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RESEARCH ARTICLE

Short-term study shows that phytate-mineralizing rhizobacteria inoculation affects the biomass, phosphorus (P) uptake and rhizosphere properties of cereal plants

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Abstract

Cereal production in southern Chile is based on volcanic soils (Andisol) that are phosphorus (P) deficient for plant nutrition. Phytate-mineralizing rhizobacteria (PMR) have been suggested as soil inoculants to improve P uptake and growth of plants cultivated in P-deficient soils. In this study, a greenhouse experiment was conducted to investigate the effects of inoculation with PMR (*Bacillus* sp. N1-19NA, *Enterobacter* sp. N0-29PA, *Pseudomonas* sp.N1-55PA and *Serratia* sp. N0-10LB) on biomass and P uptake of cereal plants (wheat, oat and barley) grown in a Chilean Andisol with out P fertilization. Results showed that inoculation with *Enterobacter* sp. N0-29PA significantly ($P \leq 0.05$) increased the biomass and Puptake of oat plants. Changes in rhizosphere properties as soil enzyme activities (acid phosphatase and urease), and auxin production potential were also produced by *Enterobacter* sp. N0-29PA inoculation. Despite the possible value, *Enterobacter* sp. N0-29PA as a soil inoculant for P-deficient soils, other PMR assayed did not consistently enhanced biomass and P uptake of plants. In addtion, polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) combined with non-metric multidimensional scaling (nMDS) analysis revealed that PMR inoculation induced changes in rhizobacterial community composition, suggesting that PMR application substantially modify the microbiological characteristics of rhizosphere. However, long-term studies at field level are still needed to practical use of PMR as inoculants in Chilean Andisols.

Keywords: Cereal, phosphorus, phytate, rhizobacteria, rhizosphere, volcanic soil

1. Introduction

Cereal production in southern Chile is established on ash-derived volcanic soils known as Andisols. Cultivated Chilean Andisols that have been fertilized often contain high amounts of total phosphorus (P), most of which is unavailable to plants due to the high rate of P fixation that occurs in soils having a low pHand high aluminum (Al) content (Mora and Canales, 1995; Borie and Rubio, 2003). To overcome this limitation, P based fertilizers must therefore be applied regularly to meet the P requirements of plants grown in these soils. In this context, there is considerable interest in possible methods to remobilize the chemically fixed phosphate by means of phosphate-solubilizing rhizobacteria (PSR), which can enhance P uptake and plant growth through production of organic acids that dissolve iron and Al phosphates (Richardson and Simpson, 2011). In addition to aluminum phosphate minerals, Chilean Andisols also contain high contents of organic P (Po) (>60% of total P), primarily in the form of phytate (42-67% of organic P) (Borie and Rubio, 2003), which represents an important pool of potentially available Pin Chilean Andisols (Menezes-Blackburn *et al*., 2013). Therefore, the application of rhizobacteria that canhydrolize and mobilize this fixed Popool could be a useful strategy to streng then the P nutrition in plants and decrease the requirement for use of P based fertilizers in Chilean Andisols.

Phytate-mineralizing rhizobacteria (PMR) play a fundamental role in the mobilization and recycling of phytate from soil P reserves (Richardson and Simpson, 2011). PMR have been isolated from the rhizosphere of pasture and cereal plants grown in Chilean Andisols and their potential use as soil inoculants to increase P availability in Chilean Andisols has been investigated (Jorquera *et al*., 2008, 2011). PMR not only have the ability to mobilize P from insoluble forms of Pi and Po but also commonly carry other traits involved in plant growth promotion, such as the production of indole acetic acid (IAA) (Acuña *et al*., 2011; Martínez *et al*., 2011). The phytohormone IAA enhances the development of the plant root system and may indirectly improve P uptake by plants (Marschner *et al*., 2011). However, the effect of PMR on P uptake, growth and fitness of plants cultivated in P-deficient soils remains unknown, especially in Chilean Andisols, and major studies are required to elucidate their efficacy as a partial substitute for P fertilizer application. Thus, the objective of this research wasto investigates the effect of previously isolated PMR on biomass and P uptake of cereal plants grown in a Chilean Andisol under P deficiency.

