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ORIGINAL PAPER

# Plant growth promotion traits of phosphobacteria isolated from Puna, Argentina

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**Abstract** The ability of soil microorganisms to solubilize phosphate is an important trait of plant growth-promoting bacteria leading to increased yields and smaller use of fertilizers. This study presents the isolation and characterization of phosphobacteria from Puna, northwestern Argentina and the ability to produce phosphate solubilization, alkaline phosphatase, siderophores, and indole acetic acid. The P-solubilizing activity was coincidental with a decrease in pH values of the tricalcium phosphate medium for all strains after 72 h of incubation. All the isolates showed the capacity to produce siderophores and indoles. Identification by 16S rDNA sequencing and phylogenetic analysis revealed that these strains belong to the genera *Pantoea*, *Serratia*, *Enterobacter*, and *Pseudomonas*. These isolates appear attractive for exploring their plant growth-promoting activity and potential field application.

**Keywords** Phosphobacteria · Phosphate solubilization · Indol acetic acid · Siderophore · 16S rDNA

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## Introduction

Phosphorus (P) is one of the major plant growth-limiting nutrients despite being abundant in soils. The free phosphorus concentration available to plants is very low even in fertile soils due to the fact that soluble P reacts with calcium (Ca), iron (Fe), or aluminum (Al) and organic compounds that lead to P precipitation (Gyaneshwar et al. 2002). This essential plant nutrient is added to soil as chemical fertilizers, but becomes insoluble and, therefore, unavailable to plants. Moreover, this practice is reaching the theoretical maximum use beyond which there will be no further increase in yields (Son et al. 2006).

Phosphobacteria have the ability to convert insoluble compounds of phosphorus into available phosphates that enhance nutrient availability to plants (Barea et al. 2005; Lugo et al. 2008; Rodríguez and Fraga 1999; Son et al. 2006; Souchie et al. 2006). Strains from the genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium*, and *Erwinia* are known phosphate solubilizers (Rodríguez and Fraga 1999). Several studies have shown that the release of organic acids, like gluconic and 2-ketogluconic acids, is one of the mechanisms responsible for solubilizing insoluble phosphate (Lin et al. 2006) since the organic acids produced by the microorganisms may reduce pH and act as chelating agents, forming complexes with Ca, Fe, or Al, and thereby releasing the phosphates to solution. Other mechanisms of solubilization comprise the release of other chelating substances and inorganic acids such as sulphideric, nitric, and carbonic acids. Secretion of phosphatase enzymes (acid and alkaline phosphatase, phytase, phosphohydrolase) by phosphobacteria is also a common mode of facilitating the conversion of insoluble forms of P to plant-available

forms and thus enhance plant P uptake and growth (Kohler et al. 2007).

Microbial phosphate solubilization may be then a solution, not only to compensates for the ever increasing costs of manufacturing fertilizers in industry but also as a way to mobilize the fertilizers added to soil. Phosphobacteria not only play a significant role in supplying P to plants, but also increase plant growth and development through other plant growth promotion activities, like nitrogen fixation, siderophores, and phytohormones production (Vassilev et al. 2006). Certain cooperative microbial activities can be exploited as a low-input biotechnology and form a basis for a strategy to help sustainable, environmentally friendly practices fundamental to the stability and productivity of both agricultural systems and natural ecosystems (Barea et al. 2005). The aim of this study was to isolate phosphobacteria from rhizospheric soil of Puna-native grasses, to identify them using molecular tools and to evaluate their phosphate solubilization activity, indol acetic acid, and siderophores production. Puna is a South American harsh biogeographical region with unique features (Lugo et al. 2008), although it is considered an alpine environment by some authors, and is a source of microorganisms with interesting characteristics.

## Materials and methods

### Isolation of phosphate-solubilizing bacteria

Rhizospheric soil samples were taken along an altitudinal gradient (3,200–3,800 masl) from Puna (northwestern Argentina), a South American stressed biogeographic region with unique features, where climate is of a desert type. The soils are superficial and immature, very poor in organic matter, sandy and rocky (Lugo et al. 2008). The samples were stored at 4°C before processing. Batch culture of soil samples with a phosphorous limited culture medium (1 μM of phosphorus) was performed, and continuous feeding at a dilution rate of 0.05 $h^{-1}$ allowed the isolation of different bacterial morphotypes.

