UNIVERSIDAD NACIONAL DE COLOMBIA

ACTA BIOLÓGICA COLOMBIANA
http://www.revistas.unal.edu.co/index.php/actabiol/index

ARTÍCULO DE INVESTIGACIÓN

In vitro DISSOLUTION OF ACIDULATED ROCK PHOSPHATE BY PHOSPHATE SOLUBILIZING MICROORGANISMS

Disolución *in vitro* de rocas fosfóricas aciduladas por microorganismos solubilizadores de fósforo

Ángela Patricia MORENO QUEVEDO[1], Nelson Walter OSORIO VEGA[1], Octavio Augusto GONZÁLEZ MURILLO[2].

[1] Universidad Nacional de Colombia, Calle 59A. n.° 63-20, of. 14-208. Medellín, Colombia.

[2] Sobiotech S.A.S. Km 25 Autopista Medellín–Bogotá. Guarne, Colombia

For correspondence. apmorenoq@unal.edu.co

Received: 20 March 2014; **Returned for revision:** 16 September 2014; **Accepted:** 24 October 2014.
Associate Editor: Francisco José Martínez Pérez.

Citation / Citar este artículo como: Moreno Quevedo AP, Osorio Vega NW, González Murillo OA. *In vitro* Dissolution of acidulated rock phosphate by phosphate solubilizing microorganisms. Acta biol. Colomb. 2015;20(2):65-71. doi: http://dx.doi.org/10.15446/abc.v20n2.42713

ABSTRACT

The low availability of phosphorus (P) in the soil and the high cost of P fertilization are factors that limit agricultural productivity. A biotechnological alternative for to handle this problem is to use soil microorganisms capable of dissolving rock phosphate (RP), thus improving its effectiveness as a P fertilizer. This study was carried out with the objective of determining the effectiveness of *Aspergillus niger* –As-, *Penicillium* sp. –Pn-, *Bacillus* sp –B-. and an unidentified actinomycete –At- in the *in vitro* dissolution of two partially acidulated rock phosphates. The treatments consisted of 2x16 factorial arrangement [2 levels of RP: either Boyaca RP or Norte de Santander RP; 16 levels of inoculum: an uninoculated control, individual inoculations (with As, Pn, B, At), dual inoculations (AsPn, AsB, AsAt, PnB, PnAt, BAt), triple inoculations (AsPnB, AsPnAt, AsBAt, PnBAt), and quadruple inoculation (AsPnBAt)]. Each treatment was replicated three times. It was found that the microbial effectiveness in the *in vitro* dissolution of RP depended on the type of RP, the composition of the inoculum used and the interaction of both factors. The best results were obtained with the Norte de Santander RP and *A. niger* used alone. When this fungus combined with the other microorganisms, its capacity to dissolve RP was significantly reduced.

Keywords: *Aspergillus niger, Bacillus* sp., biofertilizer, phosphate, *Penicillium* sp.

RESUMEN

La baja disponibilidad de fósforo (P) en el suelo y el costo de la fertilización fosfórica son limitantes para la productividad agrícola. Una alternativa biotecnológica para manejar este problema es mediante el uso de microorganismos del suelo capaces de disolver rocas fosfóricas (RP) y así mejorar su efectividad como fertilizante fosfórico. Con este fin se realizó un ensayo para determinar la efectividad microbial en la disolución *in vitro* de dos RP (Norte de Santander y Boyacá) parcialmente aciduladas. Los tratamientos consistieron en un arreglo factorial 2x16 [2 niveles de RP: Boyacá o Norte de Santander; 16 niveles de inóculo: Un control no inoculado, inóculos individuales (*Aspergillus niger* –As-, *Penicillium* sp. –Pn-, *Bacillus* sp. –B-, y un actinomiceto no identificado –At-), inóculos dobles (AsPn, AsB, AsAt, PnB, PnAt, BAt), inóculos triples (AsPnB, AsPnAt, AsBAt, PnBAt), e inóculos cuádruples (AsPnBAt)]. Cada tratamiento tuvo tres replicas. La efectividad en la disolución *in vitro* de RP fue dependiente del tipo de RP, tipo de inóculo y la interacción de ambos factores, teniendo mejores resultados con la RP del Norte de Santander y *A. niger* sólo. Cuando este hongo se combinó con otros microorganismos su capacidad para disolver RP se redujo significativamente.

Palabras clave: *Aspergillus niger*, *Bacillus* sp., biofertilizante, fosfato, *Penicillium* sp.

