular and calcium responses of *Aspergillus awamori* to external influences. Microbiology **79,** 294-299. http://dx.doi.org/10.1134/S0026261710030033

Kucey RMN. 1983. Phosphate-solubilising bacteria and fungi in various cultivated and virgin Alberta soil. Canadian Journal of Plant Science **63,** 671678. http://dx.doi.org/10.4141/cjss83-068

Londoño. 2010. Efecto de la inoculación con un hongo micorrizal y un hongo solubilizador de fósforo

en la absorción de fosfato y crecimiento de *Leucaena leucocephala* en un oxisol. Trabajo de Grado. Universidad Nacional de Colombia. Facultad de Ciencias Agropecuarias. Departamento de Biología. Medellín.

Lins ID, Cox FR. 1989. Effect of extractant and select soil properties on predicting the correct phosphorus fertilization of soybean. Soil Sciences **53,** 813-816. http://dx.doi.org/10.2136/sssaj1989.03615995005300030031x

Lynch JP, Brown KM. 2008. Root strategies for phosphorus acquisition. En: The ecophysiology of plantphosphorus interactions. 83-116 P. P.J. White, J.P. Hammond (Eds.) © Springer Science. http://dx.doi.org/10.1007/978-1-4020-8435-5_5

Marschner P. 2008. The role of rhizosphere microorganisms in relation to P uptake by plants. En: The ecophysiology of plant-phosphorus interactions. 165-176 P. P.J. White and J.P. Hammond (Eds.) © Springer Science. 296 p. http://dx.doi.org/10.1007/978-1-4020-8435-5_8

Martinez SM, Martinez GA. 2000. Efects of Phosphate Solubilization Bacteria During the Rooting Period of SUGAR Cane (*Saccharum offinarum*), Venezuela 5171 Variety, on the Grower`s Oasis Substrate. Soil and Plant Nutrition **49,** 2-9.

Mathews CK, Van Holde KE, Ahern KG. 2002. Biochemistry. Third edition. Benjamin Cummings, San Francisco. 1186 p.

Mc Spadden Gardener, 2004. Ecology of Bacillus and Paenibacillus spp. in Agricultural Systems. Symposium: The Nature and Application of Biocontrol Microbes: *Bacillus* spp. Phytophathology **94(11),** 1252-1258. http://dx.doi.org/10.1094/PHYTO.2004.94.11.1252

Moreno-Sarmiento N, Moreno-Rodríguez L, Uribe-Vélez D. 2007. Biofertilizantes para la agricultura en Colombia. 38-45. En: Izaguirre-Mayoral, M.L., C. Labandera y J. Sanjuán (eds.). Biofertilizantes en Iberoamérica: una visión técnica, científica y empresarial. Imprenta Denad Internacional, Montevideo.

Mosley MJ, Pitt D, Barnes JC. 1989. Adenine and pyridine nucleotide levels during calcium-induced conidiation in *Penicillium notatum*. Antonie van Leeuwenhoek **56,** 191-199. http://dx.doi.org/10.1007/BF00399982

Murphy J, Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chimica. Acta **27,** 31-35. http://dx.doi.org/10.1016/S0003-2670(00)88444-5

Nahas, E. 1996. Factors determining rock phosphate solubilization by microorganisms isolated from soil. World Journal of Microbiology and Biotechnology 12, 567-572. http://dx.doi.org/10.1007/BF00327716

Nahas E. 2007. Phosphate solubilizing microorganism: Effect of carbon, nitrogen and phosphorus sources. *In*: Velázquez, E.,and C. Rodríguez-Barruco (eds). First International Meeting on Microbial Phosphate Solubilization. Developments in Plant and Soil Sciences 102. Springer, The Netherlands. 111-115. http://dx.doi.org/10.1007/978-1-4020-5765-6_15

Ñústez E, Acevedo JC. 2005. Evaluación del uso de *Penicillium janthinellum* Biourge sobre la eficiencia de la fertilización fosfórica en el cultivo de la papa (*Solanum tuberosum* L. var. Diacol Capiro). Agronomía Colombiana **23(2),** 290-298.

Ocampo BM, Patiño LF, Marín MA, Salazar M, Gutiérrez P. 2012. Isolation and characterization of potential phytase-producing fungi from environmental samples of Antioquia (Colombia). Revista de la universidad Nacional de la Facultad de Agronomía **65,** 6291-6303.

Oehl F, Oberson A, Tagmann HU, Besson JM, Dubois D, Mader P, Roth H, Frossard E. 2002. Phosphorus budget and phosphorus availability in soils under organic and conventional farming. Nutrient Cycling in Agroecosystems **62,** 25– 35.

Osorio NW. 2008. Effectiveness of microbial solubilization of phosphate in enhancing plant phosphate uptake in tropical soils and assessment of the mechanisms of solubilization. Ph.D. Disertation. University of Hawaii, Honolulu, 392 p.