Serial dilutions of batch culture samples were then individually inoculated on NBRIP agar plate supplemented with 1.5% (w/v) agar (Nautiyal 1999). Phosphobacteria were identified after 7 days of incubation at 30°C because they developed clear zones around their colonies. They were picked up and further purified by replating on agar plates for qualitative estimation of phosphate solubilization index (SI). The spot inoculation was carried out using a sterile needle, and the Petri dishes were incubated at 30°C. The halo (zone of solubilization) around the bacterial colony and colony diameter were measured after incubation for 7 days. Solubilization index was evaluated according to the ratio of the total diameter (colony + halo zone) and the colony diameter (Premono et al. 1996). The test was carried out on duplicate in each case.

### Genomic DNA isolation and 16S rDNA sequencing

For phylogenetic characterization, single colonies of each strain were removed from LB plates and transferred to 2-ml microcentrifuge tubes for subsequent DNA extraction. DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method as like described by Lugo et al. (2008). Almost full-length 16S ribosomal ribonucleic acid (rRNA) gene sequences were amplified from DNA extracted with oligonucleotide primers 27f (*Escherichia coli* 16S rDNA positions 8–27) and 1492r (*E. coli* 16S rDNA positions 1,492–1,512) (Lane 1991). The PCR amplification mixture contained 0.2 mM (each) dNTP (Promega), 400 nM (each) primer (Promega), GoTaq Green Master Mix with Mg 7.5 mM 1× (Promega), and 1U GoTaq® DNA Polymerase (Promega) in a final volume of 25 μl. After a hot start at 94°C for 3 min, 30 cycles PCR reaction were run as follows: denaturation at 94°C for 1 min, annealing at 57°C for 30 s, and extension at 72°C for 1 min. In addition, a final extension at 72°C for 7 min was added. Negative controls, without DNA, were included in each experiment. PCR products were analyzed by horizontal agarose (1%) gel electrophoresis (4 V $cm^{-1}$) in Tris–borate-EDTA (TBE) running buffer (Sambrook et al. 1989). Gels were stained with ethidium bromide (0.5 mg $ml^{-1}$) for 30 min, washed three times with distilled water, and visualized on UV analyzer. The determined partial 16S rDNA sequences have been deposited in EMBL Nucleotide Sequence Database (http://www.ncbi.nlm.nih.gov), and numbers access were obtained for each strain.

### Phylogenetic analysis

Phylogenetic and molecular evolutionary analyses were conducted using the program *MEGA* version 4 (Tamura et al. 2007). The phylogenetic trees were constructed by the neighbor-joining method (Saitou and Nei 1987) using the distance matrix from the alignment. Distances were calculated using the Kimura method (1980).

### Phosphate solubilization

#### *Solubilization of tricalcium phosphate in broth culture*

Phosphobacteria were grown in LB broth overnight at 30°C with 200 rpm agitation. Flasks with 50 ml of NBRIP media were inoculated with 100 μl of LB culture ($10^2$ UFC $ml^{-1}$) and incubated on a rotary shaker at 200 rpm and 30°C. A 5-ml sample of each culture was taken at 24, 48, and 72 h

of incubation and centrifuged for 15 min at 10,000 rpm. Soluble phosphorus (P) concentrations (μg $ml^{-1}$) (Murphy and Riley 1962) and pH were measured in the supernatant for each strain. Sterile uninoculated medium was used as control. The test was carried out on triplicate in each case.