INTRODUCTION

Phosphorus (P) deficiency in tropical soils is a factor that limits plant productivity (Oberson *et al*., 2006; Cramer, 2010). For this reason, it is necessary to apply high doses of soluble fertilizers to reach the necessary concentrations of P in the soil solution (Narsian and Patel, 2000; Vassilev *et al.*, 2012). One problem of intensive agriculture is that the fertilizers applied

are expensive and their efficiency is very low (Vassilev and Vassileva, 2003; Paiva-Coutinho *et al*., 2012), making it unsustainable. Because of this, there is a pressing need to identify sustainable alternatives that may allow for a more efficient use of P for cultivated plants (Consensus Statement Declaration, 2011). Rock phosphate (RP) is a locally available and cheap alternative, but its reactivity is low due to its slow dissolution and release of P (Yusdar *et al*., 2007; Hamdali *et al*., 2010). On the other hand, it is known that the dissolution of RP improves when microorganisms capable of dissolving it are added to the system. This is the case with phosphate solubilizing microorganisms (PSM) (Sahu and Jana, 2000; Whitelaw, 2000; Galindo *et al*., 2011; Paiva-Coutinho *et al*., 2012). There have been various reports on the dissolution of RP with individual inoculates (of just one species), but little is known about the effectiveness of microbial consortia (Xiao *et al*., 2008; Oliveira *et al*., 2009; Saber *et al*., 2009; Singh and Reddy, 2011). Additionally, existing reports on the subject have focused on one particular type of RP, which has limited the results to very specific effects (Singh and Reddy, 2011; Habte and Osorio, 2012; Xuan Yu *et al*., 2012). Studies have also traditionally used non-acidulated RP, despite the fact that there are acidulated RPs available in the market whose solubility could potentially be further improved by inoculation with PSM.

The hypothesis of this investigation is that the microbial effectiveness in the *in vitro* dissolution of RP is controlled by the type of RP, the combination of PSM used as inoculates, and the interaction of these two factors. Therefore, the objective of this study was to determine the microbial effectiveness in the *in vitro* dissolution of two partially acidulated RPs (Norte de Santander and Boyaca) when inoculated (individually and combined microorganisms) with *Aspergillus niger*, *Penicillium* sp., *Bacillus* sp., and an unidentified actinomycete.

MATERIALS AND METHODS

The PSM (*Aspergillus niger* -As-, *Penicillium* sp. -Pn-, *Bacillus* sp. -B-, and an unidentified actinomycete -At-) were obtained from the Laboratory at the Sobiotech S.A.S. (6°15´07´´ N 75°25´42´´ W, altitude 2117 m) (Guarne, Antioquia, Colombia). For this study, the microorganisms were multiplied separately for three days at 28 °C in Petri dishes using the medium YMA whose composition (g/L) was: yeast extract 4, malt extract 10, glucose 4, agar 20. The medium was autoclaved at 120°C, 0.1 MPa, and 15 min. The colonies were removed from the surface of the agar with a sterilized loop and suspended in sterilized, deionized water and maintained in the refrigerator at 4 °C for later use.

Later, 1 mL of each suspension containing 10^6 colony forming units (CFU) per mL was aseptically transferred into 250 mL Erlenmeyer flask containing 75 mL of the selective medium, previously sterilized in an autoclave (120°C, 0.1 MPa, 15 min). The medium consisted of 10 g glucose, 1 g NH_4NO_3, 1 g NaCl, 0.2 g $CaCl_2.2H_2O$, 0.4 g $MgSO_4.7H_2O$ and 3.5 g RP per liter (Osorio and Habte, 2001). The RP used came from commercial mines in the country, specifically in the departments of Norte de Santander and Boyaca. These rocks were partially acidulated prior to use with concentrated sulfuric acid in the industry (Hammond *et al*., 1980). After that, the RP were passed through a 250 µm sieve and kept in a 500 µm sieve. The RP´s had a P content of 12 %, and upon preparing an aqueous suspension of 10 % the concentration of soluble P was 1.74 and 1.45 mg L^{-1}, respectively. The Erlenmeyer flasks were incubated at 28 °C in an orbital agitator at 100 rpm for seven days.

After the incubation period, 50 mL of the suspension was centrifuged at 3220 x g for 15 minutes. The supernatant was filtered through Number 42 filter paper. The pH of the solution was measured directly with a selective H^+ electrode. The concentration of P in the solution was determined using the molybdenum-blue method (Murphy and Riley, 1962). With the soluble P data obtained from this process, the RP dissolution effectiveness factor (RPDE) was calculated by dividing the concentration of soluble P by the quantity of total P in the RP and expressing this as a percentage [RPDE (%) = (soluble P/P in the RP) x 100].