Osorio NW. 2011. Effectiveness of phosphate solubilizing microorganisms in increasing plant phosphate uptake and growth in tropical soils. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management Volume III. Springer, Berlin, 65–80.
http://dx.doi.org/10.1007/978-3-642-21061-7_4

Osorno LB. 2013. Bio-acidulación de roca fosfórica bajo condiciones *in vitro*. Tesis de Maestría. Universidad Nacional de Colombia Sede Medellín.

Osorno L, Osorio N. 2014. Effect of Carbon and Nitrogen Source and Concentration on Rock Phosphate Dissolution Induced by Fungi.Journal of Applied Biotechnology **2(2),** 32-42.
http://dx.doi.org/10.5296/jab.v2i2.5475

Pandey A, Das N, Kumar B, Rinu K, Trivedi P.. 2008. Phosphate solubilization by *Penicillium* spp. isolated from soil samples of Indian Himalayan region. World Journal of Microbiology and Biotechnology **24,** 97-102.
http://dx.doi.org/10.1007/s11274-007-9444-1

Pradhan N, Sukla L B. 2005. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. African Journal of Biotechnology **5,** 850-854.

Prescott LM, Harley JP, Klein DA. 2004. Microbiología 5 ed. McGraw-Hill Interamericana. 1240 p.

Reva ON, Dixelius C, Meijer J, Priest FG. 2004. Taxonomic characterization and plant colonizing abilities of some bacteria related to *Bacillus amyloliquefaciens* and *Bacillus subtilis*. FEMS microbiology ecology **48,** 249-259.
http://dx.doi.org/10.1016/j.femsec.2004.02.003

Reyes I, Bernier L, Antoun H. 2002. Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of *Penicillium rugulosum*. Microbial Ecology **44(1),** 39-48.

Reyes I, Bernier L, Simard RR, Antoun H.. 1999a. Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. Microbial Ecology **28,** 281-290.
http://dx.doi.org/10.1111/j.15746941.1999.tb00583.x

Richardson AE. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Australian Journal of Plant Physiology **28,** 897–906.

Rodríguez H, Fraga R. 1999. Phosphate Solubilizing Bacteria and their Role in Plant Growth Promotion. Biotechnology Advances **17,** 319-339.

Rodríguez H, Fraga R, Gonzalez T, Bashan Y. 2006. Genetics of phosphate solubilization and its potential application for improving plant growth-promoting bacteria. Plant and Soil **287,** 15–21.
http://dx.doi.org/10.1007/978-1-4020-5765-6_2

Rodriguez H, Gonzalez T, Goire I, Bashan Y. 2004. Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften **91,** 552–555.
http://dx.doi.org/10.1007/s00114-004-0566-0

Rodríguez N, Rubiano ME. 2002. Aislamiento e identificación de hongos de fosfato aislados de cultivos de arroz y evaluación del p H y en

concentraciones de sacarosa y cloruro de sodio sobre su actividad solubilizadora. Trabajo de Grado. Pontificia Universidad Javeriana. Bogotá.

Roos W, Luckner M. 1984. Relationships between proton extrusion and fluxes of ammonium ions and organic acids in *Penicillium cyclopium*. Journal of General Microbiology **130,** 1007-1014. http://dx.doi.org/10.1099/00221287-130-4-1007

Sánchez PA, Salinas JG. 1983. Suelos ácidos. Estrategias para su manejo con bajos insumos en América tropical. Sociedad Colombiana de las Ciencias del Suelo (Eds). Santafé de Bogota. Colombia. 93 p.

Scervino M, Prieto M, Ivana M, Recchi M, Sarmiento N, Godeas A. 2010. Soil fungal isolates produce different organic acid patterns involved in phosphate salts solubilization. Biology and Fertility of Soils **46,** 755–763. http://dx.doi.org/10.1007/s00374-010-0482-8

Shaw BD, Hoch HC. 2007. Ions regulate spore attachment, germination, and fungal growth. *In*: R. J. Howard and N. A. R. Gow (eds). Biology of the Fungal Cell, 2nd Edition The Mycota VIII Springer-Verlag Berlin Heidelberg.

Silvieira MLA, Alleonl LRF, Guilherme LRG. 2003. Biosolids and heavy metals in soils. Scientia Agricola **60(4),** 793-806.

Souchie EL, Azcón R, Barea JM, Saggin-Júnior OJ, Ribeiro-da Silva EM. 2006. Phosphate solubilization and synergism between P-solubilizing and arbuscular mycorrhizal fungi. Pesquisa Agropecuária Brasileira **41,** 1405-1411. http://dx.doi.org/10.1590/S0100204X2006000900009

Sridevi M, Mallaiah KV. 2009. Phosphate solubilization by *Rhizobium* strains. Indian Journal of Microbiology **49,** 98-102. http://dx.doi.org/10.1007/s12088-009-0005-1

Sutton J, Peng G. 1993. Biocontrol of Botrytis cinerea in strawberry leaves. Phytopathology **83,** 615-621.