*Alkaline phosphatase production*

To determine alkaline phosphatase (AP) activity, synthetic medium (in g $l^{-1}$: Glycerol, 10; $(NH_4)_2SO_4$, 5; NaCl, 5; yeast extract, 0.5) was used with either 10 mM $K_2HPO_4$ (Pi) (excess phosphate) or 1 μM Pi (limiting phosphate). Cultures were grown overnight at 30°C, harvested, and the pellets were washed with 0.1 M $MgSO_4$ and suspended in 0.1 M Tris-hydrochloride (pH 7.4) containing 0.001 M $MgSO_4$. AP activity was determined using the method described by Ludtke et al. (1984) with p-nitrophenyl phosphate (Sigma) as substrate. One unit of alkaline phosphatase is defined as the nanomoles of p-nitrophenol produced per min per mg of cell protein. The test was carried out on triplicate in each case. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Other PGPR traits

*IAA production*

Total Indole production was determined by a colorimetric method (Glickmann and Dessaux 1995) in M9 medium with or without tryptophan (Trp) (0.1 mg $ml^{-1}$). 100 μl of LB culture ($DO_{600nm}$ 0.2) of each bacterial isolate was transferred to M9 medium. Cultures were incubated at 30°C during 48 h and then centrifuged (10,000 rpm, 15 min) to obtain the supernatant. Indoles production was determined with the Salkowski reagent. Reactions was carried out with a ratio 1:1 of supernatant to Salkowski reagent, followed by incubation in the dark at room temperature during 30 min. Total indoles were determined spectrophotometrically at 540 nm. Values were directly converted to equivalent IAA concentrations (μg per ml), using the calibration curve of Indole acetic acid. The test was carried out on triplicate in each case.

*Siderophores production*

The chrome azurol sulfonate (CAS) assay was used to determine production of siderophores (Schwyn and Neilands 1987). Ten μl of iron-free M9 pre-culture ($DO_{600nm}$ 0.2) was inoculated by triplicate in Petri dishes containing M9 solid medium supplemented with CAS. They were incubated at 30°C for 24 h, and the siderophore halo (mm) was determined by substrate colony diameter to the total diameter.

*Statistical analysis*

All statistical analyses were performed using the least significant difference (LSD) test ($P = 0.05$) to determine significant differences of the mean values between treatments. The Infostat Analytical Software (2008) for Windows was used.

## Results

Isolation of phosphate-solubilizing bacteria

After evaluating 86 isolates, only 33 showed clear halos of phosphate solubilization in NBRIP agar plate after 7 days of incubation at 30°C (Fig. 1a). PSB were further purified, and solubilization index in agar plate based on colony diameter and clear halo zone for each isolate is presented in Fig. 1b. Results showed that among PSB, EV4 was the most efficient phosphate solubilizer on NBRIP plates with SI = 6.8 ± 0.4. EV4 with other higher solubilizer isolates, EV1 (5.0 ± 1.0), EV2 (4.9 ± 0.6), EV3 (6.0 ± 0.6), IEXb (4.7 ± 0.4), and IEY (4.2 ± 0.3), were selected for further studies based on phosphate solubilization capacity and different colony characteristics.

Genomic DNA isolation and 16S rDNA sequencing of selected isolates

Identification of six selected phosphate-solubilizing bacterial strains based on 16S rDNA sequence and their phylogeny are presented in Fig. 2. Molecular analysis reveals that the sequence of EV1 showed high similitary (98.3% of identity) with type strain *Serratia marcescens subsp. sakuensis* (AB061685), two isolates EV2 and EV4 showed 99.4 and 98.8% identity with *Pantoea eucalypti* LMG 24197$^T$ (EF688009), respectively, IEXb was closely associated with *Pseudomonas tolaasii* ATCC 33618$^T$ (D84028) with 99.2% of identity, and IEY showed high similitary with *Enterobacter aerogenes* NCTC 10006$^T$ (AJ251468) with 98.9% of identity. Interestingly, EV3 isolate showed only 93.3% of identity with the closest known species in the GenBank database, in this case *Pantoea agglomerans* DSM 3493$^T$ (AJ233423). Sequences of six selected isolates were deposited in the GenBank nucleotide sequence data library under their respective accession numbers (Fig. 2).