The biomass of the microorganisms inoculated was collected through centrifugation, washed with distilled water and dried in an oven at 70°C for 12 hours. Microbial growth was expressed as dry microbial material produced per Erlenmeyer and was determined as weight lost after incineration at 500°C for five hours. This method was selected in order to avoid overestimation of weight due to the adherence of the microorganisms to the RP (Reyes *et al.,* 1999; Habte and Osorio 2012; Mendes *et al*., 2014).

A completely randomized experimental design was employed, the treatments consisted of 2x16 factorial arrangement [two levels of RP: either Boyaca RP or Norte de Santander RP; 16 levels of inoculum: an uninoculated control, individual inoculations (with As, Pn, B, At), dual inoculations (AsPn, AsB, AsAt, PnB, PnAt, BAt), triple inoculations (AsPnB, AsPnAt, AsBAt, PnBAt), and quadruple inoculation (AsPnBAt)]. Each treatment was replicated three times.

The data obtained was statistically analyzed using analysis of variance and the Duncan multiple range test to determine the separation of media. The level of significance (p) was ≤ 0.05. For all cases, the statistical analysis was performed using the statistical program Statgraphics Centurion XV (StatPoint, Inc., Herdon, Virginia, EE.UU.).

RESULTS

Microbial effectiveness in the *in vitro* dissolution of partially acidulated RP was affected by the treatment. The pH of the medium was significantly reduced (p<0.05) by both individual and combined inoculation (As: *A. niger*, At: actinomycete; B: *Bacillus* sp.; Pn: *Penicillium* sp.) (Fig. 1). The control treatment (uninoculated) had a pH of 5.09, which

was significantly greater than the pH of all of the inoculated samples. Significantly lower pH values were obtained when the medium was inoculated with As alone (3.58) or in combination with other microorganisms (3.63 – 3.74). Inoculation with the *Bacillus* sp. (B) resulted in the highest pH value (4.53) of the inoculated treatments (Fig. 1). On the other hand, neither the type of RP nor the interaction of RP x microorganism significantly affected the pH of the medium.

The concentration of soluble P was significantly affected by the treatments (RP x inoculum). The concentration of P was significantly greater (p<0.05) when the Norte de Santander RP was used (P: 42.3 mg L^{-1}) than with the Boyaca RP (P: 35.2 mg L^{-1}) (Fig. 2), independent of the effect of inoculation with the microorganisms.

The concentration of soluble P (mg L^{-1}) in the control treatment (uninoculated) was 17 mg L^{-1}. After inoculation with *A. niger* alone (As), this significantly increased to 93.5 mg L^{-1}, which represents a 5.5-fold increase. This was the highest value detected for this variable (Fig. 2). Inoculation with As in combination with the other microorganisms presented soluble P values that fluctuated between 39.5 and 76.5 mg L^{-1} (significant differences were found among these). These were significantly greater than the control, but lower than with inoculation with as alone. Inoculation with other microorganisms did not produce significant differences from the uninoculated control (Pn: 20.7 mg L^{-1}, B: 12.8 mg L^{-1}, At: 7.5 mg L^{-1}). Combined inoculation with these last two microorganisms did not differ from the control either.

In regards to the interaction of RP type and the type of microorganism(s) used as inoculates, there were significant differences found in the concentration of soluble P (mg L^{-1}) in some cases. For example, with the Boyaca RP, the best results were obtained with the B, PnxB, and PnxAt inoculations. For the Norte de Santander RP, the best results were seen with Pn, AsxPn, AsxAt, AsxPnxB, AsxPnxAt, and AsxBxAt. With the rest of the inoculates, there were no significant differences according to the type of RP used (Fig. 2).

Upon comparing the variables of soluble P concentration and the pH of the medium corresponding to each type of microorganism(s) used as inoculates, an inverse relationship was found between the variables. That is to say, at lower pH values, there was a greater concentration of soluble P. The lowest pH and the highest concentration of soluble P occurred when the medium was inoculated with just As. This was followed by the group of treatments that included As in the inoculation in combination with other microorganisms. Inoculation with the microorganisms Pn, B, At and combinations of the three did not show differences with the control in regards to the concentration of soluble P, despite a decrease in pH (~4.0) (Fig. 3).

