ISSN 0719-3882 print
ISSN 0719-3890 online

EFFECT OF IMMOBILIZED *Serratia* sp. BY SPRAY-DRYING TECHNOLOGY ON PLANT GROWTH AND PHOSPHATE UPTAKE

EFECTO DE *Serratia sp.* INMOBILIZADA POR LA TECNOLOGÍA DE SECADO POR ASPERSIÓN EN EL CRECIMIENTO DE PLANTAS Y ABSORCIÓN DE FOSFATOS

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ABSTRACT

A study was done to investigate the efficiency of rhizobacteria on solubilization of insoluble phosphate in liquid culture medium and its assimilation by wheat plants in quartz sand potted experiments. Serratia sp. was selected to investigate the variation on pH values, enzymatic activity and phosphate solubilization in Pikovskaya liquid medium. A relation between pH diminution, phosphatase production and P solubilization was found. After 60 days of plant assay, root, shoot and plant height did not respond to inoculation of *Serratia* sp. However, immobilized by *Serratia* sp. had beneficial effects on P uptake. The results demonstrated that inoculation of the immobilized rhizobacteria is a promising option for microbial inoculant to increase P level in tissues of wheat plants and could be an innovative technique for application in agricultural industry.

Key words: microbial inoculant, rhizobacteria, bioencapsulation, rock phosphate, insoluble phosphate, phosphate solubilizing bacteria.

RESUMEN

Un estudio fue realizado para investigar la eficiencia de rhizobacterias en la solubilización de fosfatos insolubles en medios de cultivo líquidos y su asimilación por plantas de trigo en experimentos en macetas con arena de cuarzo. Una cepa de *Serratia* sp. fue seleccionada para investigar la variación en el pH, actividad enzimática y solubilización de fosfatos en el medio de cultivo Pikovskaya. Se encontró una relación entre la disminución del pH, la producción de fosfatasas y solubilización de P. Después de 60 días de ensayo con plantas, las raíces, tallos y el largo de la planta no respondieron a la inoculación de *Serratia* sp. Sin embargo, la inmobilización de *Serratia* sp. ha tenido efectos benéficos en la absorción de P. Los resultados demuestran que la inoculación con rhizobacterias inmobilizadas es una opción prometedora para los inoculantes microbianos para aumentar los niveles de P en plantas de trigo y podría ser una técnica innovadora para la aplicación en la industria de la agricultura.

Palabras clave: inoculantes microbianos, rhizobacterias, bioencapsulación, roca fosfórica, fosfato insoluble, bacteria solubilizadora de fosfato.

Recibido: 27 mayo 2013. Aceptado: 14 agosto 2013.

INTRODUCTION

The deficiency of phosphorus (P) in soil is one of the most important chemical factors that restrict the growth of plants. Moreover, P is the second most important macronutrient after nitrogen, which plays an important role in plant development (Sashidhar and Podile, 2010). However, this element is in low concentration in many type of soils. A large part of the phosphate, approximately 95-99%, is present as insoluble forms of P and therefore cannot be used by plants (Vassileva et al., 1998). This is mainly due to the fact that soluble P applied to the soil as fertilizer is absorbed by the colloidal fraction and there is little availability to plants (Daniels et al., 2009). Depending on the charged density, P ions have a tendency to precipitate and to form complexes such as $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$. Thus, large quantities of phosphates fertilizers are applied to increase plant productivity. However, the applied soluble forms of P are easily precipitated into insoluble forms, which lead to excess application of P fertilizer (Omar, 1998). This unmanaged excess of P application is known to cause environmental and economic disadvantages. It is well known, that the excess of P application leads to pollution, soil erosion and runoff water containing large amounts of soluble P (Brady, 1990).

Nowadays there is a great interest to study many soil microorganisms that have been identified to solubilize the insoluble phosphate such as rock phosphate, and make it available to plants (Tripura et al., 2007). Several groups of bacteria known as phosphate solubilizing bacteria (PSB) help plants in providing soluble forms of phosphate (Sashidhar and Podile, 2010). The PSB improve phosphate solubilization and fix the complexes into the soil, increasing the efficiency of the chemical fertilizers used. Among the soil complex groups, bacteria of the genus *Serratia* are highly efficient in solubilizing phosphate forms (Ben Farhat et al., 2009; Goldstein, 2000). Also, the association between PSB and plant roots plays a key role in the nutrition of many agro-ecosystems, particularly in P deficient soils (Goldstein, 2007; Jorquera et al., 2008). The mechanisms used by PSB to transform the phosphate that is present in insoluble forms to soluble forms, are mainly chelating secretion of organic acids and/or decrease the pH of the medium by extrusion of H^+ (Turan et al., 2006). The studies of the role of PSB in sustainable agriculture have provided a biotechnological solution; in this sense PSB could play an important role in supplying phosphate to plants and is an alternative for improving the efficiency of chemical fertilizers (Khan et al., 2007).