2. Materials and Methods

2.1. Strains

Bacillus sp. N1-19NA, *Enterobacter* sp. N0-29PA, *Pseudomonas* sp. N1-55PA and *Serratia* sp. N0-10LB, were previously isolated from rhizosphere soil and selected for their abilities to mineralize phytate (phytic acid dodecasodium salt hydrate), solubilize phosphate (tricalcium phosphate), and produce auxin-like substances (Martínez *et al*., 2011).

2.2. Soil

The soil selected for this research was an Andisol belonging to the Piedras Negras (PN) series, which contains high total P, most of which is unavailable to plants as determinated by P_{Olsen} method (see below). This soil has been used as a model for studies on P-deficiency in southern Chile (Paredes *et al*., 2011),

and regular applications of P fertilizers are required to sustain the crop productivity. The soil samples were collected from the top 20 cm of the soil profile inthe Osorno province of southern Chile (40 º20'S; 72º35'W). Soil was air-dried and passed through a 2-mm sieve to homogenize the samples. Fractionation of soil P yielded (mg kg^{-1}): total P (Pt) 1124, inorganic P (Pi) 131 and organic P (Po) 993, P_{Olsen} 3. Other chemical properties indicated a K content of 109 mg kg^{-1}, pH_{H20} 5.4, organic matter 17 % and Al saturation 15.2 %, cation exchange capacity 2.95 cmol+ kg^{-1}.

2.3. Greenhouse experiment

A greenhouse experiment was conducted to evaluate the effects of PMR on biomass and P uptake of cereal plants without P fertilization. Similar economic-relevant cereal plants (wheat, *Triticum aestivum* L. cv. Fritz; oat, *Avena sativa* L. cv. Rayen; barley, *Hordeum vulgare* L. cv. Pincoya) were used in this assay. The data were complemented with measurements of diverse rhizosphere properties, such as root-surface phosphatase activity, phosphorus availability, auxin production potential, soil enzyme activities (urease and acid phosphatase) and bacterial community structure.

Firstly, cereal plants were germinated on wet filter paper for 7 d, after which 10 seedlings were transplanted per pot containing 0.95 kg of the PN soil. Each plant was inoculated with one milliliter of PMR suspension (~1×10^9cfu mL^{-1}) at 1, 30 and 55 days (Fernández *et al*., 2007), and periodically irrigated to maintain 60% of the maximum water holding capacity. The inoculation treatments included: (1) control (uninoculated), (2) *Bacillus* sp. N1-19NA, (3) *Enterobacter* sp.N0-29PA, (4) *Pseudomonas* sp. N1-55PA, and (5) *Serratia* sp. N0-10LB.The plants were maintained under greenhouse conditions for 75 days and N fertilization (200 kg urea ha^{-1}) was carried out at day-30.No P fertilization was applied.

The assays were carried out in completely randomized design, and the data were analyzed statistically with one-way ANOVA and LSD (least significant differences) post hoc test using JMP statistical software (SAS Institute, Inc.). Differences with $P\leq0.05$ were considered significant.

2.4. Plant biomass and P uptake

Plants were carefully removed from the pots and washed with distilled water toremove soil adhering to the roots. The plants were then dried at 65 ºC for 48 h to determine total plant biomass as dry weight (kg pot^{-1}). After plant dry biomass determination, the P contents (mg kg^{-1}) in the plant tissues were analyzed by the molybdovanadate method as described by Sadzawka *et al.* (2007). Briefly, the samples were dry-ashed in a muffle furnace at 500 °C for 8 h and digested with 2 M HCl. The acid digests were filtered and P in the extracts was quantified by spectrophotometry at 466 nm using a premeasured calibration curve to determine P content. Finally, the P uptake (mg pot-^{1}) was estimated as P content (mg kg^{-1}) × plant dry biomass (kg pot^{-1}).

2.5. Rhizosphere properties

2.5.1. Phosphorus availability

Plant available P in the rhizosphere soil was estimated by P_{Olsen} method as described by Sadzawka *et al.* (2006). Briefly, available P was extracted from rhizospheric soil with a bicarbonate ($NaHCO_3$) solution at pH 8.50. Phosphorus in the extracts was quantitatively measured at 880 nm by a formation of an antimony–phosphate–molybdate complex reduced with ascorbic acid to form a blue-colored complex.

2.5.2. Root surface phosphatase activity

Root surface phosphatase activity was determined according to the procedure described by Rubio *et al.* (1990) and Paredes *et al.* (2011), which uses *p*-nitrophenyl phosphate (*p*-NPP) substrate to measure enzyme activity. Roots were submerged in *p*-NPP solution and incubated at 20 ºC for 30 min. Acid phosphatase activity was calculated as the micrograms of *p*-nitrophenyl (*p*-NP) released per hourper gram root fresh weight.

