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Screening phosphate solubilizing actinobacteria isolated from the rhizosphere of wild plants from the Eastern Cordillera of the Colombian Andes

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Colombia is a tropical country with high diversity and an agricultural economy, yet their soils are characterized by low pHs and poor phosphorus concentrations. Soil supplementation with chemical fertilizers containing soluble phosphorus is a costly and contaminating practice and for this reason, the aim of this study was to isolate actinobacteria that are able to release soluble phosphate from wild plants of the Eastern Cordillera of Colombia and select strains with high phosphorus solubilizing activity. To screen the isolates of actinobacteria, we used two qualitative assays to determine the efficiency of solubilization by measuring the halo of hydrolysis in a Pikovskaya's agar plate (PVK). A second assay was performed on broth with National Botanical Research Institute's phosphate growth (NBRIP) medium containing bromophenol blue (BPB). Finally, the released soluble phosphate by actinobacteria was quantified using insoluble $Ca_3(PO_4)_2$ or $AlPO_4$ as sole sources of P. Only five of the tested strains were the best solubilizing strains in the two qualitative assays. The strains T1C, T1H, T3A, T3C, P3E, F1A, F2A and V2B solubilized significantly more phosphorus than the other strains, which was shown for the quantitative assay. Strains T1C, T3A, T3C and F1A are candidates for future studies and to evaluate other plant growth promoting activities.

Key words: Screening, phosphate solubilizing, actinobacteria, Colombia.

INTRODUCTION

Colombia is one of the five countries in the world with the largest diversity of genes, species, and ecosystems. The Colombian Andes are located in one of the world biodiversity hotspots and the savannahs of the Llanos are listed as one of the world eco-regions with rare, rich and biologically important habitats (Myers et al., 2000; Herzog et al., 2011). Soils within these areas are characterized by low pHs and low phosphorus concentrations (Rao et al., 2004; Fassbender and Bornemisza, 1994; Malagon et al., 2003). Soil phosphorus dynamics is characterized by physicochemical (sorption-desorption) and biological (immobilization-mineralization) processes. Phosphate anions can be immobilized by precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} providing a high phosphorus fixation capacity to soils (Oliveira et al., 2009; Khan et al., 2009). In Colombia's agricultural tradition, to prepare soils by adding manure, chemical fertilizers or organic amendments, any of them is supplemented with phosphate in order to compensate the phosphorus deficiency is a common practice (León, 1991; Guimaraeset

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et al., 2001). Soil supplementation with chemical fertilizers containing soluble phosphorus is thus a costly and conta-minating practice, not only because of their way of use but because of the highly polluting mode in which they are industrially produced, requiring the use of sulphuric acid at high temperatures. Furthermore, wrong fertilizer applications can cause problems of eutrophication and erosion (Whitelaw, 2000; Vassilev et al., 2006). Many studies have been done on phosphate solubilizing micro-organisms (PSM) as an alternative in the prevention of environmental and agricultural issues mentioned above (Rodriguez and Fraga, 1999; Krishnaraj and Goldstein, 2001; Kumar et al,. 2010; Bhattacharyya and Jha, 2012).

There are reports on studies with microorganisms capable of solubilizing phosphate, especially bacteria and some fungi, which have experimentally demonstrated their capacity to improve phosphorus availability to plants in laboratory, greenhouse and field experiments (Rudresh et al., 2005; Deubel et al., 2005; Pandey et al., 2008; Hamdali et al., 2012). To a lesser extent, actinobacteria have been reported as microorganisms with the capacity to release phosphorus into the soil (Mba, 1994, 1997; Hamdali et al., 2008, El-Tarabily et al., 2006). Actinobacteria have special interest because these filamentous sporulating bacteria are able to thrive in extremely different soils, play important ecological roles in soil nutrient cycling and are recently being regarded as plant growth promoting rhizobacteria (Jiang et al., 2005; Pathom-Aree et al., 2006; Franco-Correa et al., 2010). The selection of PSM is carried out by rapid qualitative methods. The most common method is to determine the efficiency of solubilization by measuring the halo of hydrolysis in a Pikovskaya's agar plate (1948), but results are not always consistent and in some cases this method is insufficient to detect all the PSM (Nautiyal 1999; Mehta and Nautiyal, 2001; Rashid et al., 2004). Mehta and Nautiyal (2001) developed a system that includes a broth - the National Botanical Research Institute's phosphate growth medium (NBRIP) medium and a qualitative assay in liquid medium, which is more accurate and reliable in the selection of PSM and therefore makes the screening process more quick, efficient and with low cost (Khan et al., 2009).