Phosphate solubilization

*Solubilization of tricalcium phosphate in broth culture*

In order to quantify phosphate-solubilizing activity, EV1, EV2, EV3, EV4, IEXb, and IEY isolates were inoculated in

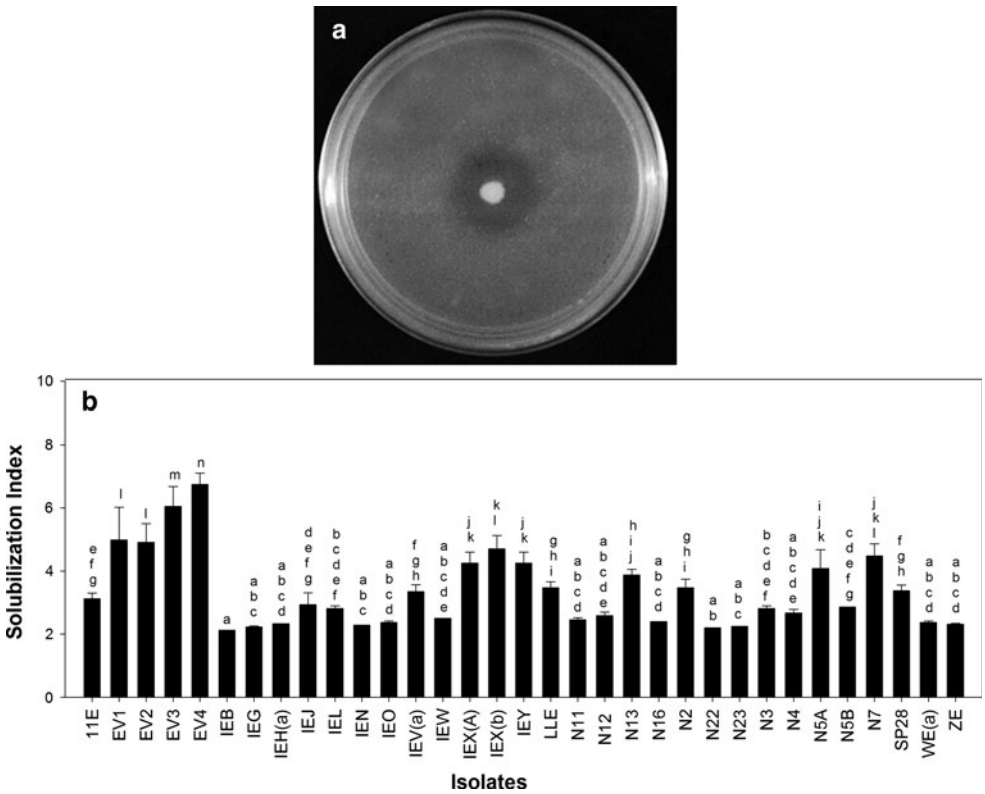


**Fig. 1** **a** Clear halo of solubilization around colony of PSB isolate after 7 days of incubation. **b** Phosphate solubilizing activity of 33 isolates in NBRIP agar plate (mm) after 7 days of incubation at 30°C. Data are the means of two determinations, and the *error bars* indicate standard deviation (SD). *Different letters* indicate significant differences at $P = 0.05$ according to LSD test