On the other hand, the microbial dry mass (mg) was significantly different depending on the type of RP used; a better result was obtained with the Boyaca RP (27.8 mg)

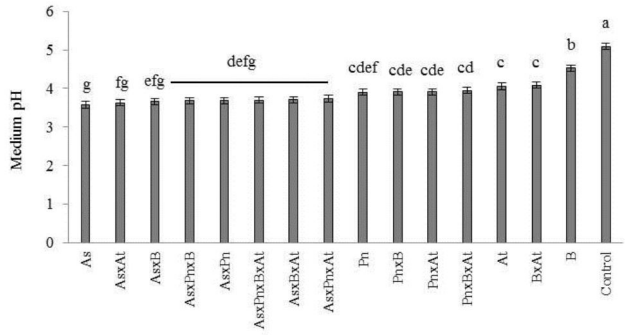

Fig. 1. Medium pH as a function of individual and combined inoculation (As: *A. niger*, At: actinomycete; B: *Bacillus* sp.; Pn: *Penicillium* sp.). The bars represent standard error. The columns with different letters showed significant differences among them, according to the Duncan multiple range test ($p \leq 0.05$).

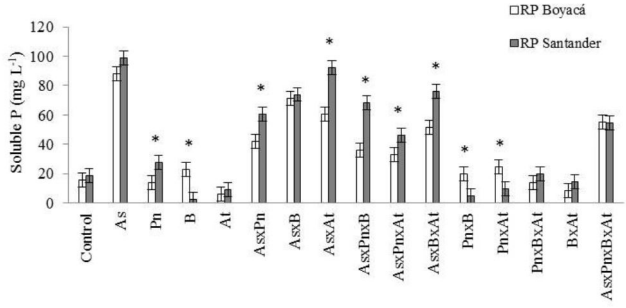

Fig. 2. Concentration of soluble P (mg L^{-1}) as a function of the type of RP used and the individual and combined inoculations (As: *A. niger*, At: actinomycete; B: *Bacillus* sp.; Pn: *Penicillium* sp.). The bars represent standard error. The columns with the asterisk (*) indicate significant differences in this concentration between the different RP, based on the inoculation given (minimum significant difference according to Fisher, $p \leq 0.05$).

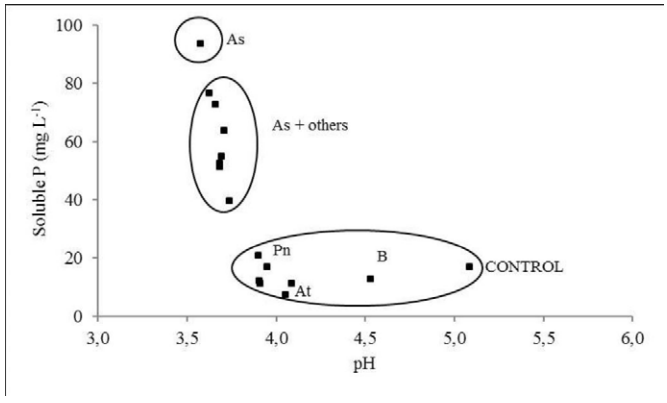

Fig. 3. Relationship between the concentration of soluble P and the medium pH. The letters indicate the type of microorganism(s) employed as inoculates (As: *A. niger*, At: actinomycete; B: *Bacillus* sp.; Pn: *Penicillium* sp.).

than with the Norte de Santander RP (23.2 mg), independent of the type of microorganism used as an inoculate (Fig. 4).

The microbial dry mass was also affected by the type of microorganism applied to the medium. Upon examining the dry mass for each of the individual inoculates, the following order was found: As 36.1 mg > Pn 21.0 mg > At 14.7 mg > B 9.0 mg. In general, the treatments that included As in combination with other inoculates had a greater mass (25.3 – 38.3 mg), while the other combined treatments resulted in a smaller mass (Fig. 5). The results indicate that the interaction of type of RP with type of inoculate did not have a signficant effect on the variable of microbial dry mass.

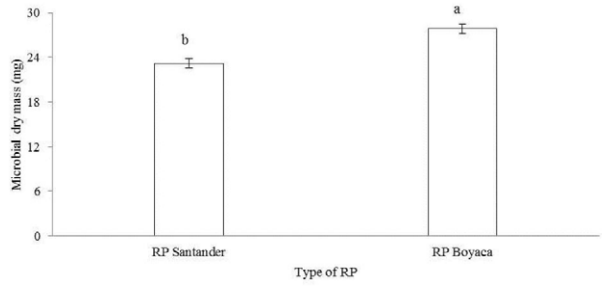

Fig. 4. Microbial dry mass (mg) as a function of the type of RP used. The bars represent standard error. The columns with different letters showed significant differences among them based on the Duncan multiple range test ($p \leq 0.05$).