2.5.3. Soil enzyme activities

Acid phosphatase and urease were chosen as indicators of soil capacity to mineralize Po and N compounds, respectively. Soil acid phosphatase activity was measured using the method described by Rubio *et al*. (1990) for soil with high organic matter content. Samples of 1 g in 4 mL buffer (0.1 M Tris, pH 5.5) were incubated with 0.115M *p*-NPP for 1 h in a 20 ºC water bath. The reaction was stopped by the addition of 1 mL of 0.5 M $CaCl_2$, and the mixture was filtered and centrifuged at 2,500 ×*g* for 10 min. The *p*-NP concentration in the supernatant was measured at 400 nm in a spectrophotometer. Urease activity was assayed by the modified method of Nannipieri *et al.* (1980). Four mL of 0.1 M phosphate buffer (pH 7.1) and 1 mL of 1.067 M urea were added to 1 g soil samples. The samples were incubated at 20 ºC for 2 h, and thereafter, 5 mL of 2 M KCl were added to terminate the reaction. The $N\text{-}NH_4^+$ was determined by an ion selective electrode.

2.5.4. Auxin production potential

To determine the auxin-like substances production potential in the rhizosphere, the method described by Benítez *et al.* (2004) was used. Two grams of fresh rhizosphere soil (without roots) were aseptically collected and placed in a 50 mL flask. Six mL of phosphate buffer (pH 7.5) with glucose (1 g glucose in 100 ml^{-1} phosphate buffer) and 4 mL of 4 mM L-tryptophan were added. Soil suspensions were mixed and incubated at 37 ºC for 24 h in the dark. Two milliliters of 5% trichloroacetic acid solution to stop the enzymatic conversion of tryptophan to auxin, and 1 mL of 0.5 M $CaCl_2$ solution was added. An aliquot was transferred to a 1.5 mL centrifuge tube and centrifuged at 5,000 ×*g* for 5 min. Then, a 1-mL aliquot of the supernatant was mixed vigorously with 2 mL of Salkowski's reagent and allowed to stand at room temperature for 20 min, after which the absorbance at 535 nm was measured. The concentration of IAA-equivalents in each culture medium was determined by comparison with an IAA standard curve.

2.5.5. Rhizobacterial community composition

The effect of PMR on the composition of rhizobacterial communities was evaluated by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) of 16S rRNA genes in DNA extracts from the soils. PCR–DGGEwas carried out as described previously by Jorquera *et al.* (2010). Total DNA was extracted using a Power Soil Soil DNA Isolation Kit (Mo–Bio Laboratories, Inc.), after which 16S rRNA genes were amplified using the primer set EUBf933-GC and EUBr1387. The PCR–DGGE analysis was performed in a 9% (w/v) polyacrylamide gel with a gradient of 30% and 55% (urea and formamide).The electrophoresis was run for 12 h at 100 V, after which

the gels were stained with SYBR Gold (Molecular Probes, Invitro gen Co.) for 30 min and photographed on an UV transilluminator. PCR–DGGE band profiles were compared and dendrograms were generated using Phoretix 1D analysis software (TotalLab Ltd.). Similarities in community composition among samples were calculated by Bray-Curtis method and visualised using non-metric multidimensional scaling (nMDS) utilizing PAST freeware (http://folk.uio.no/ohammer/past/).

3. Results

3.1. Plant biomass and P uptake

Inoculation of wheat and barley plants with PMR did not significantly ($P \leq 0.05$) increased the biomass and P uptake in tissues compared with uninoculated controls (Table 1), except for oat plants inoculated with *Enterobacter* sp. N0-29PA, which significantly ($P \leq 0.05$) increased the plant biomass (3.21g pot^{-1}) and P uptake (2.61 mgpot^{-1}) compared with the uninoculated controls (2.49g pot^{-1} and 1.77 mgpot^{-1}) (Table1).

Table 1. Effect of phytate-mineralizing rhizobacteria inoculation on plant biomass, P content and P uptake of cereal plants without P fertilization.