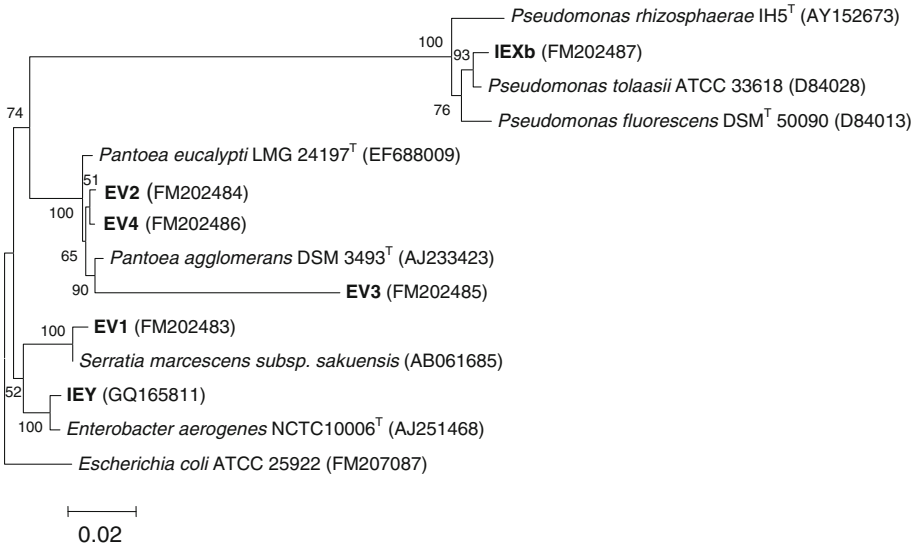


**Fig. 2** Phylogenetic tree showing the relationships among the PSB isolates and between representatives strain of other related taxa. The tree was constructed by using the MEGA 4 after aligning the sequences with Clustal*W* and generating evolutionary distance matrix inferred by the neighbor-joining method using Kimura parameter 2. The *numbers* at the nodes indicate the levels of bootstrap support based on data for 500 replicates. *Scale bar* indicates 0.02 substitutions per nucleotide position. *Escherichia coli* ATCC 25922 was used as the outgroup. Accession numbers of 16S rDNA sequences are given in *parentheses*

NBRIP broth and incubated for 7 days. The values of pH and amounts of soluble P at 24, 48, and 72 h in the medium are presented in Table 1. The solubilization of $Ca_3(PO_4)_2$ in liquid medium by different strains was accompanied by a decrease in pH from the initial pH 7 after 72 h. No relevant changes were observed in the blanks. In general, a wide range of values of phosphate solubilization among isolates was observed. The maximum P solubilization at 24 h was recorded by isolate EV1 (470.4 μg $ml^{-1}$), 471.7 μg $ml^{-1}$ at 48 h, and 469.6 μg $ml^{-1}$ at 72 h with a

drop in the final pH to 3.3. Soluble P production was approximately ninefold higher than uninoculated NBRIP medium (50.0 μg $ml^{-1}$). Among the isolates, the minimum concentration of soluble P (227.4 μg $ml^{-1}$) was observed in the cultures of IEY at 24 h and the pH of the medium was relatively higher (5.3). Even though a larger decrease in the pH value was generally associated with higher levels of P solubilization, in some cases, for example, IEXb, where pH value decreased only to 4.7, comparatively higher amounts of soluble P (445.3 μg $ml^{-1}$) were detected in the medium.

### *Alkaline phosphatase production*

The AP production by six selected isolates in limiting and excess of phosphate was measured in cell extracts by incubation with *p*-nitrophenyl phosphate as substrate. It is known that synthesis of periplasmic AP is repressed at high phosphate concentrations and derepressed in limiting conditions of Pi (Yashphe et al. 1990). Table 2 shows specific activities of AP of our isolates in both conditions. It was evident the inhibitory effect of high concentration of phosphate (10 mM) respect to the P-limiting condition (1 μM) in enzyme production. With 1 μM of phosphate, EV1 and IEXb isolates produced higher levels of AP (82.1 and 77.3 U/mg protein, respectively), EV4 and IEY were lower producers with 4.1 and 6.0 U/mg of protein, respectively, while EV2 and EV3 produced values under detection limit of AP. On the other hand, values of the enzyme with 10 mM of phosphate were undetectable for all isolates assessed here, which should be consequence of the inhibitory effect at high levels of phosphate in enzyme production.

**Table 1** Solubilization of inorganic phosphate and pH by selected isolates in broth using National Botanical Research Institute's phosphate growth medium (NBRIP) medium with 5 g $l^{-1}$ of $Ca_3(PO_4)_2$ at 24, 48 and 72 h

| Isolates | P conc. (μg $ml^{-1}$) | | | pH | | |
|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| Control | $50.0^{a}$ | $49.8^{a}$ | $49.7^{a}$ | 7.0 | 6.9 | 6.9 |
| EV1 | $470.4^{e}$ | $471.7^{f}$ | $469.6^{e}$ | 4.1 | 3.9 | 3.3 |
| EV2 | $317.2^{d}$ | $438.1^{e}$ | $445.4^{d}$ | 4.6 | 4.5 | 3.3 |
| EV3 | $351.6^{d}$ | $370.5^{c}$ | $387.1^{c}$ | 4.9 | 3.8 | 3.3 |
| EV4 | $273.9^{c}$ | $451.2^{e}$ | $458.1^{e}$ | 4.5 | 4.5 | 3.1 |
| IEXb | $330.8^{d}$ | $391.5^{d}$ | $445.3^{d}$ | 4.6 | 4.6 | 4.7 |
| IEY | $227.4^{b}$ | $311.5^{b}$ | $350.0^{b}$ | 5.3 | 5.0 | 5.0 |