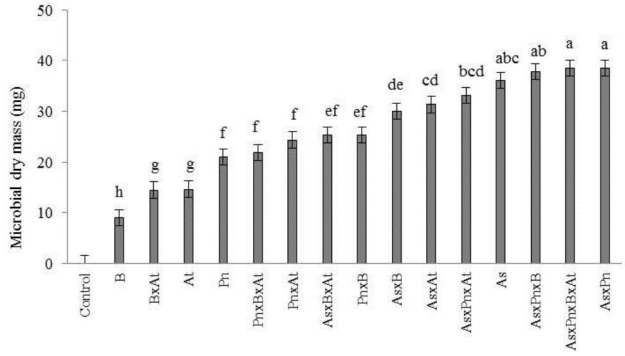

Fig. 5. Microbial dry mass (mg) as a function of individual and combined inoculation (As: *A. niger*, At: actinomycete; B: *Bacillus* sp.; Pn: *Penicillium* sp.). The bars represent standard error. The columns with different letters showed significant differences between them based on the Duncan multiple range test ($p \leq 0.05$).

DISCUSSION

The results obtained allowed us to clearly demonstrate the hypothesis that microbial effectiveness in dissolving partially acidulated RP depends on the type of inoculate used, the type of RP and the interaction of both factors. The dissolution of RP by microorganisms through acidification has been well documented. This acidification is caused by the extrusion of protons associated with the assimilation of ammonium or the production of organic acids (Kang *et al*., 2008; Sharan *et al*., 2008; Ben Farhat *et al*., 2009; Oliveira *et al*., 2009; Habte and Osorio 2012; Selvakujmar *et al*., 2013). Evidence of these mechanisms in the present study is seen in the decrease in pH from an initial value of 5.09 to 3.58 with the use of *A. niger*. In regards to this fungus, there are authors who speak of the production of organic acids, particularly citric, oxalic and gluconic acids, as its dissolution mechanism (Sperber, 1958; Illmer and Schinner, 1995; Reyes *et al*., 1999; Seshadri *et al*., 2004).

Narsian and Patel (2000), Reddy *et al*., (2002), Barroso and Nahas (2005) and Mittal *et al*., (2008) reported that relative efficiency in the dissolution of RP depends on the type of RP and the type of microorganism, as well as the quantity and quality of the acid secreted into the medium. Chuang *et al*., (2007) report that, regarding *A. niger*, when Ca-P was used as the source of P, they detected gluconic acid; when the phosphoric source was Fe-P or Al-P, they detected oxalic acid. Goenadi *et al*., (2000) and Bojinova *et al*., (2008), meanwhile, detected principally citric acid, among other acids.

When the RPDE of the individual inoculates was examined, the following order was found among the microorganisms evaluated: As 21.9 % > Pn 4.8 % > B 3.0 % > At 1.8 %. In the uninoculated control, the RPDE was 4.0 %. Based on this, it is worth highlighting the high effectiveness of *A. niger* in dissolving RP. These results corroborated those obtained by Achal *et al.,* (2007) and Mittal *et al.,* (2008), who found that *Aspergillus* spp. increased soluble P by dissolving tricalcium phosphate and RP (4.7 y 248 mg L^{-1}, respectively). Similarly, Saber *et al.,* (2009) reported that the maximum *in vitro* dissolution of RP was obtained with *A. niger* (67 mg L^{-1}), followed by *Penicillium* spp. (46.2 mg L^{-1}). In contrast, Jayasinghearachchi and Seneviratne (2006) found that *Penicillium* spp. produced significantly greater RP dissolution than *Aspergillus* spp., which does not agree with our results, likely due to differences in microbial species and laboratory conditions (shaking, type of RP, time of incubation, among others).

On the other hand, Reyes *et al.,* (1999) and Yadav and Tarafdar (2003) found a negative correlation between microbial dry mass and the concentration of soluble P obtained from the dissolution of RP. This coincides with the results obtained in the present study, since the RP with the greatest dissolution (Norte de Santander RP) generated a lower microbial dry mass than that found with the Boyaca RP. These authors suggest the presence of an H^+ pumping mechanism in the dissolution of small quantities of RP, which allows for improved biomass development. They also suggest that the microbial isolates with a lower biomass can transfer carbon in order to produce a higher quantity and diversity of organic acids that dissolve more RP, which leads to a drainage of C and could explain the lower biomass (Mathews *et al*., 2000).