Wheat	Plant biomass ($\times 10^{-3}$ kg pot^{-1})	P content in tissues (mg kg^{-1})	P uptake* (mg pot^{-1})
Uninoculated control	2.90 ±0.06** a	845 ±28 ab	2.45 ±0.11 a
Bacillus N1-19NA	2.33 ±0.17 b	787 ±12 b	1.83 ±0.11 b
Enterobacter N0-29PA	2.76 ±0.24 ab	863 ±16 ab	2.39 ±0.25 ab
Pseudomonas N1-55PA	2.50 ±0.04 ab	921 ±31 a	2.31 ±0.10 ab
*Serratia*N0-10LB	2.75 ±0.04 ab	883 ±36 ab	2.43 ±0.11 a
Oat	Plant biomass ($\times 10^{-3}$ kg pot^{-1})	P content in tissues (mg kg^{-1})	P uptake* (mg pot^{-1})
Uninoculated control	2.49 ±0.12 b	713 ±43 ab	1.77 ±0.14 b
Bacillus N1-19NA	2.82 ±0.16 ab	759 ±31 ab	2.15 ±0.20 ab
Enterobacter N0-29PA	3.21 ±0.02 a	812 ±44 a	2.61 ±0.15 a
Pseudomonas N1-55PA	2.73 ±0.10 b	667 ±41 b	1.83 ±0.17 b
*Serratia*N0-10LB	2.74 ±0.12 b	745 ±21 ab	2.05 ±0.15 ab
Barley	Plant biomass ($\times 10^{-3}$ kg pot^{-1})	P content in tissues (mg kg^{-1})	P uptake* (mg pot^{-1})
Uninoculated control	1.37 ±0.09 a	656 ±16 a	0.89 ±0.04 a
Bacillus N1-19NA	1.24 ±0.10 a	659 ±22 a	0.82 ±0.08 a
Enterobacter N0-29PA	1.30 ±0.15 a	703 ±7 a	0.92 ±0.11 a
Pseudomonas N1-55PA	1.48 ±0.10 a	698 ±14 a	1.03 ±0.06 a
*Serratia*N0-10LB	1.20 ±0.01 a	674 ±17 a	0.81 ±0.02 a

*calculated as P content (mg kg^{-1}) × Plant dry biomass (kg pot^{-1})

**values represent mean ± standard error (average of three repeats). Different letters in the same column denote significant difference ($P \leq 0.05$).

3.2. Rhizosphere properties

3.2.1. Phosphorus availability and root surface phosphatase activity

Rhizosphere soil collected from wheat and oat plants inoculated with *Enterobacter* sp. N0-29PA had significantly ($P\leq0.05$) greater available P (4.8 mg kg^{-1} in wheat and 3.3 mg kg^{-1} for oat) than rhizosphere soil from uninoculated plants (2.8 mg kg^{-1} and 2.7 mg kg^{-1}, respectively) (Figure 1, A and B). In contrast, in the same treatments the root phosphatase activities were significantly ($P\leq0.05$) lower (907 mg *p*-NP g^{-1} FW h^{-1} for wheat and 906 mg *p*-NP g^{-1} FW $h^{-1}$for oat) as compared to the uninoculated controls (1562 *p*-NP g^{-1} FW h^{-1} for wheat and1351 *p*-NP g^{-1} FW h^{-1} for oat). In oat and barley plants, inoculation with *Bacillus* sp. N1-19NA also significantly ($P\leq0.05$) increased plant available P (3.25 mg kg^{-1} and 4.29 mg kg^{-1}) compared to the uninoculated control (2.7 mg kg^{-1} and 3.5 mg kg^{-1}) and decreased (937 *p*-NP g^{-1} FW h^{-1} and 1718 *p*-NP g^{-1} FW h^{-1}) the activity of root surface phosphatase compared to the uninoculated controls (1351*p*-NP g^{-1} FW $h^{-1}$and 2498*p*-NP g^{-1} FW h^{-1}) (Figure 1, B and C).