Values within same column followed by the same letter are not significantly different according to Fisher's protected LSD (least significant differences)

**Table 2** Alkaline phosphatase activities of six selected isolates in M9 medium with limiting (1 μM) or excess (10 mM) concentrations of phosphate

| Isolates | Alkaline phosphatase activity[a] | |
|---|---|---|
| | Limiting phosphate | Excess phosphate |
| EV1 | 82.1 ± 1.0 | N.D. |
| EV2 | N.D. | N.D. |
| EV3 | N.D. | N.D. |
| EV4 | 4.1 ± 0.2 | N.D. |
| IEXb | 77.3 ± 1.0 | N.D. |
| IEY | 6.0 ± 0.8 | N.D. |

[a] Activity is expressed in units per mg of protein cell

*N.D.* not detectable

## Other PGPR traits

### *IAA production*

Considering the phytohormone production as one of the features for bacteria as PGPR, biosynthesis of total indoles in selected isolates in M9 medium supplemented or not with Trp was studied. Figure 3 shows the values of total indoles detected in each case. It can be seen that the presence of Trp has a significant incidence in the biosynthesis of total indoles in all the isolates. In general, a variation of indolic compounds values among isolates was observed. The strain IEXb was the higher producer in absence (5.9 ± 0.3 μg IAA $ml^{-1}$) or presence of Trp (16.8 ± 2.9 μg IAA $ml^{-1}$) (Fig. 3).

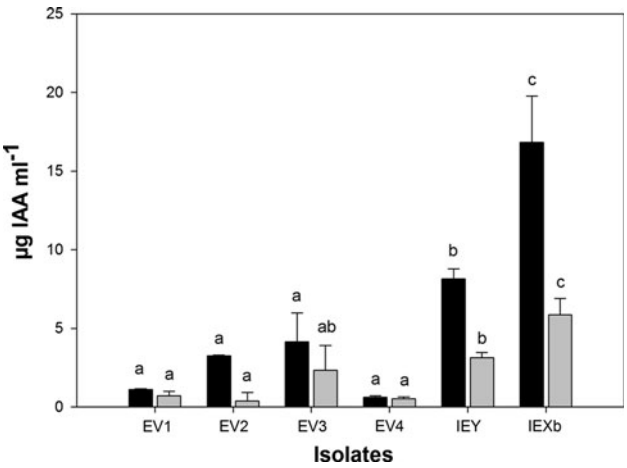


**Fig. 3** Total indoles production of selected isolates grown in M9 medium supplemented (*black bar*) or not (*gray bar*) with tryptophan (Trp) (0.1 mg $ml^{-1}$) after 48 h of incubation at 30°C in rotatory shaker. Data are the means of three determinations, and the *error bars* indicate SD. *Different letters* indicate significant differences according to LSD test ($P = 0.05$)

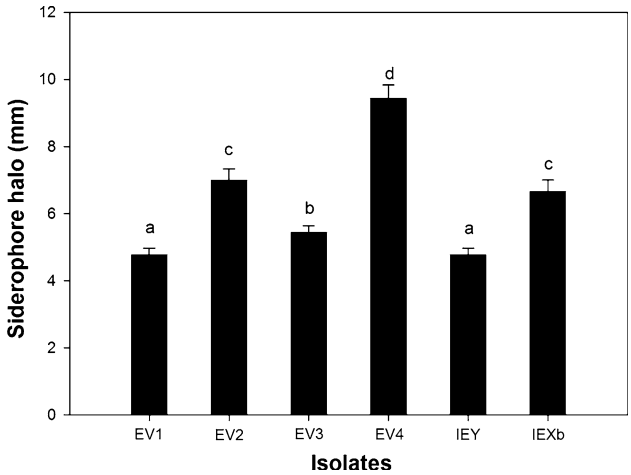


**Fig. 4** Siderophore production (halo size in mm) by selected isolates after 24 h of incubation at 30°C in CAS agar plate. Data are the means of three determinations, and the *error bars* indicate SD. *Different letters* indicate significant differences according to LSD test ($P = 0.05$)

