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Indole acetic acid and phytase activity produced by rhizosphere bacilli as affected by pH and metals

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Abstract

The abilities to produce indole acetic acid (IAA) and mineralize organic phosphorus by phytase are desirable traits in plant−growth promotion rhizobacteria (PGPR) particularly in Chilean Andisols which are characterized by low pH and high total P. However, little is known about the influence of soil properties that are specific to Andisol (low pH and metal toxicity) on the effectiveness of PGPR. Here, we assessed the effect of pH and metal cations on IAA and phytase activity of cell-associated proteins produced by two bacilli strains isolated from the rhizosphere of pasture plants. The production *in vivo* of IAA by *Paenibacillus* sp. SPT−03 was significantly increased (7−fold) when incubated in tenfold diluted culture medium, compared to the full–strength medium. At low pH (pH<5), phytase activity of cell−associated proteins and IAA production of *Bacillus* sp. MQH−19 was decreased, whereas they were increased in *Paenibacillus* sp. SPT−03. Moreover, phytase activity *in vitro* of cell−associated proteins and IAA production in both bacilli strains were significantly inhibited by 30−100% and 44−70% by concentrations of 10 mM and 350 µM Fe^{3+} and Al^{3+}, respectively. At 350 µM Mn^{2+} IAA production was inhibited by 30−100% in both strains but there was no effect on phytase activity. This study shows that certain properties of Andisol may differentially affect some mechanisms related with PGPR efficiency.

Keywords: Andisol, *Bacillus*, indole acetic acid, phytase, rhizobacteria, rhizosphere.

1. Introduction

In the rhizosphere, defined as the soil influenced by roots, bacterial species that carry out functions which promote growth of plants have been defined as plant−growth promotion rhizobacteria (PGPR) (Martínez-Viveros *et al.*, 2010). The enhancement of plant growth by members of bacilli strains, such as Bacillus and Paenibacillus, has been well documented (McSpadden, 2004; Ona, 2003). They promote plant growth by a number of mechanisms, including the solubilization of phosphorus and production of phytohormones, such as indole acetic acid (IAA) (Choudhary and Johri, 2009; Lal and Tabacchioni, 2009). Phytohormones such as IAA may indirectly improve P acquisition by plants by increasing root growth (Marschner *et al.*, 2011). Moreover, Bacillus and Paenibacillus are also able to produce endospores which enhances their persistence and viability in soils (Lal and Tabacchioni, 2009; Nicholson, 2002). Hence, PGPR bacilli strains which have multiple mechanisms by which they promote plant growth have attracted considerable interest by microbiologists; biofertilizers containing Bacillus and Paenibacillus strains have been developed and commercialized. However, successful application of PGPR in the field is limited by a lack of knowledge on how environmental factors affect their survival and functionality in the plant rhizosphere.

Volcanic ash−derived soils (Andisols) in southern Chile have high contents of organic phosphorus (mainly as inositol phosphate, also known as phytate) (Borie and Rubio 2003). Rhizobacteria with the ability to mineralize phytate have been isolated and may improve P uptake by plants in these soils (Jorquera *et al.*, 2008; Patel *et al.*, 2010; Unno *et al.*, 2005). However, these soils are also characterized by low pH (<5.5) as a consequence of natural acidification and long−term application of acidifying N fertilizers (particularly urea) (Mora *et al.*, 2004). The low pH limits plant growth because the concentrations of metals (Al^{3+} and Mn^{2+}) in the soil solution can reach toxic levels. It is known that soil pH and metal cations may affect many processes occurring in the rhizosphere (Greiner, 2004; Idris *et al.*, 2007). Recently, Martinez *et al.* (2011) demonstrated that N fertilisation and factors present in Chilean Andisols (such as organic acids and metals) can have a relevant role in the occurrence and performance of culturable IAA-producing rhizobacteria. However, our knowledge if these factors also influence the effectiveness of soil inoculants in Chilean Andisols is still limited.

The main objective of this study was to assess the effect of pH and metal cations on the IAA and phytase activity produced by two bacilli strains isolated from the rhizosphere of pasture plants growing on an Andisol.