3.2.2. Soil enzyme activities

Acid phosphatase and urease activities in the rhizosphere were altered by inoculation, with variable responses depending on the plant species and PMR (Table2). In soils containing wheat plants inoculated with *Pseudomonas* sp. N1-55PA, the acid phosphatase activity was significantly ($P\leq0.05$) lower (446 mg *p*-NP g^{-1} soil h^{-1}) compared tothe soils from other treatments (569-683 mg *p*-NP g^{-1} soil h^{-1}) and uninoculated controls (614 mg *p*-NP g^{-1} soil h^{-1}). In contrast, the application of the *Pseudomonas* sp. N1-55PAto soils planted with oat plants significantly ($P\leq0.05$) increased acid phosphatase (887 mg *p*-NP g^{-1} soil h^{-1}) and urease (6.65 µmol NH_3 g^{-1} soil h^{-1}) activities in rhizosphere soil as compared to the uninoculated controls (789 mg *p*-NP g^{-1} soil h^{-1} and 5.04 µmol NH_3 g^{-1}soil h^{-1}). In barley plants, inoculation with *Enterobacter* sp. N0-29PA resulted ina significant ($P\leq0.05$) increase in acid phosphatase activity (703 mg *p*-NP g^{-1} soil h^{-1}); whereas inoculation with *Serratia* sp. N0-10LB resulted in a significant ($P\leq0.05$) decrease (6.7 µmol NH_3 g^{-1}soil h^{-1}) inurease activity compared with uninoculated controls (605 mg *p*-NP g^{-1} soil h^{-1} and 8.9 µmol NH_3 g^{-1} soil h^{-1}, respectively).

3.2.3. Auxin production potential

The potential for auxin production was increasedin the rhizosphere following inoculation with PMR as compared to uninoculated plants. Values for inoculated wheat plants ranged from 6.5–8.4 µg IAA-equivalents g^{-1} h^{-1} as compared to the uninoculated control plants which had 3.7 µg IAA-equivalents g^{-1} h^{-1} (Figure 2, A). In oat plants, the treatments with PMR had a higher (10.8–12.8 µg IAA-equivalents g^{-1} h^{-1}) auxin production but not significant ($P\leq0.05$) compared with uninoculated control (8.4µg IAA-equivalents g^{-1} h^{-1}) (Figure 2, B). Similarly to wheat plants, the inoculation of barley plants with *Enterobacter* sp. N0-29PA, *Pseudomonas* sp. N1-55PA and *Serratia*sp. N0-10LB significantly ($P\leq0.05$) increased (17.5-19.2 µg IAA-equivalents g^{-1} h^{-1}) IAA production in the rhizosphere (control 11.3 µg IAA-equivalents g^{-1} h^{-1}) (Figure 2, C).

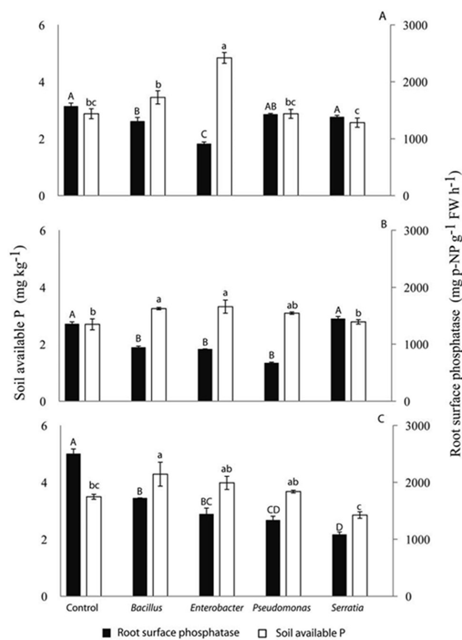

Figure 1. Rhizosphere phosphorus availability and root surface phosphatase activity in A) wheat, B) oat and C) barley plants inoculated with phytate-mineralizing rhizobacteria without P fertilization. Values represent means ± standard error (n=3). Different upper case (or lower case) letters indicate differences (P≤0.05).

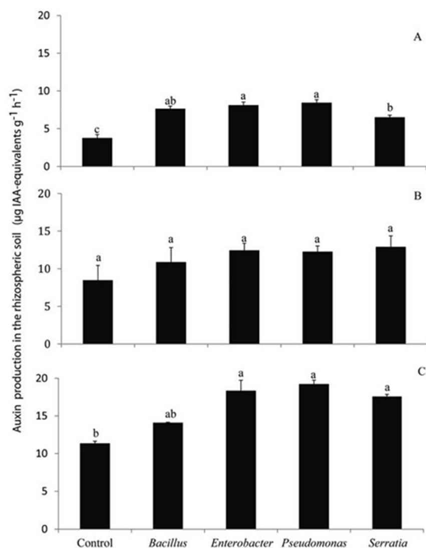

Figure 2. Auxin production potential in the rhizosphere of A) wheat, B) oat and C) barley plants inoculated with phytate-mineralizing rhizobacteria without P fertilization.Values represent mean ± standard error (n=3). Different letters denote significant difference (P≤0.05).