It is clear that combining a microorganism that is efficient at dissolving RP, such as *A. niger*, with other less efficient or inefficient microorganisms (Pn, B and At) significantly reduces the microorganism's capacity to dissolve RP, with respect to its individual effect.

It is possible that the lower concentration of soluble P is due to (i) competition for the C source (glucose) and other nutrients (p.e., Ca, Mg, Fe, etc.) and/or (ii) that the other microorganisms are consuming the P that has been released by *A. niger*. Regardless of what the reason is, it is evident that the potential use of these co-inoculations in the presence of cultivated plants is not ideal, given that they produce less soluble P. It is preferable to use the most efficient microorganism by itself. This result could be further investigated in the future in order to test this conclusion.

The fact that the RP used in this study was acidulated is very important. The uninoculated controls presented soluble P values of 17 mg L^{-1}, while in other studies using the same methods, the values have been ~1 mg L^{-1} (Osorio and Habte, 2001). The acidulation of RP has been proposed as a way of improving the effectiveness of RP as a fertilizer (FAO, 2007). Our results suggest that the RP solubilizing microorganisms can further accelerate the dissolution of this material, thus improving its capacity to release P for cultivated plants. In the review performed of the relevant literature, and in other recent reviews (Daza, 2010; Londoño, 2010; Habte and Osorio, 2012), no reports were found that studied the microbial dissolution of previously acidulated RP; for this reason, we believe this is the first study performed.

Future investigations should center on the current sustainability of agricultural systems, focusing particularly on the RPDE of *A. niger* under field conditions and in combination with viable, simple and easily applied biotechnological alternatives, such as micorrhyzal fungi that positively impact the interaction of plant and soil in different plant species.

CONCLUSIONS

The microbial effectiveness in dissolving partially acidulated RP depends on the type of inoculum used, the type of RP and the interaction between the two factors. In general, *Aspergillus niger* was more effective in dissolving partially acidulated RP. The coinoculation of *A. niger* and other microorganisms evaluated did not generate better benefits in dissolving partially acidulated RP. The use of phosphate solubilizing microorganisms can further accelerate the dissolution of partially acidulated RP.

ACKNOWLEDGEMENTS

We would like to thank the Universidad Nacional de Colombia and Soluciones Biotecnológicas y Agroambientales (Sobiotech) for the support they provided during our investigation. We thank the English translation and grammar corrections made by Marguerite Cawley.

REFERENCES

Achal V, Savant VV, Reddy MS. Phosphate solubilization by a wild type strain and UV-induced mutants of *Aspergillus tubingensis*. Soil Biol Biochem. 2007;39:695-699. Doi: http://dx.doi.org/10.1016/j.soilbio.2006.09.003

Barroso CB, Nahas E. The status of soil phosphate fractions and the ability of fungi to dissolve hardly soluble phosphates. Appl Soil Ecol. 2005;29:73-83. Doi: http://dx.doi.org/10.1016/j.apsoil.2004.09.005

Ben-Farhat M, Jordan MJ, Chaouech-Hamada R, Landoulsi A, Sotomayor JA. Variations in essential oil, phenolic compounds, and antioxidant activity of Tunisian cultivated *Salvia officinalis* L. J Agr od Chem. 2009;57:10349-10356. Doi: http://dx.doi.org/10.1021/jf901877x

Bojinova D, Velkova R, Ivanova R. Solubilization of Morocco phosphorite by *Aspergillus niger*. Bioresource Technol. 2008;99:7348-7353. Doi: http://dx.doi.org/10.1016/j.biortech.2007.08.047

Chuang CC, Kuo YL, Chao CC, Chao WL. Solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger*. Biol Fertil Soils. 2007;43:575-584. Doi: http://dx.doi.org/10.1007/s00374-006-0140-3

Consensus Statement Declaration. Sustainable phosphorus summit. Tempe, Arizona, USA: ASU School of Life Sciences; 2011. 9 p.

Cramer MD. Phosphate as a limiting resource: introduction. Plant Soil. 2010;334:1-10. Doi: http://dx.doi.org/10.1007/s11104-010-0497-9

Daza PC. Aislamiento de microorganismos de la rizosfera de *Leucaena leucocephala* y evaluación de su uso potencial en la rehabilitación de suelos degradados [tesis de maestría]. Medellín: Facultad de Ciencias, Universidad Nacional de Colombia; 2010. 50 p.