2. Material and methods

2.1. Bacilli strains

The bacilli strains used in this study were *Bacillus* sp. MQH−19 and *Paenibacillus* sp. SPT−03. Both strains were isolated from pasture containing graminaceous plants (*Lolium* sp., *Festuca* sp., *Dactylis* sp.) by Jorquera *et al.*, (2011). *Bacillus* sp. MQH−19 was isolated from pasture located at the experimental station Maquehue (Andisol Freire series; 38°50' S, 72°41' W) of La Frontera University, which had a history of intensive annual fertilization with urea (300 kg ha^{-1}), triple super phosphate (400 kg ha^{-1}), potassium magnesium sulfate (300 kg ha^{-1}), and calcium carbonate (500 kg ha^{-1}). In contrast, *Paenibacillus* sp. SPT−03 was isolated from a natural pasture at San Pablo de Tregua (Andisol Liquiñe series; 39°36' S, 72°3' W), which had not received any chemical or manure inputs for 60 years. Our recent studies have demonstrat-

ed that both strains carry genes encoding β−propeller type phytases and have the ability to degrade phytate (Jorquera *et al.*, 2011).

2.2. Biochemical and enzymatic characterization

Substrate utilization and enzyme release of *Bacillus* sp. MQH−19 and *Paenibacillus* sp. SPT−03 was evaluated by using commercial API® kits (20NE, 20E and ZYM; bioMérieux) which are commonly used for bacterial characterization. *Bacilli* strains were taken from overnight cultured in Luria−Bertani (LB) broth (g l^{-1}): 1 tryptone, 0.5 yeast extract, 0.5 NaCl and pH 7.0. Cells were washed, suspended in sterile saline solution (0.9% w/v of NaCl) and then inoculated into each test tray following the procedure recommended by manufacturer.

The production of siderophores, iron (Fe) chelating agents which may prevent the proliferation of plant pathogenic bacteria in the rhizosphere (Siddiqui, 2006), was evaluated on universal chrome azurol 'S' (CAS) medium as described by Alexander and Zuberer (1991). After incubation for 4 days, the appearance of orange zones around the colonies was taken as an indicator of siderophore production.

To test the ability to release phosphate from insoluble inorganic and organic P forms, the strains were cultured under shaking (120 rpm) for 2 days at 30°C in NBRIP (National Botanical Research Institute's phosphate growth medium) (Nautiyal, 1999) and PSM (phytase−screening medium) (Kerovuo *et al.*, 1998) broths, containing Ca−phosphate ($Ca_3(PO_4)_2$) and Na−phytate ($C_6H_{18}O_{24}P_6 \cdot xNa^+ \cdot yH_2O$) as sole P sources, respectively. Un−inoculated broths served as controls. Phosphate released in liquid media was measured at 355 nm using the ammonium molybdate method (Heinonen and Lahti, 1981) and quantified by comparison with a standard curve prepared with known concentrations of PO_4^{2-}.

2.3. Phytase activity in vitro

Bacillus sp. MQH−19 and *Paenibacillus* sp. SPT−03 were grown in 50 ml PSM broth (pH 7.0) for 2 days at 30°C and extracellular and intracellular phytase activity was measured in broth and protein extracts as follows. Bacterial cells and supernatant were separated by centrifugation (3,600 rpm for 5 min) and the supernatant was subjected to ammonium sulfate (0−85%) precipitation. The cell pellet was treated with lysozyme (5 mg ml^{-1}) and sonicated (20 kHz constant frequency for 2 min) to induce cell lysis. The cell debris was centrifuged (5,000 rpm for 5 min) and the supernatant was also subjected to ammonium sulfate precipitation. Both pellets were suspended and stored in Tris−HCl buffer pH 7.0 at −20°C. The phytase activity was assayed according to Greiner *et al.*, (2004). Ten µl of crude protein extract was incubated with 270 µl of Na−phytate solution (2.5 mM of phytate in Tris−HCl buffer pH 7.0) for 30 min at 37°C. The reaction was stopped by addition of 1,150 µl of a 2:1:1mixture of fresh acetone, sulfuric acid, ammonium molybdate (10 mM) and 80 µl of citric acid (1 M). Then the solution was centrifuged (5,000 rpm for 5 min), the absorbance of the supernatant was measured at 355 nm and compared with standard curve of PO_4^{2-}. One unit of phytase activity is equivalent to 1 µM P released in 1 min. Blanks were performed by adding the stop solution prior to substrate addition. Phytase activity under acidic condition (sodium acetate buffer pH 4.5) was also measured.