*Siderophores production*

Additionally, the production of siderophores by selected strains was tested in CAS agar. After 24 h of incubation, considering the halo size, EV4 was the larger siderophore producer, with 9.4 ± 0.4, followed by EV2 (7.0 ± 0.3), IEXb (6.7 ± 0.3), EV3 (5.4 ± 0.2), EV1 (4.8 ± 0.2), and IEY (4.8 ± 0.2) mm (Fig. 4). All the bacterial strains employed in our study produced the same change in color (from blue to orange), as reported by the literature (Milagres et al. 1999; Rosas et al. 2006).

## Discussion

Phosphate is one of the major nutrients limiting plant growth, and frequent application of high amounts of P-fertilizer leads to fixation of phosphate in soil. Phosphate-solubilizing microorganisms produce a major contribution to overall plant P nutrition and growth and have increased yields of many crops. They help to minimize the application of P-fertilizer, reduce environmental pollution, and promote sustainable agriculture (Vikram and Hamzehzarghani 2008). In this study, 33 rhizobacteria were isolated based on their inorganic phosphate-solubilizing capability. Several isolates were stronger solubilizers, and values of solubilization index in agar plate were higher than those observed by other authors (Fig. 1b). The $Ca_3(PO_4)_2$ solubilization by these isolates in agar plate ranged from 2.2 to 6.8 of SI values compared with 1.5–6.0 of isolates of peat soils from Indonesia (Sitepu et al. 2007). Alam et al. (2002) have reported SI of 3.3 by the best bacterial strain isolate from maize rhizosphere of Pakistan soils. Six isolates, EV1, EV2, EV3, EV4, IEXb, and IEY, were selected due to their high solubilization activity. In NBRIP broth culture, an inverse correlation between the pH value of the culture and the released P was observed in all cases (Table 1). This fact may indicate that phosphate solubility was directly correlated with the organic acids produced. There are reports in the literature indicating that PSB released many kinds of organic acids, but the type of acid produced is dependent on the microorganism (Chen et al. 2006; Kohler et al. 2007; Lin et al. 2006; Pandey et al. 2006; Rodríguez and Fraga 1999; Son et al. 2006). In fact, an inverse relationship between pH and soluble phosphate was reported by the same authors.

Production of enzyme like phosphatases is other mechanism of phosphate solubilization (Rodríguez and Fraga 1999). Two isolates, IEXb and EV1, showed high activity of AP at 1 μM of P; however, the production of this enzyme was under detection limit in excess of phosphate (10 mM) compared to limiting condition, which could explained that the synthesis of alkaline phosphatase by these bacteria was inducible in low Pi, while it was repressed in high concentration. These results are in concordance with solubilization activity in NBRIP broth, where both isolates were strong P solubilizers. Interestingly, IEXb strain produced a smaller drop in pH value compared to others isolates. This might suggest that this strain is capable to solubilize phosphate by other ways than the production of organic acid. Therefore, we found a positive correlation between phosphate-solubilizing capacity and phosphatase enzyme activity.