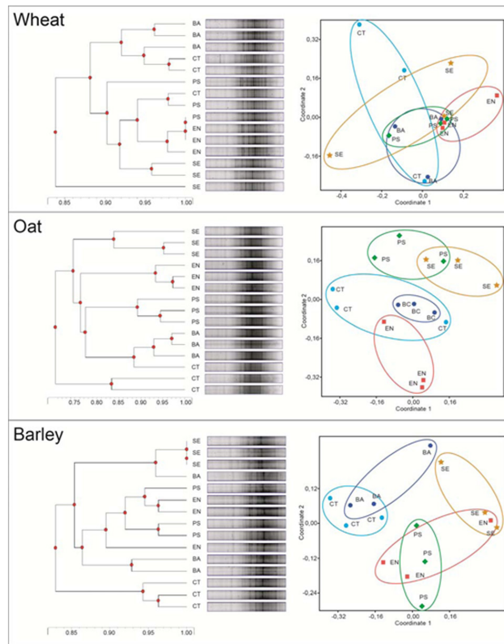

Figure 3. Effect of phytate-mineralizing rhizobacteria on rhizobacterial communities associated with wheat, oat and barley plants without P fertilization, and visualized as dendrogram and non-metric multidimensional scaling (nMDS) analysis of DGGE profiles (16S rRNA gene).

3.3. Rhizobacterial community composition

The dendrograms and nMDS analysis describing the effect of PMR inoculation on compositions of the bacterial communities in the rhizosphere of cereal plants are shown in Figure 3. In wheat, the nMDS analysis did not show any significant change in the bacterial communities of uninoculated and inoculated plants. In contrast, inoculation ofoat plants with either *Pseudomonas* sp. N1-55PA and *Serratia* sp. N0-10LB was associated with significant changes in bacterial community structure compared to the uninoculated control orto plants inoculated with *Bacillus* sp. N1-19NA and *Enterobacter* sp. N0-29PA. Likewise, inoculation of barley plants with either *Enterobacter* sp. N0-29PA, *Pseudomonas* sp. N1-55PA or *Serratia*s p. N0-10LB induced significant changes compared with the uninoculated control and plants inoculated with *Bacillus* sp. N1-19NA. In all cases, *Bacillus* sp. N1-19NA did not produce significant changes in rhizosphere bacterial communities in comparison to the uninoculated control.

4. Discussion

In general, this study showed that inoculation with PMR did not consistently enhanced biomass and P uptake of plants grown in Andisols. These results are similar to those reported by Krey *et al.* (2011) and Ramírez and Kloepper (2010) for non sterile soil and low P available (<10 mg P available kg^{-1} soil) conditions. Krey*et al.* (2011) did not observe significant effects on the plant growth of maize and oil seed rape by the application of two P-mobilizing

strains (*Pseudomonas fluorescens* DR54 and *Enterobacter radicincitans* DSM 16656) alone and mixed. Ramírez and Kloepper (2010) also did not observe an effect on shoot P content of Chinese cabbage by phytase-producing bacilli (*Bacillus amyloliquefaciens* FZB45) inoculation compared with the control. However, our results also showed an increase (~14%) in P content in tissues of oat plants inoculated with *Enterobacter* sp. N0-29PAin PN soil. Moreover, oat plants inoculated with *Enterobacter* sp. N0-29PA increased significantly the plant biomass (~29%) and P uptake (~47%) (Table1). These results reveal the potential of *Enterobacter* sp. N0-29PA as inoculant to improve biomass and P nutrition of oat plants cultivated in P-deficient soils, such as Chilean Andisols. Assays have showed that inoculation with PSR and PMR (*Enterobacter*, *Pseudomonas* and *Bacillus*) significantly increase the biomass and P uptake of plants grown in P deficient soils, even in the absence of P fertilization, suggesting that PSR and PMR may effectively substitute a part of P fertilization (Kumar *et al*., 2013; Viruel *et al*., 2014). In many cases, inoculation of plants with PSR or PMR did not result in an increase of the crop parameters monitored. Several actors are attributed to inconsistent performance; one factor receiving increased attention is the effect of inappropriate method for selected PSR based on their ability to solubilization tricalcium phosphates (Bashan *et al*., 2013).