2.4. Production of IAA in vivo

The production of IAA by the bacilli strains was determined by colorimetric measurement at 530 nm using Salkowski's reagent as described by Patten and Glick (2002). Bacterial cells were grown under shaking (120 rpm) for 2 days at 30ºC in LB broth and tenfold diluted LB broth (dLB) at pH 5.0 and pH 7.0

supplemented with tryptophan (1 mg ml^{-1}) as IAA precursor. After incubation, the cells were centrifuged (3,000 rpm for 10 min at 4°C) and 1 ml of supernatant was combined with 2 ml of Salkowski's reagent (150 ml of 95-98% H_2SO_4, 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$, and 250 ml distilled water) and incubated for 30 min at room temperature. The quantification of IAA was carried out using a standard curve with known concentrations of pure IAA (Sigma−Aldrich, Co.).

Under acidic condition, the effect of several amino acids as precursors for production of IAA by bacilli strains was also assayed. Bacterial cultures in LB and dLB at pH 5.0 were supplemented with the following amino acids (1 mg ml^{-1}): proline, phenylalanine, alanine, cysteine and methionine. Cultures supplemented with tryptophan (1 mg ml^{-1}) were used as positive controls and uninoculated broths as negative controls. Tryptophan resulted in the highest IAA concentration and was therefore used for further assays.

2.5. Effect of pH and metal cations on phytase activity in vitro

Crude protein extracts from *Bacillus* sp. MQH−19 and *Paenibacillus* sp. SPT−03 grown in PSM were obtained as described above. The buffer used to determine the effect of pH on phytase activity of cell−associated proteins had the following composition: 100m M sodium acetate−acetic acid (pH 3.5, 4.5 and 5.5), 100 mM sodium acetate−HCl (pH 6.5), and 100 mM Tris−HCl (pH 7.0).

The *in vitro* effect of metal cations on phytase activity of cell−associated proteins was tested according to method described by Greiner *et al.*, (2004). The total crude protein was incubated for 15 min at 37ºC with Fe^{3+}, Al^{2+} and Mn^{2+} at a final concentration of 10 mM at the optimal pH for cell−associated phytase activity in each strain: pH 7.0 for *Bacillus* sp. MQH−19 and pH 4.5 for *Paenibacillus* sp. SPT−03 .

2.6. Effect of pH and metal cations on production of IAA in vivo

To test the effect of pH, *Bacillus* sp. MQH−19 and *Paenibacillus* sp. SPT−03 were grown under shaking (120 rpm) for 2 days at 30ºC in dLB supplemented with tryptophan at pH 5.0, 6.0 and 7.0.

To evaluate the *in vivo* the effect of metal cations on IAA production, bacilli strains were cultured for 2 days at 30ºC in dLB (at pH 5.0) supplemented with the following metals (350 µM): Fe^{3+}, Al^{2+} and Mn^{2+}. This concentration represents the higher range of metal concentrations in the soil solution of Chilean Andisols (Mora *et al.*, 2009; Rosas *et al.*, 2007) Cell density (O.D. 600 nm) measurements showed that bacterial growth was not significantly affected by any treatment (data no shown). The IAA production was quantified as described above at point 2.4.

2.7. Analysis of data

Each experiment was performed in triplicate and repeated at least twice. Statistical analysis was performed by using the statistical software JMP®, version 5.0 (SAS Institute, Inc.). Before statistical analysis, the data were tested for normality. The significance of each treatment was established by one way ANOVA and the means were separated by Tukey's test ($p \leq 0.05$).

3. Results

3.1. Characterization of bacilli strains

The biochemical and enzymatic characterization of *Paenibacillus* sp. SPT−03 and *Bacillus* sp. MQH−19 is shown in the Table 1. Both strains showed common characteristics, such as production of α−glucosidase, β−glucosidase, acetoin, gelatinase, esterase, acid phosphatase and naphthol phosphohydrolase; and utilization/assimilation of D−glucose, D−mannitol, D−

mannose, N−acetyl−glucosamine, D−maltose, potassium gluconate, malate and citrate. Also, both strains were able to reduce nitrate and to produce siderophores on agar. In contrast to *Bacillus* sp. MQH−19, *Paenibacillus* sp. SPT−03 was able to produce β−galactosidase, ornithine decarboxylase and α−galactosidase; and utilize/assimilate rhamnose, D−melibiose and phenylacetic acids. Furthermore, *Paenibacillus* sp. SPT−03 produced all peptid hydrolases assayed (α−chymotrypsin, trypsin, valine arylamidase, cystine arylamidase and leucine arylamidase).