Phosphobacteria are capable of producing physiologically active auxins that may have pronounced effects on plant growth (Vassilev et al. 2006). Bacterial strains were able to produce IAA in broth culture, and this production increased in the presence of a physiological precursor, L-tryptophan. IEXb was the isolate that showed the highest level of IAA, both in presence or absence of Trp (Fig. 3), and these values were higher to those recorded by other researchers. Recently, the IAA production in presence of the amino acid in the culture media of 9.88 μg $ml^{-1}$ by *Pseudomonas fragi* CS11RH1 and 7.4 μg $ml^{-1}$ by *Pantoea dispersa* strain 1A isolated from a high-altitude Himalayan rhizosphere has been reported (Selvakumar et al. 2008b, 2009). Ali et al. (2010) report auxin production ranged from 0.05 to 0.84 and 1.16 to 8.22 μg $ml^{-1}$ in the absence and presence of L-tryptophan, respectively. On the other hand, production of siderophores in agar CAS was found in all isolates, being EV4 isolate the highest producer (Fig. 4). Siderophore production is beneficial to plants by solubilizing iron formerly unavailable to the plant and has also some biocontrol properties because it helps a particular microorganism to compete effectively against other organisms for available iron, especially pathogenic fungi

(Deepa et al. 2010). Indole-3-acetic acid (IAA) and siderophores, which are among the most frequently studied metabolites with plant growth promotion capability, are found to be released by microorganisms that express P-solubilizing activity (Selvakumar et al. 2008a, 2009). These characteristics could give an added value as PGPR to the bacterial strains assessed here.

The 16S rDNA partial sequence analysis placed EV1, EV2, EV3, EV4, and IEY isolates at the genus level in *Enterobacter*, *Serratia*, and *Pantoea*, which are grouped under one family, Enterobacteriaceae. Only one isolate IEXb belong to Pseudomonaceae family. Pseudomonas species are known P-solubilizer (Nautiyal 1999; Peix et al. 2003; Selvakumar et al. 2009; Rosas et al. 2006), while several reports have described strains of *Serratia marcescens* (Chen et al. 2006; Farhat et al. 2009; Selvakumar et al. 2008a), *Pantoea agglomerans* (Chung et al. 2005; Son et al. 2006; Sulbarán et al. 2009), and *Enterobacter aerogenes* (Deepa et al. 2010; Fernandez et al. 2007; Wu and Zhou 2005) as good phosphate solubilizers. Thus, the EV1, EV3, and IEY strains are similar to other organisms already related to phosphate solubilizing. However, EV2 and EV4 isolates were identified as *Pantoea eucalypti* strains, a novel species recently described by Brady et al. (2008) isolated from soils of Argentina, Colombia, Uruguay, South Africa, and Uganda, and IEXb was identified as *Pseudomonas tolaasii* strain, none described previously as P solubilizers.

The properties of these six strains which include solubilization of insoluble phosphate, siderophore, and phytohormones (IAA) production could be useful for technological applications in the area of plant production. The growth promoter effect in plants gives extra value to the isolates, since they may not only be increasing the bioavailability of one of the most important plant nutrient, like phosphorus, but also would release substances that have an antibiotic activity and improve iron nutrition for the plant, in case of siderophores, and increased lateral and root hair growth, in the case of IAA production.

The exogenous introduction of phosphobacteria in agriculture soils would help to decrease the use of chemical fertilizer while increasing yields. This would mean that a low-cost ecotechnology engineered through specific bacteria responsible for solubilization of rock phosphate could be of considerable economic importance in the developing countries. The good results obtained in vitro cannot always be dependably reproduced under field conditions. It is expected that inoculation with rhizobacteria containing PGP characteristics consequently promotes root and shoot growth. Further, evaluation of the isolates exhibiting multiple plant growth-promoting (PGP) traits on soil–plant system is needed to uncover their efficacy as effective PGPR. The present study is important in view of identification of native bacterial strains with strong potential for development as bioinoculants.

## Conclusion

In conclusion, the strains *Serratia marcencens* EV1, *Enterobacter aerogenes* IEY, *Pantoea agglomerans* EV3, *Pantoea eucalypti* EV2, *Pantoea eucalypti* EV4, and *Pseudomonas tolaasii* IEXb solubilized larger amounts of P in NBRIP liquid medium, compared with other native isolates. All the selected isolates showed to produce siderophores and indoles, two important characteristics within the PGPR group. Organic acid production was, perhaps, not the only possible reason for phosphate solubilization in case of IEXb. These isolates with high phosphate-solubilizing ability appear attractive for exploring their plant growth-promoting activity toward the development of microbial inoculants.

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