The aim of this study was to isolate actinobacteria able to release soluble phosphate from wild plants of the Eastern Cordillera of Colombia and to select strains with high phosphorus solubilizing activity with the purpose of suggesting those microorganisms as potential biofertilizers and hence help to use less chemical fertilizers in agricultural practices.

MATERIALS AND METHODS

Soil samples and isolation of Actinobacteria

Soil samples were collected from various localities from the Eastern Cordillera between 2010 and 2011. Diverse habitats at different altitudes were selected for the isolation of actinobacteria strains (Figure 1). These habitats included the rhizospheres of wild plants growing in natural protected areas and forests; samples were taken from forest species associated with *Vallea*, *Weinmannia*, *Vaccinium*, *Drimys*, *Rosmarinus*, plus samples from legumes grasses (*Rye Grass* or *Bromus*) and clovers (*Trifolium repens* and *Trifoliumpratense*). Soil pH, total P, available P, and organic matter were characterized (Table 1). The samples were taken at a depth of 15-30 cm and were placed in polyethylene bags, closed tightly and stored in a refrigerator at 4°C. Actinobacteria were isolated by dilution plate method using oat-meal agar. These media were supplemented with nistatin (0.1 % V/V). Plates were incubated at 26°C, and monitored daily. Subcultures led to purified bacterial colonies that's howed an actinobacteria-like appearance. Gram staining indicated that they were Gram positive mycelia sporulating bacteria. A classical approximation was used for the characterization like aerial mass colour, reverse side pigments, melanoid pigments, soluble pigments. We did determination of micro morpho-logical characteristics of the spore-bearing hyphae, the number of spores at the end of mature hyphae and the spore chain Morphology. The strains were stored in 20% sterile glycerol at -20°C.

Screening of phosphate-solubilizing actinobacteria

The solid plate assay was done to measure the halo zone formed surrounding the bacteria after being inoculated on agar and incubated for 72 h at 26°C on PVK growth medium containing 5 g of tricalcium phosphate as sole phosphorus source and pH 7.2 (Pikovskaya 1948). Halo-forming colonies were recorded as positive. The solubilization index was calculated as the ratio between the total diameter (colony + halo) and the colony diameter (Kumar and Narula, 1999). A second assay was performed on broth with NBRIP medium containing BPB following the protocol of Mehta and Nautiyal (2001). In brief, 5 ml of NBRIP-BPB medium in a 30-ml test tube was inoculated with the actinobacterial strain (50 ml inoculum with approximately 3 X 10^7cfu/ml), and the isolates were grown for 4 days at 26°C with continuous agitation at 120 rpm. At the end of the incubation period, the final OD-600 values were subtracted from the initial values. The medium pH was measured by immersing a glass electrode into the culture broth.

Release soluble phosphate

Solubilization of P by Actinobacteria was quantified using insoluble 5 g/L of $Ca_3(PO_4)_2$ or 1 g/L of $AlPO_4$ as sole sources of P in the NBRIP broth medium. In each autoclaved flask, 2 ml of bacterial suspension (approximately 3 X 10^7cfu/ml) were transferred to 100 ml Erlenmeyer flask containing 20 ml of NBRIP broth medium. The actinobacteria cultures were placed on a rotary shaker at 120 rpm for 4 days and pH was measured after incubation with a pH meter. Suspensions were centrifuged to remove bacterial cells and other insoluble materials. The resulting supernatants were passed through a 0.45 µm filter and the inorganic phosphate content of the culture filtrate was determined by the molybdenum blue method (Murphy and Riley, 1962). The available phosphorous was determined using a spectrophotometer at 880 nm and calibrated with a standard KH_2PO_4 curve.