In relation to P release, *Bacillus* sp. MQH−19 had a higher phytate mineralization capacity, whereas *Paenibacillus* sp. SPT−03 showed a greater ability to solubilize inorganic P.

3.2. Phytase activity and production of IAA

The phytase activity assays showed that both bacilli strains had cell−associated phytase activity. After 30 min of incubation with phytate at pH 7.0, *Bacillus* sp. MQH−19 showed a mean value of 141 mU mg^{-1} protein, whereas no phytase activity was detectable in the crude protein extract from cells of *Paenibacillus* sp. SPT−03. However, at low pH (4.5), both *Bacillus* sp. MQH−19 and *Paenibacillus* sp. SPT−03 showed phytase activities with average values of 35 and 82 mU mg^{-1} protein, respectively. Phytase activity was not detected in the pellets of either bacilli strain.

Both bacteria were able to produce IAA when supplemented with tryptophan (Figure 1). However, IAA production was significantly higher ($P \leq 0.05$) in *Paenibacillus* sp. SPT−03 grown in dLB (32−37 μg ml^{-1}) compared with LB (4−6 μg ml^{-1}), and compared with *Bacillus* sp. MQH−19 grown in dLB and LB (3−6 μg ml^{-1}). Under acidic conditions (pH 5.0), IAA production was higher in LB and dLB supplemented with tryptophan compared to the other amino acids assayed, particularly in *Paenibacillus* sp. SPT−03 (Table 2).

3.3. Effect of pH and metal cations on phytase activity and production of IAA

The strains differed in optimal pH for phytase activity (Figure 2). Cell−associated phytase was highest at pH 7.0 for *Bacillus* sp. MQH−19 and at pH 4.5 for *Paenibacillus* sp. SPT−03. IAA production by *Bacillus* sp. MQH−19 was highest at pH 6.0 and decreased by 62% at pH 5.0 (Figure 2). For *Paenibacillus* sp. SPT−03, IAA production was highest at pH 5.0 and decreased by 42% at pH 7.0.

Ten mM Fe^{3+} and 350 μM Al^{3+} significantly decreased cell−associated phytase activity and IAA production (Figure 3). Compared to the controls, the cell−associated phytase activity of *Bacillus* sp. MQH−19 in full-strength LB was inhibited by 100 and 54% in the presence of Fe^{3+} and Al^{3+}, respectively. Cell−associated phytase activity from *Paenibacillus* sp. SPT−03 was inhibited by 70 and 30% by Fe^{3+} and Al^{3+}, respectively. The metals had similar negative effects on production of IAA in dLB (Figure 4). Compared to the control, presence of Fe^{3+} and Al^{3+} reduced IAA production in *Bacillus* sp. MQH−19 by 57−70% and in *Paenibacillus* sp. SPT−03 by >90%. In addition, 350 μM Mn^{2+} inhibited production of IAA by 44−50% in both bacilli strains, but did not affect the phytase activity of the crude protein extracts. Additionally, we carried out a chemical speciation analysis (GeoChemEZ) to evaluate the possible interaction of metals on phytase activity. The analysis revealed that 98% Al and 100% Fe form complexes with phytate while 32% of Mn could be found as Mn^{2+}.

3.4. Discussion

Rhizobacteria can enhance plant growth by a number of mechanisms, hence it is likely that plant growth promotion by rhizobacteria is not the result of a single mechanism but rather the combined result of several mecha-

