Bacillus-like phosphobacteria in agronomic volcanic soils from Chile

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Abstract
Here we evaluated the occurrence of *Bacillus*-like phosphobacteria (BLP) in rhizospheric soils from diverse grasslands established on agronomic volcanic soils of Southern Chile. BLP were grown on culture media containing insoluble phytate and phosphate as sole phosphorus (P) source. Additionally, phytase gene was detected by PCR using a degenerate primer set for *Bacillus* phytases. Sequencing of DNA fragments amplified by PCR, revealed the presence of *Paenibacillus* carrying phytase gene close to 3-phytase. This study reveals that Chilean volcanic soils are a reservoir of BLP populations which can present mechanisms involved in the expression of phytases. To our knowledge, this is first report on detection and characterization of *Bacillus* phytase gene in rhizospheric soils.

Key Words
Bacillus. phytase, phytate, rhizosphere, phosphobacteria, volcanic soils

Introduction
The biofertilizers are live microorganisms that are able to colonize the rhizosphere or internal tissues of plants, promoting the growth of host plant through increase supply or availability of nutrients, such as nitrogen and P (Richardson *et al.* 2009). Bacteria with capacity to release phosphate from insoluble P forms by organic acid production and enzymatic activity (i.e. phytase), also known as phosphobacteria, are currently studied and commercialized to improve the growth, yield, and quality of crops as well as for biotechnological applications in other fields, such as animal and human nutrition, aquaculture and environmental protection (Jorquera *et al.* 2008). In this context, *Bacillus* is one of most isolated and studied phosphobacterial groups and commonly is suggested as potential biofertilizers. However, the occurrence, genomics and dynamics of BLP in agronomic soils is clearly unknown and poorly studied. The objectives of the present study were i) to evaluate the occurrence of BLP in rhizospheric soils from diverse grasslands established on agronomic volcanic soils of Southern Chile, and ii) to evaluate the presence of genetic traits involved in the expression of phytase which is a enzyme capable to release phosphates from phytate in soils.

Methods
Sampling
Rhizospheric soil samples were taken from three grasslands located in the La Araucania and Los Ríos regions from Southern Chile, and inmediatelly transported to laboratory for their analysis. The chemical properties of rhizospheric soils are shown in the Table 1.

BLP isolation
Bacillus-like were isolated according to standard protocol (Holt and Gerhardt, 1994). Rhizospheric soil samples were treated at 80°C for 10 min and aliquotes were plated on phytase-screening medium (Kerovou *et al.* 1998) to detect *Bacillus*-like isolates with phytate-mineralization capacity.

Phytase gene detection by PCR
Based on current literature and public database, a degenerate primer set for *Bacillus* phytases described by Tye *et al*. (2002) was chosen and used to detect phytase gene in rhizospheric isolates of BLP (Figure 1, B). The isolates showing positive phytase signal were selected and characterized by 16S rRNA gene sequencing. The presence of phytase gene was also confirmed by sequencing. The DNA sequences obtained in this study were compared with those present in Genbank database by BLAST tools.

Results and Discussion
Despite of significant differences in the chemical properties among the soils sampled (except % organic matter), the results showed that between 77-91% of cultured *Bacillus*-like showed capacity to solubilise phosphate and/or mineralize phytate on agar (Figure 1, A). Two grasslands showed high percentages of

Bacillus-like isolates with phytate mineralization capacity, 66 and 75% for grassland no.1 and no.2, respectively. In contrast, the higher percentage in grassland no.3 was observed in *Bacillus*-like isolates with both phytate mineralization and phosphate solubilization activity. *Bacillus*-like isolates with capacity to utilize insoluble P forms (phosphates and phytates) have commonly isolated from rhizosphere of different plants and suggested as plant-growth promoting rhizobacteria (Idriss *et al.* 2002; Richardson *et al.* 2009). The analysis of DNA sequences revealed the presence of *Paenibacillus* carrying phytase gene close to 3-phytase reported in *Bacillus* strains (Figure 1, C). The great biotechnological potential of *Paenibacillus* in different industrial processes and in sustainable agriculture has been recently revised (Lal and Tabacchioni, 2009). On other hands, some studies have failed in detection of bacterial phytase in environmental isolates, especially *Bacillus* (Hill *et al.* 2007). The specific detection of phytase gene will allow further studies focused on the dynamics and expression of phytase gene in the rhizosphere plants, with the consequent importance in the application of *Bacillus*-based biofertilizers to improve the P nutrition in plants. To our knowledge, this is first report on detection and characterization of *Bacillus* phytase gene in rhizospheric soils.

Table 1. Average values of selected chemical properties of rhizospheric soils.

	Grassland		
	1	2	3
pH H_2O	5.0-5.1	5.3-5.8	5,7-5,9
P (ppm)	5	36	8
K (ppm)	70	454	224
Organic matter (%)	20	19	19
Al Saturation (%)*	30.8	2,8	0,5

*Calculated as = Al/cation exchange capacity [∑(K, Ca, Mg, Na and Al)]×100

Tabla 2. Average values of *Bacillus*-like isolated from diverse grassland.

Grassland	SP (%)	MP (%)	SPMP (%)	NSM (%)	TOTAL (%)	TOTAL BLP (%)
1	1.4	65.9	11.3	21.4	100	77.2
2	5.2	74.7	15.8	4.3	100	90.5
3	2.5	17.5	66.0	14.0	100	83.5

SP=*Bacillus*-like that solubilize phosphate; MP= *Bacillus*-like that mineralize phytate; SPMP= *Bacillus*-like that solubilize phosphate and mineralize phytate; NSM= *Bacillus*-like that no solubilize phosphate and/or mineralize phytate.

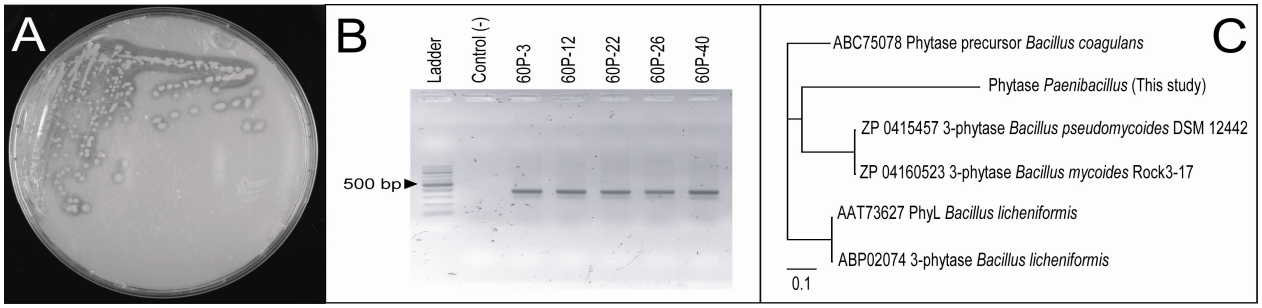

Figure 1. (A) Phytate-mineralization on agar by BLP. (B) PCR amplification of phytase gene in environmental isolates of BLP. (C) Neighbor-Joining tree showing phylogenetic affiliation of detected phytase and the closest relative phytases in Genbank database.

Conclusions

This study shows the high occurrence of *Bacillus*-like phosphobacteria in rhizospheric soils from diverse grasslands established on agronomic volcanic soils of Southern Chile. Also, this study gives evidence for the presence in these soils of *Paenibacillus* spp. carrying mechanisms involved in the expression of phytases.

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