Statistical analysis

All of the experiments were performed with four replicates. The data were analyzed with a One Way Analysis of Variance (ANOVA) and a Duncan's multiple range test to determine any significant differences between groups at $p < 0.05$. All the statistical analyses were performed using the SPSS 11.0 for Windows® software.

location (number of isolates)	Temperature and humidity	coordinates N	coordinates W	Elevation m.s.n.m
TOTA (17)	18ºC y73%	5° 30`23.7``	72° 57`58.1``	3042
	18ºC y69%	5° 30`24.2``	72° 57`58.4``	3033
	20ºC y50%	5° 30`39.4``	72° 57`58.9``	3032
	15ºC y70%	5° 30`28.3``	72 58`58.2``	3031
	21ºC y58%	5° 30`24.5``	72° 57`56.0``	3031
PAIPA (15)	15ºC y86%	5° 47`57.3``	73° 0.7`49.7``	2882
	14ºC y86%	5° 47`52.1``	73° 0.7`40.7``	2845
	17ºC y82%	5° 47`36.6``	73° 0.7`45.2``	2663
	17ºC y84%	5° 44`39.9``	73° 0.5`58.8``	2634
	18ºC y82%	5° 44`20.9``	73° 0.4`17.9``	2543
	15ºC y84%	5° 45`31.2``	73° 0.6`40.7``	2534
FUSAGASUGA (9)	26ºC y56%	4° 22`46.4``	74° 18`11.1``	2355
	26ºC y60%	4° 22`49.4``	74° 18`31.7``	2214
	25ºC y62%	4° 22`51.8``	74° 18`33.9``	2155
	27ºC y55%	4° 22`10.6``	74° 21`41.3``	2060
	27ºC y88%	4° 21`37.3``	74° 21`13.2``	1844
LA VEGA (6)	24ºC y83%	5° 01`44.8``	74°19`38.0``	1466
	23ºC y77%	5° 01`46.9``	74°19`37.0``	1369
	24ºC y76%	5° 01`45.3``	74°19`37.6``	1367
	23ºC y79%	5° 01`49.5``	74°19`41.3``	1341
	24ºC y78%	5° 01`47.9``	74°19`42.7``	1329
VILLETA (8)	30ºC y54%	5° 01`42.5``	74°28`33.0``	1194
	31ºC y49%	5° 01`09.5``	74°28`31.9``	939
	29ºC y51%	5° 01`04.3``	74°28`27.4``	900
	26ºC y68%	5° 01`0.35``	74°28`26.0``	889
MANÍ (2)	27ºC y88%	4° 53`39.1``	72° 21`41.2``	215
	27ºC y86%	4° 53`49.1``	72° 21`24.0``	213
	29ºC y87%	4° 54`07.0``	72° 21`22.0``	211
	28ºC y88%	4° 54`21.8``	72° 21`05.5``	210
	28ºC y87%	4° 53`50.1``	72° 21`39.3``	210
	26ºC y92%	4° 54`14.8``	72° 21`15.2``	208

Figure 1. Map of Colombia with coordinates, altitude, humidity and temperature of the localities sampled.

Table 1. Characteristics of the soil from the different regions in Colombia.

Region	Soil pH	Total P (mg/kg)	Available P (mg/kg)	Organic matter %
Tota*	5.06 ± 0.3	360 ± 283.6	118.4 ± 126.5	13 ± 16.9
Paipa*	5.52 ± 1.0	614 ± 311.1	8 ± 2.6	5.96 ± 5.3
Mani*	4.8 ± 0.5	407.2 ± 408.6	17.04 ± 31.3	2.2 ± 1.3
Fusagasuga*	5.1 ± 1.4	2830.6 ± 1554.0	55.52 ± 56.8	13.52 ± 13.5
La vega*	4.06 ± 0.6	1504.4 ± 417.7	86.52 ± 171.4	4.6 ± 0.6
Villleta*	5.9 ± 1.6	949.25 ± 880.8	8.725 ± 7.6	2.95 ± 1.7
Andina	5.5 ± 0.2	922 ± 1143	83 ± 83	8 ± 3.2
Caribe	6 ± 1.1	1343 ± 1143	101 ± 123	9 ± 1.8
Pacific	4.5 ± 2.1	315 ± 456	37 ± 28	8.5 ± 5.5
Orinoquia	7 ± 2.4	541 ± 626	116 ± 84	2.8 ± 4.5
Amazonica	5 ± 0.8	815 ± 717	43 ± 86	3 ± 4.6

*Data of this work. 30 soil cores from random location. Values are Average ±standard deviation.