Torres-Rubio MG, Valencia-Plata SA. Bernal-Castillo J, Martinez-Nieto P. 2000. Isolation of enterobacteria, *Azotobacter* sp. and *Pseudomonas* sp. Producers of indole-3-acetic acid and siderophores, from Colombian rice rhizosphere. Revista Latinoamericana de Microbiología **42,** 171–176.

Thomas GV, Shantaram MV, Saraswathy N. 1985. Occurrence and activity of phosphate-solubilizing fungi from coconut plantation soils. Plant Soil **87,** 357-364. http://dx.doi.org/10.1007/BF02181903

Trolove SN, Hedle YMJ, Kirk GJD, Bolan NS, Loganathan P. 2003. Progress in selected areas of rhizosphere research on P acquisition. Australian Journal of Soil Research **41,** 471– 499. http://dx.doi.org/10.1071/SR02130

Valencia H, Sánchez J, Valero N. 2005. Producción de ácido indolacético por microorganismos solubilizadores de fosfato presentes en la rizósfera de *Espeletia grandiflora* y *Calamagrostis effusa* del Páramo el Granizo. p. 177-193. En: Bonilla, M. (ed.). Estrategias adaptativas de plantas de páramo y del bosque altoandino en la cordillera oriental de Colombia. Unibiblos, Bogotá.

Valencia H, Sánchez J, Vera D, Valero N, Cepeda M. 2007. Microorganismos solubilizadores de fosfatos y bacterias fijadoras de nitrógeno en páramos y región cálida tropical (Colombia) p. 169-183. En: Sánchez, J. (ed.). Potencial biotecnológico de microorganismos en ecosistemas naturales y agroecosistemas. Universidad Nacional de Colombia, Bogotá.

Valero N. 2003. Potencial biofertilizante de bacterias diazotrofas y solubilizadoras de fosfatos asociadas al cultivo de arroz (*Oryza sativa* L.). [Tesis

de maestría] Maestría Interfacultades en Microbiología, Universidad Nacional de Colombia.

Valero N. 2007. Determinación del valor fertilizante de microorganismos solubilizadores de fosfato en cultivos de arroz. p. 169-183. En: Sánchez, J. (ed.). Potencial biotecnológico de microorganismos en ecosistemas naturales y agroecosistemas. Universidad Nacional de Colombia, Bogotá.

Vassilev N, Vassileva M, Fenice M, Federici F. 2001. Immobilized cell technology applied in solubilization of insoluble inorganic rock phosphates and P plant acquisition. Bioresource Technology **79,** 263– 271. http://dx.doi.org/10.1016/S0960-8524(01)00017-7

Venkateswardu B, Rao AV, Raina P. 1984. Evaluation of phosphorus solubilization by microorganisms isolated from Aridisols. Journal of the Indian Society of Soil Science **32,** 273-277.

Vera D, Perez H, Valencia H. 2002.Aislamiento de hongos solubilizadores de fosfatos de la rizosfera de Arazá (*Eugenia stipitata,* Myrtaceae). Acta Biologica Colombiana **7(1),** 33-40.

Vessey JK. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571-586.

Wakelin S, Warren A, Harvey R, Ryder H. 2004. Phosphate solubilization by *Penicillium* sp. closely associated with wheat roots. Biology and Fertility of Soils **40,** 36–43. http://dx.doi.org/10.1007/s00374-004-0750-6

Whitelaw MA. 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. Advances in Agronomy **69,** 99-151. http://dx.doi.org/10.1016/S0065-2113(08)60948-7

Useche Y. 2003. Contribución al conocimiento de bacterias y hongos solubilizadores de fosfato bajo tres usos de suelo en el sur del Trapecio Amazónico. Trabajo de Grado. Universidad Nacional de Colombia, Facultad de Ciencias. Departamento de Biología. Bogotá.

Xiao C, Chi R, He H, Qiu G, Wang D, Zhang W. 2009. Isolation of phosphate-solubilizing fungi from phosphate mines and their effect on wheat seedling growth. Applied Biochemistry and Biotechnology **159,** 330-342. http://dx.doi.org/10.1007/s12010-009-8590-3

Zambrano C. 1989. Efecto de la concentración de inóculo de *Trichoderma harzianum* sobre el desarrollo de *Macrophomina phaseolina*. p. 56. En: Resúmenes XI Seminario Nacional de Fitopatología. Sociedad Venezolana de Fitopatología. 19 al 23 de Noviembre 1989. Trujillo, Venezuela.

Zhang F, Zhu Z, Yang X. 2013. *Trichoderma harzianum* T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere. Applied Soil Ecology **72,** 41-48. http://dx.doi.org/10.1016/j.apsoil.2013.05.016

Zúñiga D, Hoyos R, Afanado L. 2010. Evaluación de plántulas de cardamomo (*Elettaria cardamomum* (L.) Maton) por su resistencia *in vitro* al filtrado de cultivo de *Fusarium oxysporum* Link. Vitae **17(2),** 155-164.