FAO. Utilización de las rocas fosfóricas para una agricultura sostenible. Boletin FAO fertilizantes y nutrición vegetal No. 13. Roma; 2007. 177 p.

Galindo T, Sánchez J, Polanía J. Capacidad solubilizadora de fosfatos de microorganismos rizosféricos asociados a dos manglares del Caribe colombiano. Acta biol. Colomb. 2011;11(1):153-154.

Goenadi DH, Siswanto, Sugiaro Y. Bioactivation of poorly soluble phosphate rocks with a phosphorus-solubilizing fungus. Soil Sci Soc Am J. 2000;64:927-932. Doi: http://dx.doi.org/10.2136/sssaj2000.643927x

Habte M, Osorio NW. Effect of Nitrogen Form on the Effectiveness of a phosphate-solubilizing fungus to dissolve rock phosphate. J Biofertil Biopestici. 2012;3:1-4. Doi: http://dx.doi.org/10.4172/2155-6202.1000127

Hamdali H, Smirnov A, Esnault C, Ouhdouch Y, Virolle MJ. Physiological studies and comparative analysis of rock phosphate solubilization abilities of actinomycetales originating from Moroccan phosphate mines and of *Streptomyces lividans*. Appl Soil Ecol. 2010;44:24-31. Doi: http://dx.doi.org/10.1016/j.apsoil.2009.09.001

Hammond LL, Chien SH, Polo JR. Phosphorus availability from partial acidulation of two phosphate rocks. Fert Res. 1980;1(1):37-49. Doi: http://dx.doi.org/10.1007/BF01073563

Illmer P, Barbato A, Schiner F. Solubilization of hardly soluble AlPO4 with P-solubilizing microorganisms. Soil Biol Biochem. 1995;27:265-270. Doi: http://dx.doi.org/10.1016/0038-0717(94)00205-F

Jayasinghearachchi HS, Seneviratne G. Fungal solubilization of rock phosphate is enhanced by forming fungal-rhizobial biofilms. Soil Biol Biochem. 2006;38:405-408. Doi: http://dx.doi.org/10.1016/j.soilbio.2005.06.004

Kang SC, Pandey P, Khillon R, Maheshwari DK. Process of rock phosphate solubilization by *Aspergillus* sp. PS 104 in soil amended medium. J Environ Biol. 2008;29:743-746. Doi: http://www.ncbi.nlm.nih.gov/pubmed/19295075

Londoño AA. 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 [tesis de maestría]. Medellín: Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia; 2010. 74 p.

Mathews CK, Van-Holde KE, Ahern KG. Biochemistry. 3rd edition. Benjamin/Cummings. San Francisco; 2000. 1186 p.

Mendes GO, Moreira De Freitas AL, Pereira OL, Da Silva IR, Vassilev NB, Costa M D. Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. Ann Microbiol. 2014;64(1): 239-249. Doi: http://dx.doi.org/10.1007/s13213-013-0656-3

Mittal V, Singh O, Nayyar H, Kaur J, Tewari R. Stimulatory effect of phosphate-solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield. Soil Biol Biochem. 2008;40:718-727. Doi: *http://dx.doi.org/10.1016/j.soilbio.2007.10.008*

Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 1962;27:31-36. Doi: http://dx.doi.org/10.1016/S0003-2670(00)88444-5

Narsian V, Patel HH. *Aspergillus aculeatus* as a rock phosphate solubilizer. Soil Biol Biochem. 2000;32:559–565. Doi: http://dx.doi.org/10.1016/S0038-0717(99)00184-4

Oberson A, Bunemann EK, Friesen DK, Rao IM, Smithson PC. Improving phosphorus fertility in tropical soils through biological interventions. In: Uphoff N, editor. Biological approaches to sustainable soil systems. Taylor and Francis Group. Boca Raton, Florida: CRC Press,; 2006. p. 531-546.