RESULTS

Soil samples and isolation of Actinobacteria

We isolated 57 strains of actinobacteria from six different sampling areas from October 2010 to July 2011. Soil characterization showed pH ranges from 4.0 to 5.9, total P from 360 to 2830 mg/kg, available P from 8.7 to 118.4 mg/kg, and organic matter from 2.95 to 13.52% (Table 1). Figure 2 shows a greater abundance of actinobacteria in localities at the highest elevations: 56% of the isolates belong to Tota and Paipa, 40% to the towns of Fusagasuga, La Vega and Villeta; and the remaining 4% of the isolates were from Mani, a locality at a lower elevation that is located on tropical plains. The use of oatmeal agar allowed the recovery of large numbers of actinobacteria that showed growth and sporulation results after 7 days of culture. Preliminary morphological characterization of the isolates showed typical structures, like aerial and substrate mycelium, conidia chains, some

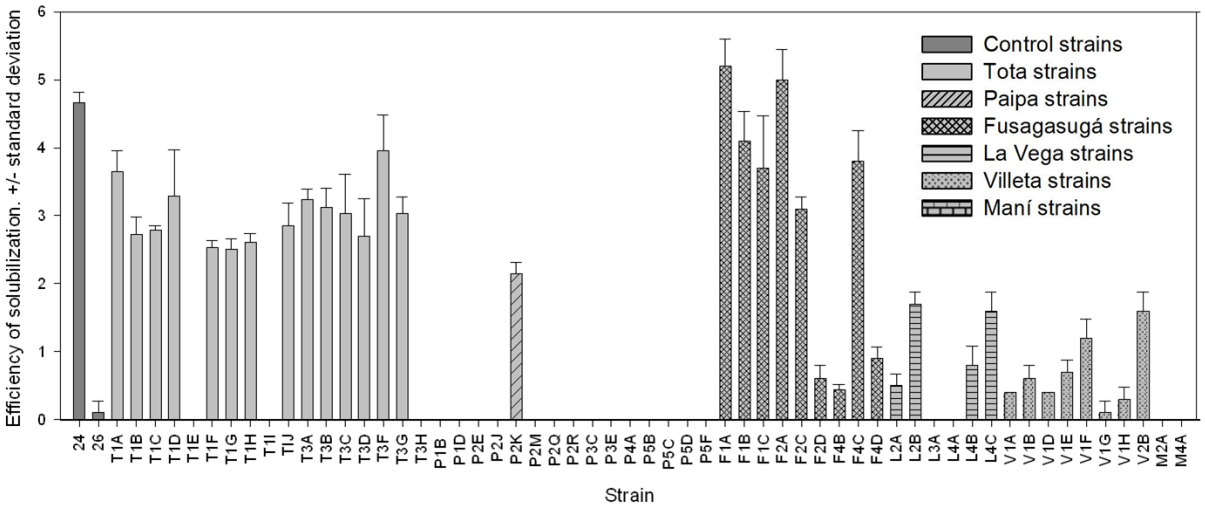

Figure 2. Efficiency of solubilization of the isolates. A clear zone around a growing colony indicate phosphate solubilization and was measured as phosphate solubilazation index.

mycelium with coccoid elements, and spore chain morphologies namely Rectiflexibiles, Retinaculiaperti and Spirales. In addition, other characteristics as melanoid pigments, reverse side pigments and soluble pigments were observed. Macroscopically, all have a dry appearance granular, powdery or velvety, characteristic of the actinobacteria. Additionally, there were several colors: white (T1B, T3B, P2R, L4C and M2A), heavy or light gray (T3A, T3C, T3D, L3A, P3E, F2A), beige (F1A, F2C), blue-green (V1B) and pink (V2B and V1E), and diffusible pigment production (F1A, F2C). Finally, we recognized the smell of wet soil, representative characteristic of this group of microorganisms for the production of geosmina. Some genera of actinobacteria were identified as *Streptomyces*, *Nocardia* and *Actinomadura,* among unidentified isolates.