nisms acting simultaneously (Martínez-Viveros *et al.*, 2010). In the present study, we showed that *Bacillus* sp. MQH−19 and *Paenibacillus* sp. SPT−03 are able to produce diverse enzymes and utilize various substrates, and are able to utilize P from insoluble organic and inorganic P forms. Chilean Andisols are characterized by high concentrations of total and organic P (Borie and Rubio, 2003) and *Pseudomonas*, *Enterobacter* and *Pantoea* with ability to mineralize/solubilize P and produce phosphorus hydrolases have previously isolated from pasture plants grown in Chilean Andisol (Jorquera *et al.*, 2008). We have previously reported phytase activity in *Bacillus* sp. MQH−19 (1.9×10^{-10} kat mg^{-1}) protein and *Paenibacillus* sp. SPT−03 (5.9×10^{-10} kat mg^{-1} protein) (Jorquera *et al.*, 2011), but here we also show that our bacilli strains are capable to produce siderophores and synthesize IAA. Bacilli strains that can solubilize P and produce siderophore and IAA have widely been reported (Raddadi *et al.*, 2008; Trivedi and Pandey, 2008). Previous studies have reported IAA productions between 12−48 μg ml^{-1} in *Bacillus* (Ali *et al.*, 2009) and 4.6−44.6 μg ml^{-1} in *Paenibacillus* (Lebuhn *et al.*, 1997). These values are similar to that observed for *Paenibacillus* sp. SPT−03 (4−37 μg IAA ml^{-1}), but higher than that of *Bacillus* sp. MQH−19 (3−6 μg IAA ml^{-1}). Bacilli strains that can solubilize P and produce IAA have been shown to promote the growth of maize and wheat (Beneduzi *et al.*, 2008; Trivedi and Pandey, 2008)

The stimulation of IAA release by tryptophan found in the present study is in accordance with previous reports (Ali *et al.*, 2009; Lebuhn *et al.*, 1997). In *Bacillus*, the production of IAA has been described as tryptophan-dependent (Ali *et al.*, 2009; Idris *et al.*, 2007). However in the absence of tryptophan, the bacilli strains were also able to synthesize small amounts of IAA, suggesting that bacteria may use other aromatic amino acids as substrates for IAA production (Moat *et al.* 2002). Biosynthesis of IAA via tryptophan−independent pathways has been reported previously (Baca and Elmerich, 2007; Spaepen *et al.*, 2006). In agreement with our results, IAA production by *Pantoea agglomerans* was lower in the presence of other aromatic amino acids than with tryptophan (Sergeeva *et al.*, 2007).

Interestingly, IAA production by *Paenibacillus* sp. SPT−03 was greater in the tenfold diluted LB broth than in the full-strength medium. It has been reported that IAA production by *Azospirillum brasilense* requires depletion of the carbon source in the growth medium (Ona *et al.*, 2003) and is enhanced by N limitation (Malhotra and Srivastava, 2008).

The effect of pH and metals on phytase activity and IAA production differed between the *Bacillus* strains. Maximal phytase activity and IAA production were found at pH>5.0 in *Bacillus* sp. MQH−19 but at pH<5.0 in *Paenibacillus* sp. SPT−03. The studied Bacilli strains contain phytase−encoding genes with high similarity to β−propeller phytases of *Bacillus* (Jorquera *et al.*, 2011). The optimal pH for β−propeller phytases is in the neutral and alkaline range (Fu *et al.*, 2008). Hence the cell−associated proteins from *Paenibacillus* sp. SPT−03 seems to be an unusual β−propeller type phytase or contain another phytase types which we have not been identified, yet. However, a β−propeller phytase with maximal activity at acid pH has been reported for *Bacillus licheniformis* (Tye *et al.*, 2002).

The effect of pH on IAA production is in agreement with studies with *Azospirillum brasilense* (Ona *et al.*, 2003; Vande Broek *et al.*, 2005). Maximal IAA production in *A. brasilense* was at pH 6.2 (Malhotra and Srivastava, 2008) and at pH 8 in *Klebsiella pneumoniae* (Sachdev *et al.*, 2009). In the present study, 10 mM Al^{3+} and Fe^{3+} inhibited phytate hydrolysis in both strains, whereas Mn^{2+} had no effect. Inhibition of phytate hydrolysis by Al^{3+} and Fe^{3+} but not by Mn^{2+} were reported by Greiner *et al.*, (2004) in phytase from *Pantoea agglomerans* suggesting similarity between phytase from *Pantoea* and studied *Bacilli* strains. Nevertheless, the chemical speciation results indicated that

metals might not be phytase inhibitors, but the hydrolysis was in fact affected by a phytate-ligand sequestration effect, which is in accordance to previous studies with fungal phytases (Dao, 2004)

The cations (Fe^{3+}, Al^{+3} and Mn^{2+}) inhibited IAA production, which is in agreement with Dimkpa *et al.*, (2008), who demonstrated a negative effect of Fe^{3+} and Al^{+3} on auxin production by *Streptomyces* strains. Siderophores may reduce the toxic effect of metal cations by chelation (Dimkpa *et al.*, 2008; 2009), but although the Bacillus strains can produce siderophores, this mechanism did not seem to be effective in our study.