Oliveira CA, Alves VMC, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, et al. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol Biochem. 2009;41(9):1782-1787. Doi: http://dx.doi.org/10.1016/j.soilbio.2008.01.012

Osorio NW, Habte M. Synergistic influence of an arbuscular mycorrhizal fungus and P solubilizing fungus on growth and plant P uptake of *Leucaena leucocephala* in an Oxisol. Arid Land Res Mgmt. 2001;15:263-274. Doi: http://dx.doi.org/10.1080/15324980152119810

Paiva-Coutinho F, Wagner-Pereira F, Mayumi A, Yano-Melo. Solubilization of phosphates *in vitro* by *Aspergillus* spp. and *Penicillium* spp. Ecol Eng. 2012;42:85-89. Doi: http://dx.doi.org/10.1016/j.ecoleng.2012.02.002

Reddy MS, Kumar S, Babita K. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus niger*. Bioresource Technol. 2002;84:187-189. Doi: http://dx.doi.org/10.1016/S0960-8524(02)00040-8

Reyes I, Bernier L, Simard RR, Antoun H. Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV- induced mutants. FEMS Microbiol Ecol. 1999;28:291-295. Doi: http://dx.doi.org/10.1016/S0168-6496(98)00119-6

Saber WIA, Ghanem KM, El-Hersh MS. Rock phosphate solubilization by two isolates of *Aspergillus niger* and *Penicillium* sp. and their promotion to mung bean plants. Res J Microbiol. 2009;4:235-250. Doi: http://dx.doi.org/10.3923/jm.2009.235.250

Sahu SN, Jana BB. Enhancement of the fertilizer value of rock phosphate engineered through phosphate solubilizing bacteria. Ecol Eng. 2000;15:27-39. Doi: http://dx.doi.org/10.1016/S0925-8574(99)00013-0

Selvakumar G, Joshi P, Suyal P, Kumar-Mishra P, Krishna-Joshi G, Venugopalan R, et al. Rock phosphate solubilization by psychrotolerant *Pseudomonas* spp. and their effect on lentil growth and nutrient uptake under polyhouse conditions. Ann Microbiol. 2013. Doi: http://dx.doi.org/10.1007/s13213-012-0594-5

Seshadri S, Ignacimuthu S, Lakshminarasimhan C. Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different *Aspergillus niger* strains. Chem Eng Commun. 2004;191:1-10. Doi: http://dx.doi.org/10.1080/00986440490276308

Sharan A, Shikha, Darmwaln S, Gaur S. *Xanthomonas campestris*, a novel stress tolerant, phosphate-solubilizing bacterial strain from saline-alkali soils. World J Microbiol. Biotechnol. 2008;24:753-759. Doi: http://dx.doi.org/10.1007/s11274-007-9535-z

Singh H, Reddy MS. Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils. Eur J Soil Biol. 2011;47:30-34. Doi: http://dx.doi.org/10.1016/j.ejsobi.2010.10.005

Sperber JI. Solution of apatite by soil microorganisms producing organic acids. Aust J Agr Res. 1958;9:778-781. Doi: http://dx.doi.org/10.1071/AR9580782

Vassilev N, Eichler-Löbermann B, Vassileva M. Stress-

tolerant P-solubilizing microorganisms. Appl Microbiol Biotechnol. 2012;95:851-859. Doi: http://dx.doi.org/10.1007/s00253-012-4224-8

Vassilev N, Vassileva M. Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. Appl Microbiol Biot. 2003;61:435-440. Doi: http://dx.doi.org/10.1007/s00253-003-1318-3

Whitelaw MA. Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv Agron. 2000;69:99-151. Doi: http://dx.doi.org/10.1016/S0065-2113(08)60948-7

Xiao CQ, Chi RA, Huang XH, Zhang WX, Qiu GZ, Wang DZ. Optimization for rock phosphate solubilization by phosphate-solubilizing fungi isolated from phosphate mines. Ecol Eng. 2008;33:187-193. Doi: http://dx.doi.org/10.1016/j.ecoleng.2008.04.001

Xuan Y, Xu L, Tian-Hui Z, Guang-Hai L, Cui M. Co-inoculation with phosphate-solubilizing and nitrogen-fixing bacteria on solubilization of rock phosphate and their effect on growth promotion and nutrient uptake by walnut. Eur J Soil Biol. 2012;50:112-117. Doi: http://dx.doi.org/10.1016/j.ejsobi.2012.01.004

Yadav RS, Tarafdar JC. Phytase and phosphatase producing fungi in arid and semi-arid soils and their efficiency in hydrolyzing different organic P compounds. Soil Biol Biochem. 2003;35:1-7. Doi: http://dx.doi.org/10.1016/S0038-0717(03)00089-0

Yusdar H, Anuar AR, Hanafi M, Azizah H. Analysis of phosphate rock dissolution determining factors using principal component analysis in some acid Indonesian soils. Commun Soil Sci Plant Anal. 2007;38:273-282. Doi: http://dx.doi.org/10.1080/00103620601094239