Screening of phosphate-solubilizing actinobacteria

Figures 2 and 3 show which isolates belong to strains with the best phosphorus solubilizing capacity as well as two control strains deposited at the Pontificia Universidad Javeriana: *Streptomyces* sp. MCR26 labelled as negative control and *Streptomyces* sp. MCR24 as positive control. These control strains were characterized as Plant growth-promoting rhizobacteria (PGPR) (Franco-Correa et al., 2010). The plate assay revealed that strains with the highest values of phosphorus solubilization are F2A, F1A, F1B, F1C, F4C, T3F, T1A, T1D, and T3A. These strains have the same activity as that of the control strain *Streptomyces* sp. MCR24. On the other hand, the liquid evaluation exhibited that isolates with higher solubilizing capacity are those that produce changes greater than 1.5 optical density units at 600 nm, which were F1A, F1B, F1C, F2D, F4B, F4D, M2A, M4B, T1B, T1C, T1D, T1G, T1H, T3A, T3B and T3C. In addition to the activity results obtained with the test, the pH present in the culture medium was low for the majority of the strains selected. The best solubilizing strains with low pH are from the town of Tota, which was the sampling zone with highest elevation and lowest temperature in addition to a relatively low moisture percentage. In this area, a large number of actinobacteria were isolated from five samples collected in the surroundings of the lake, near an area known as Playa Blanca where the few remnants of wild forests are influenced by agriculture and tourism. The town Fusagasuga has strains with high activity but not a decrease in pH; this locality has a lower humidity and higher temperatures.

Release soluble phosphate

The results of the two qualitative assessments are not totally consistent. Seven of the tested strains F1A, F1B, F1C, F4C, T1A, T1D and T3A were the best solubilizing strains in both the solid and liquid evaluation media. We made a quantitative assessment to find which of the strains has the highest solubilizing capacity and which of the two methods is more reliable. Figure 4 shows that the strains T1C, T1H, T3A, T3C,P3E, F1A, F2A and V2B are as good as *Streptomyces* sp. MCR24 for $Ca_3(PO_4)_2$ and that these strains solubilized significantly more phosphorus than the other strains. Strains T1H, T1C, T3A, T3C and F1A are present only in the selection obtained with the methodology reported by Mehta and Nautiyal (2001) suggesting that this test can choose more strains with true solubilizing ability and for this reason, it is more reliable. These strains also lowered pH at around 4 or less with exception of F1A. This result is supported by the change in coloration in the medium caused by the presence of BPB pH indicator,which turns from purple to

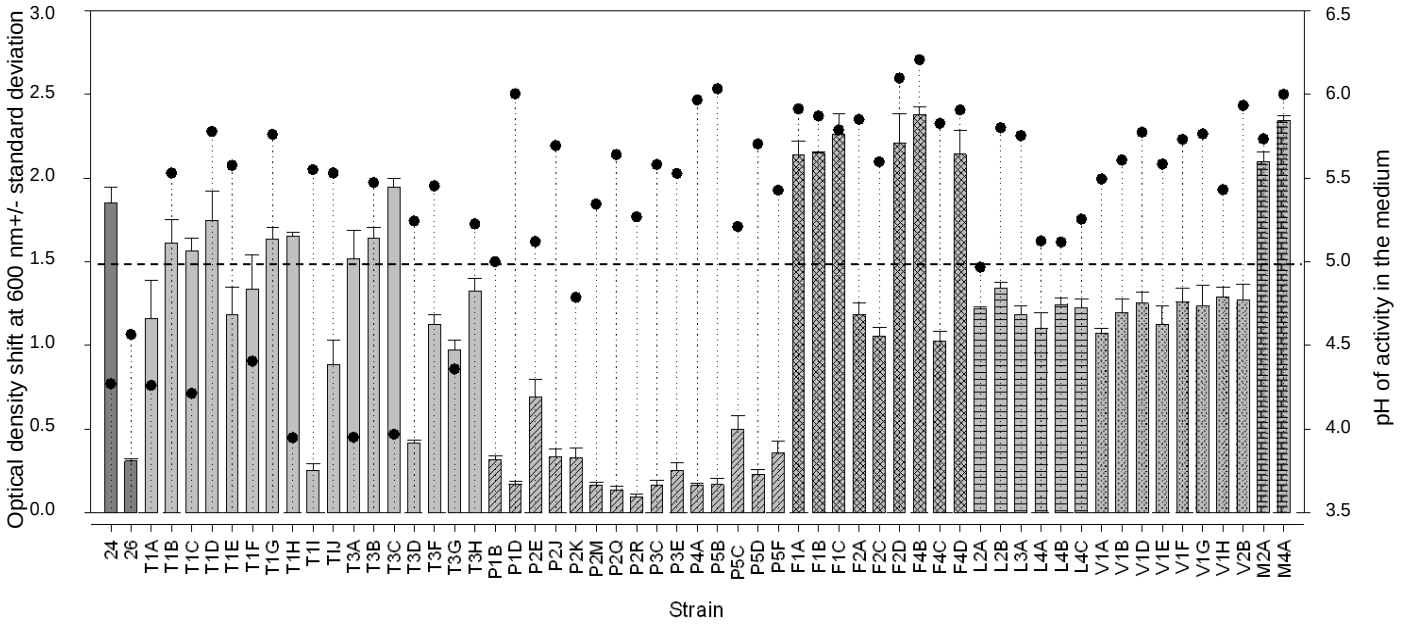

Figure 3. Change in optical density at 600 nm. The dotted line indicates the cut-off for the selection of microorganisms with high tricalcium phosphate solubilizing capacity.