Table 1. Biochemical and enzymatic characterization of *Bacillus* sp. MQH-19 and *Paenibacillus* sp. SPT-03

	Bacilli strains				
	MQH-19	SPT-03		MQH-19	SPT-03
Production of:			Glycosidases		
β−galactosidase	−	+	α−fucosidase	−	−
β−glucosidase	+	+	α−mannosidase	−	−
Arginine dihydrolase	−	−	α−galactosidase	−	+
Lysine decarboxylase	−	+/−	α−glucosidase	+	+
Ornithine decarboxylase	−	+	N−acetyl−β−glucosaminidase	−	−
H2S	−	−	β−glucuronidase	−	−
Urease	−	−			
Tryptophane deaminase	+/−	+	Peptid hydrolases		
Indole	−	+/−	α−chymotrypsin	−	+
Acetoin	+	+	Trypsin	−	+/−
Gelatinase	+	+	Valine arylamidase	−	+
			Cystine arylamidase	−	+
Utilization/assimilation of:			Leucine arylamidase	−	+
D−glucose	+	+			
D−mannitol	+	+	Ester hydrolases		
D−mannose	+	+	Lipase	−	+/−
Inositol	−	−	Esterase/lipase	+	+/−
D−sorbitol	+/−	+	Esterase	+	+
L−rhamnose	−	+			
D−sacarose	+/−	+	Phosphoric hydrolases		
D−melibiose	−	+	Alkaline phosphatase	+/−	+
Amygdalin	+/−	+	Acid phosphatase	+	+
L−arabinose	+/−	+	Naphthol phosphohydrolase	+	+
N−acetyl−glucosamine	+	+			
D−maltose	+	+	P releasing (μM ml−1)		
Potassium gluconate	+	+	PSM	2.6±0.1	N.D.
Capric acid	−	−	NBRIP	0.3±0.1	3.2±0.3
Adipic acid	−	+/−			
Phenylacetic acid	−	+	Siderophore production	+	+
Malate	+	+	Nitrate reduction	+	+
Citrate	+	+			

+ : positive reaction; +/–: weak positive reaction; –: negative reaction. N.D.: not detected. PSM: phytase–screening medium. NBRIP: national botanical research institute's phosphate growth medium.

Table 2. Effect of aminoacids on production of IAA by *Bacillus* sp. MQH−19 and *Paenibacillus* SPT−03 in LB broth and tenfold diluted LB broth (dLB) at pH 5.0.

	Bacilli strains			
	MQH-19		SPT-03	
IAA Production (μg ml^{-1})	LB	dLB	LB	dLB
Control	1.4 ± 0.003	0.5 ± 0.002	1.9 ± 0.003	0.9 ± 0.004
Proline	0.6 ± 0.003	1.2 ± 0.004	2.1 ± 0.005	1.2 ± 0.001
Phenylalanine	0.5 ± 0.004	0.5 ± 0.002	1.1 ± 0.009	1.2 ± 0.005
Alanine	0.4 ± 0.002	0.6 ± 0.006	2 ± 0.005	0.7 ± 0.002
Cysteine	1.8 ± 0.018	0.4 ± 0.007	2.1 ± 0.006	1.4 ± 0.005
Methionine	1.2 ± 0.021	0.4 ± 0.001	0.6 ± 0.015	1.1 ± 0.150
Tryptophan	9.4 ± 0.018 [a]	2.7 ± 0.040 [b]	10.4 ± 0.008 [b]	31.2 ± 0.026 [a]

Samples (n=6). Numbers without letter are not significantly different (Tukey, $p \leq 0.05$)