In relation to P available in the rhizosphere, inoculation with either *Bacillus*sp. N1-19NA or *Enterobacter* sp. N0-29PA significantly increased the concentrations of P_{Olsen} in the rhizosphere as compared to soils inoculated with the other strains or the uninoculated controls (Figure 1). One explanation might be attributed to the phytases that are actively secreted into soil by phytate-mineralizing microorganisms, releasing phosphates from fixed Po in soils, which can then be taken up by plants (Richardson and Simpson, 2011). Another explanation could be that P is released during death and turnover of the bacteria along the older root parts behind the root apices as postulated by Marschner *et al.* (2011). Investigations have shown that available P levels can be increased after soil bacterial inoculation (*Bacillus megaterium*, *Pseudomonas striata*), but have not investigated the mechanism by which this occurs (Sundara *et al*., 2002; Viruel *et al*.,2014). Sundara *et al*. (2002) showed an increase in P_{Olsen} in the rhizosphere of sugarcane inoculated with *Bacillus megatherium* var. *phosphaticum*. Still other processes by which bacteria might enhance plant P uptake could involve stimulation of root hair formation, or increased rates of organic acids exudation by microorganisms or plant roots (Richardson and Simpson, 2011). A recent study reports that the inoculation of cattle manure with phytate-mineralizing *Bacillus* increased the Pi released from phytase-labile P pool contained in the manure, however, applying inoculated manure to soil did not significantly increase yield or P uptake by wheat plants (Menezes-Blackburn *et al*., 2014).

One of the most interesting observations from this research is the inverse relationship between the level of available P in the rhizosphere and the phosphatase activity of the roots in plants inoculated with *Bacillus* sp. N1-19NA and *Enterobacter* sp. N0-29PA (Figure 1). This phenomenon was previously reported by Paredes *et al*. (2011), in which they found that the root surface phosphatase was inhibited at least 65% in response to increased available P in the rhizosphere. Under P deficiency, it would be hypothesized that the root plants secrete phosphatases to increase P mobilization in the rhizosphere. However, the regulation of root surface phosphatase activity with respect to plant available P in the rhizosphere is not yet well understood. Moreover, the possible suppression of this enzyme in the presence of bacteria that putatively enhances P availability requires further explanation. One possibility is that local elevated P

contents in the soil may suppress the induction of the surface enzyme activity even while the plant shoot suffers from P deficiency.

In relation to soil enzyme activities in the rhizosphere, inoculation with *Pseudomonas* sp. N1-55PA increased acid phosphatase and urease activities in the rhizosphere of oat plants (Table 2). This result is in agreement with Mäder *et al*. (2011) who reported that combined application of *Pseudomonas* strains (*P. jessenii* R62 and *P. synxantha* R81) and arbuscular mycorrhizal fungi increased soil enzyme activities of alkaline phosphatase, acid phosphatase, urease and dehydrogenase in the rhizosphere of wheat. In contrast, inoculation with *Pseudomonas* sp. N1-55PA decreased acid phosphatase in the rhizosphere of wheat plants (Table 2). This observation also suggets varied effects on soil enzyme activities according to plant types and strains inoculated.

Table 2. The effect of phytate-mineralizing rhizobacteria inoculation on soil enzyme activities of cereal plants without P fertilization.

Parameter	Treatments				
	Control	*Bacillus* N1-19NA	*Enterobacter* N0-29PA	*Pseudomonas* N1-55PA	*Serratia* N0-10LB
Wheat					
Acid phosphatase (mg *p*-NP g^{-1} soil h^{-1})	614 ±40* ab	683±17 a	649±17.8 ab	446±25.7 c	569.3±5 b
Urease (μmol NH_3 g^{-1}soil h^{-1})	4.05±0.3 ab	3.38±0.03 b	4.14±0.17 a	3.64±0.01 ab	3.81±0.04ab
Oat					
Acid phosphatase (mg *p*-NP g^{-1} soil h^{-1})	789.1±12 b	824.2±18 ab	836±0 ab	887±27 a	832±10 ab
Urease (μmol NH_3 g^{-1}soil h^{-1})	5.04±0.33 b	5.02±0.4 b	6.19±0.37 ab	6.65±0.63 a	6.57±0.16 a
Barley					
Acid phosphatase (mg *p*-NP g^{-1} soil h^{-1})	604.7±6 b	601.4±15 b	702.7±29 a	665.5±6 ab	632±9 ab
Urease (μmol NH_3 g^{-1}soil h^{-1})	8.9±0.8 a	8.6±0.09 ab	8.5±0.55 ab	9.2±0.24 a	6.7±0.16 b

*values represent mean ± standard error (average of three repeats). Different letters in the same row denote significant difference ($P \leq 0.05$).