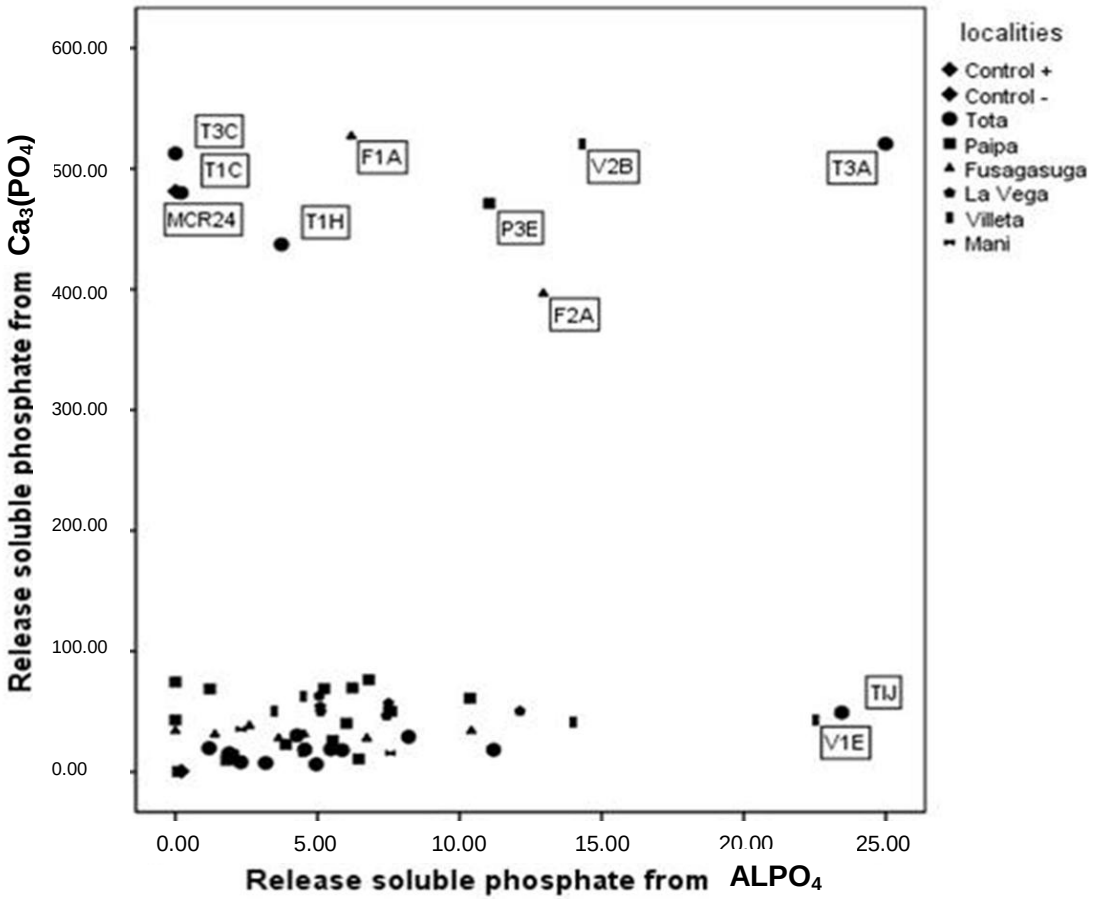

Figure 4. Released soluble phosphate. Activity with $Ca_3(PO_4)_2$ 5g·L^{-1} source is shown in Y and activity with $AlPO_4$ 1g·L^{-1} source is shown in X.

green, a fact related to the secretion of organic acids by actinobacteria. No tests were made with aluminum phosphate because Mehta and Nautiyal (2001) evaluation methodology was designed for tricalcium phosphate. We observed that only in the quantitative assessment strains T1J and T3A can solubilize phosphorus significantly from $AlPO_4$. In addition, the control strain *Streptomyces* sp. MRC24 had a very low activity with this source of phosphorus.