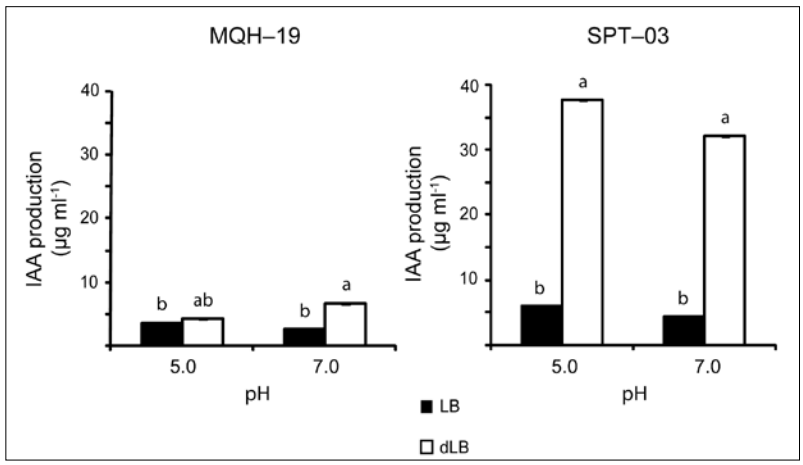

Figure 1. Production of IAA by *Bacillus* sp. MQH−19 and *Paenibacillus* SPT−03 in LB broth and tenfold diluted LB broth (dLB) at pH 5.0 and 7.0 (n=6). Bars with the same letter are not significantly different (Tukey, $p \leq 0.05$).

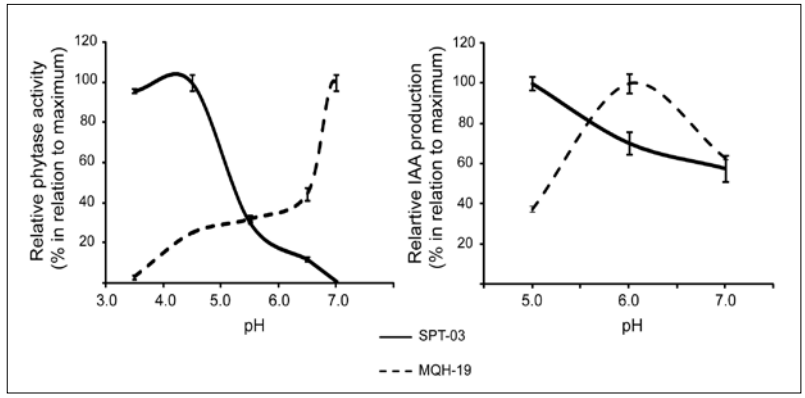

Figure 2. Effect of pH on phytase activity of cell−associated proteins (left graph) and production of IAA (right graph) by *Bacillus* sp. MQH−19 and *Paenibacillus* SPT−03 in dLB broth (n=6). Vertical lines represent standard error.

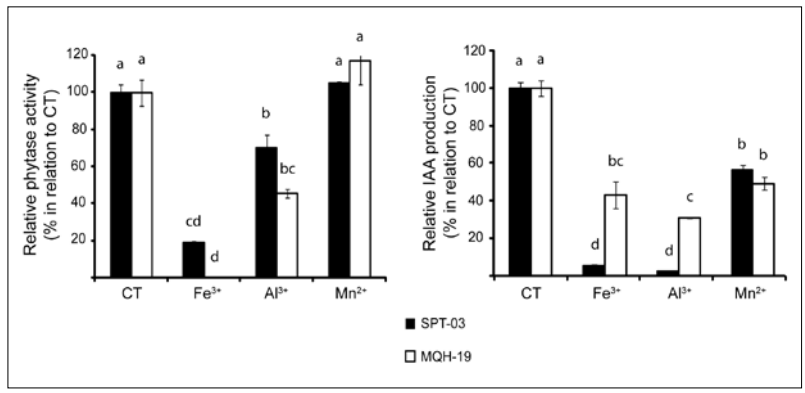

Figure 3. Effect of metal cations on phytase activity of cell−associated proteins (left graph) and production of IAA (right graph) by *Bacillus* sp. MQH-19 and *Paenibacillus* sp. SPT-03 in dLB broth at pH 5.0. CT= control; Fe= iron; Al= aluminum; Mn= manganese (n=6). Bars with the same letter on bars are not significantly different (Tukey, $p \leq 0.05$).

4. Conclusions

Our study showed that environmental factors, including nutrient and amino acid availability, pH and metal cations, may influence the phytase activity and/or IAA production by bacilli strains present in Chilean Andisols. Differences between two bacilli strains were observed, suggesting that *Paenibacillus* sp. SPT−03 may be more an efficient inoculant for Chilean Andisol, due to its higher phytase activity and IAA production under acidic condition. These differences between rhizobacteria should be taken into account to maximize the effectiveness of an inoculant when are applied in the different soil types, particularly in acidic soils.

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