As expected, inoculation of plants with the PMR strains enhanced the potential production of auxin, indirectly suggesting an effective colonization of the roots by the introduced strains (Figure 2). Production of auxin by rhizobacteria has been suggested as one of the most important factors affecting plant growth and may indirectly affect Puptake by modifying of architecture and morphology of roots or by altering plant root exudation (Marschner *et al*., 2011). However, in general terms a correlation between auxin productionpotential in the rhizosphere and plant growth was not observed in this study.

One of the most difficult questions in the development of soil inoculants is their interaction with the native rhizosphere microbial community. Bacterial inoculants that are introduced into soils must compete with indigenous rhizobacteria that are well adapted to the local environment. In this research, we found that the compositions of bacterial communities present in the rhizosphere of oat and barley were significantly affected by PMR inoculations (*Enterobacter* sp.N0-29PA, *Pseudomonas* sp. N1-55PA and *Serratia* sp. N0-10LB) but not in the rhizosphere of wheat (Figure 3). These results are similar to those reported in previously published studies. Grandlic *et al*. (2009) evaluated the effect of plant growth-promoting bacteria (*Clavibacter* sp., *Rhodanobacter* sp. and *Arthrobacter* sp.) inoculations on *Buchloe*

dactyloides rhizosphere microbial community structure. In that work, PCR–DGGE and nMD Sanalysis of the 16S rRNA gene profiles showed that rhizosphere community structures from rhizobacteria-inoculated treatments were different from both uninoculated tailings rhizosphere profiles and profiles from the compost used to amend the tailings. Likewise,de-Bashan *et al*. (2010) reported changes in rhizosphere (*Atriplex lentiformis*) community structure in response to *Azospirillum brasilense* Sp6 inoculation by PCR–DGGE analysis. They suggested that soil inoculants not only could persist and stimulate plant growth, but also can directly or indirectly influence rhizobacterial community development. Here we observed inconsistent effects of inoculants on rhizosphere community structure. Inoculation with *Bacillus* sp.N1-19NA did not cause changes in rhizobacterial community structure for any of the three plants assayed, whereas inoculation with *Pseudomonas* sp. N1-55PA and *Serratia* sp. N0-10LB resulted in shifts in community structures in oat and barley. Many reports suggest that inoculants do not have noticeable effects on community structure using low-resolution methods based on 16S rRNA gene profiles. Herschkovitz *et al*. (2005) reported that *A. brasilense* inoculation did not alter or disrupt the microbial structure at the group-specific level in maize rhizosphere as revealed by PCR–DGGE finger prints analysis. Likewise, Lerner *et al*. (2006) reported that no prominent effect of *A. brasilense* inoculation was observed on the bacterial communities of plant roots grown in two different soils by PCR–DGGE and ARISA (automated ribosomal intergenic spacer analysis). Piromyou *et al*. (2011) inoculated forage corn rhizosphere with *Pseudomonas* sp. SUT 19 and *Brevibacillus* sp. SUT 47 and they reported that dominant species in microbial community structure were not altered by PGPR strains.

5. Conclusions

The greenhouse experiment conducted here showed that the effects of inoculation with phytate-mineralizing rhizobacteria (PMR) were variable depending on the introduced bacterial strain and the cereal plant species. However, the PMR inoculation clearly influenced plant and rhizosphere properties, such plant biomass, P content in tissues, P uptake, root-surface phosphatase activity, phosphorus availability, auxin production potential, and soil enzyme activities. Particularly, *Enterobacter* N0-29PA significantly increased plant biomass and Puptake of oat plants in volcanic soil without P fertilization. However, the evaluation of *Enterobacter* N0-29PA as a complement to or partial substitute for P fertilization in cultivated Andisol requires major studies and analysis before recommendations can be made for its use as a biofertilizer. In addtion, PMR inoculation induced substantial changes in rhizobacterial community composition as revealed by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) and non-metric multidimensional scaling (nMDS) analysis. This result shows that PMR application substantially modified the bacterial communities in the rhizosphere. However, long-term studies are required to confirm if these changes are maintained by PMR inoculants at field level in Chilean Andisols.

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