DISCUSSION

Soil samples and isolation of Actinobacteria

The abundance of the isolates in the locality of Tota can be explained by the environmental conditions of this area, such as an average temperature of 18°C, sandy soils with low water content, and pH of 5.0 to 7.2; characteristics that facilitate growth and colonization of actinobacteria. On the other hand, the towns of Mani and La Vega have clay soils with low organic matter content, low oxygen tension and pH below 5.0 (Table 1). These soil characteristics can decrease the abundance of actinobacteria (Goodfellow and Williams, 1983; Alexander 1977; El-Tarabily and Sivasithamparam, 2006). Actinobacteria occurs in a wide range of environments, but soil is the most common ecological niche because the role of these microorganisms is to recycles oil nutrients. Isolates reflect abundant numbers and variety of morphologies in each of the localities sampled, but due to the isolation methodology used, the predominant genus was *Streptomyces* spp. (Xu et al., 1996*;* Ghodhbane-Gtari et al., 2010). Actinobacteria isolates show that fertile soils with high concentrations of nitrogen and carbon are not always necessary to isolate these microorganisms. Although growth of these microorganisms is optimum with pH close to neutrality; our findings show that they can be isolated from sandy soils with low pH and high aluminum concentrations; results that are consistent with those of Shirokikh et al. (2002) and Norovsuren et al. (2007).

Screening of phosphate-solubilizing actinobacteria

In order to perform the screening for an adequate selection of phosphorus solubilizing actinobacteria from Colombian native soils, the isolates were subjected to the traditional test used by PVK and the test reported by Mehta and Nautiyal (2001). Although both qualitative tests use different measurement units, there is a slight correlation pattern between them. Figure 2 shows high bars for the locality of Tota and locality of Fusagasuga, respectively. These results are similar to those in Figure 3, where the highest strain bars belong to the same localities with the greatest activity strains shown in Figure 2. The two methodologies are used, but the assay by Mehta and Nautiyal (2001) is currently more reported, because it reveals strains with good phosphorus-solubilizing capacity which is not detected in the plate assay (Leyval and Barthelin, 1989; Louw and Webley, 1959; Gupta et al., 1994; Mehta and Nautiyal, 2001; Liu et al., 2011).

In our work, the advantage of the liquid assay methodology was observed with strains Tota, Fusagasuga, La Vega and Villeta, which showed a major shift in the NBRIP-BPB broth decolorization and a high release of soluble phosphorus in the quantitative assessment. Figures 2, 3 and 4 show these strains are not equally distributed in all the six sampled localities; on the contrary, they are found mostly in the locality of Tota. This locality is a lake of 56.2 km^2 surrounded by crop fields of onions, potatoes, beans, peas and carrots, and a few small high Andean forests. The constantly chemically fertilized crops are affecting forests and causing eutrophication in water bodies. The soil analysis of the Tota locality showed a 13% of organic matter, a cation-exchange capacity of 6 cmol/kg, a pH around 5 or less, and a total phosphorus concentration of 360 mg/kg with only 118 mg/kg available phosphorus. These results suggest that only 33% of phosphorus applied to the soil may be taken up by plants, on the other hand the remaining 67% is not used and can cause environmental problems. The latter phosphorus parameters reveal excessive chemical fertilization. Probably, excess phosphorus is adsorbed or precipitated and organic matter is removed as the crop itself, so microorganisms are forced to use mainly inorganic forms of phosphorus.

Release soluble phosphate

Perez et al. (2007) propose that isolates causing a shift of > 1.5 units be selected for further studies. In order to confirm the usefulness of this cut-off point proposed by Perez et al. (2007) and therefore to select the best strains, we implemented the quantitative assay measuring the release of soluble phosphorus in the NBRIP broth (Baig et al., 2010). Figure 4 shows that strains T1C, T1H, T3C, P3E, and V2B have a significantly higher activity to other isolates, a result that was not observed in the plate assay possibly because one or more acids involved in the process did not diffuse into the agar so that there was no presence of a solubilization halo. The evaluation in NBRIP-BPB broth revealed that isolates decolorizing the broth more than 1.5 units were also more efficient in the quantitative assay. Also, the assay by Mehta and Nautiyal (2001) contribute to reduce costs and efforts in the search of microorganisms with biofertilizing potential. Studies based on physiology of actinobacteria in Colombia are scarce, especially those focused on agriculture (Joaquín et al., 2006; Cardona et al., 2009; Franco-Correa et al., 2010); in addition, there has been little research on biofertilizer-based clean technologies as a valuable input for agricultural development (Burbano and Silva, 2010).

Developing native biofertilizers contributes to the management of tropical soils with high absorption and fixation of phosphorus due to their high cation concentrations, usually low pH and clay texture, characteristics that have traditionally been amended with an increased use of che-

mical fertilizers resulting in a negative impact on ecosystems. Strains T1C, T1H, T3A, T3C, P3E, F1A, F2A and V2B have phosphorus solubilizing capacities between 396 and 520 µg/ml with tricalcium phosphate; these results are good when compared with other bacteria from similar soils reported as phosphorus solubilizers,s uch as *Bacillus* spp., and *Azotobacter* spp (Narula et al., 2000; Chatli et al., 2008; Saharan and Nehra, 2011). Using similar soil isolates, Perez and co-workers reported an activity with tricalcium phosphate as high as 97 µg/ml by *Burkholderia* and with iron phosphate of 42 µg/ml by *Serratia.* Regarding actinobacteria, El-Tarabily (2008) reported that *Micromonospora* has an activity of 218 µg/ml with phosphate rock and Gupta (2010) reported that *Streptomyces* has an activity of 46 µg/ml with tricalcium phosphate. The values obtained are similar to the results of Chen et al. (2006) that evaluated this activity in actinobacteria belonging to the genera *Rhodococcus* sp. and *Arthrobacter* sp. which had 186 and 519 activities µg/ml, respectively. Those activities reported show that our isolates have a great potential and an acceptable activity.

Differences in the reported values by other authors may be due to the measurement techniques employed or to the source of phosphorus evaluated. Occasionally, the measurements with rock phosphate may overestimate the values of *in vitro* solubilization because this source of phosphorus is composed of several insoluble as well as soluble forms of phosphorus (Zapata and Roy, 2007). One of the most valuable achievements of our research is having isolated an actinobacteria with the ability to solubilize both tricalcium phosphate and aluminum phosphate; the latter is a source difficult to solubilize because aluminum phosphate is rapidly absorbed in acid soils. Its fixation rate per unit area is twice the rate in neutral or calcareous soils. Aluminum is also the leading cause of phosphorus precipitation in acid soils (Olsen and Watanabe, 1957; Holford, 1983). The source of phosphorus in the $AlPO_4$ test had a concentration of 1 g/L in NBRIP broth because this compound is highly toxic to many microorganisms. The greatest *in vitro* resistance to aluminum by actinobacteria reported in the scientific literature is of 5 g/L $AlPO_4$ (Sayed et al., 2000); therefore, several authors have been working with lower concentrations (Illmer, 1995; Prijambada et al., 2009). In addition, it is known that $AlPO_4$ toxicity varies by the complexity of the soils and that actinobacteria have good resistance to aluminum thus their soil colonization is facilitated in presence of this element (Shirokikh et al., 2002; Rueda et al., 2009). Strains with improved phos-phorus solubilizing activity lowered the pH probably due to the production of organic acids, which is one of the phosphorus releasing mechanisms reported in scientific literature (Khan et al., 2009; Saharan and Nehra 2011). Soils with these characteristics predominate in the Colombian tropics, hence strain T3A is important because it can release phosphorus from different sources. All the facts mentioned above, render these strains good candidates for the production of new bio-fertilizers with the ability of colonizing crop rhizospheres in tropical soils.

Strains T1C, T3A, T3C and F1A are candidates for future studies. These strains have similar activity to the positive control strain *Streptomyces* sp. MCR24, which was previously characterized by Franco-Correa et al. (2010) who reported a broad variety PGPR activities besides phosphorus solubilizing capacity. Prior studies have reported that strain swith good phosphorus solubilizing capacity have also a variety of mechanisms to promote plant growth like the strain *Streptomyces* sp. MCR24. For this reason, further studies with these strains designed to evaluate other plant growth promoting activeties, like nitrogen fixation, degradation of complex carbohydrates, synergistic activities, antagonisms and production of plant growth promoting hormones, can make an important contribution to agriculture.

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