Introduction of PSB into soil have demonstrated that some inoculants can improve plant uptake of nutrients, increasing the efficiency of applied chemical fertilizers (Adesemoye and Kloepper, 2009). However, liquid inoculation of PSB into soil affects cells survival, because of a variety of environmental stressors and competitors (Wu et al., 2012). Bioencapsulation of active compounds achieves certain desirable effects, such as stabilization and protection (Schoebitz et al., 2012; 2013). Variability of PSB inoculation on plant is mainly due to the quality in the inoculants formulations containing an effective bacterial strain and can determine the success or failure of a biological agent.

Spray-drying is widely used in large-scale production of encapsulated since is economical and adaptable, and produces an excellent product quality. This method involves the dispersion of homogenized microorganisms in maltodextrin followed by atomization and spraying of the mixture into a warm chamber (Watanabe et al., 2002) leading to evaporation of the solvent and consequently the development of microcapsules. The main advantages of the process are to manage on a continuous basis, low operating cost, and high quality of particles, also rapid solubility of the capsules, small size and high stability capsules.

Research on PSB have been focused mainly on liquid media or peat to introduce bacteria into the soil, although a few have been carried out for immobilized bacteria, being this method a satisfactory alternative to biologically solubilize rock phosphate. A major role of inoculant carrier is to provide more suitable microenvironment for the prolonged survival into the soil (Schoebitz et al., 2013). High cell concentrations of inoculant to improve survival during storage period ensure good protection of bacteria in soil is the key factor to ensure positive response on plant inoculation (Rekha et al., 2007). Bioencapsulation of microorganisms in biopolymer matrices by spray drying is a valuable alternative to produce formulations with extended shelf life (Muñoz-Celaya et al., 2012). *Serratia* sp. is a PSB that has been studied because of its ability to dissolve rock phosphate and produce acid and alkaline phosphatases (Schoebitz et al. 2013). The aim of this study was to measure the effects of encapsulated rhizobacteria by spray-drying technology on solubilization of insoluble inorganic phosphate forms and their assimilation by wheat plants in potted experiments.

MATERIALS AND METHODS

Microorganisms and culture conditions

Serratia sp. was provided by the Instituto de Producción y Sanidad Vegetal, Universidad Aus-

tral de Chile. Was grown in 100 mL of sterile trypticase soy broth (casein peptone 15 g L^{-1}; soy peptone 5 g L^{-1}; sodium chloride at 5 g L^{-1}) adjusted to pH 7.0. Liquid cultivation was performed on a rotary shaker (160 rpm) at 25°C to harvest after 24 h of growth.

Mineral P solubilization in liquid media

The efficiency of *Serratia* sp. was measured with Pikovskaya liquid media (PVK) containing (g L^{-1}): 10.0 glucose, 0.2 NaCl, 0.5 $(NH_4)SO_4$, 0.1 $MgSO_4$, 0.1 $MnSO_4$, 0.5 yeast extract and 5.0 P (as $Ca_3(PO_4)_2$, $FePO_4$ or $AlPO_4$). Phosphate minerals were used as sole source of P for initial behavior of *Serratia* sp. The pH of each medium was adjusted to 5.8 before autoclaving. 200 mL-Erlenmeyer flasks containing 50 mL of PVK with the purified bacterial strain were used. The flasks were incubated during 3, 5 and 7 days at 25°C at 160 rpm. The *Serratia* sp. culture was centrifuged during 10 min at 8700 *g*, and the supernatant was removed for phosphate and enzyme analysis. Quantitative spectrophotometric analysis of the soluble phosphate was measured according to the standard protocol described by Murphy and Riley (1962).

Enzyme activity

Phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. For the assay, 2 mL of 0.5 M sodium acetate buffer adjusted to pH 6.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 mL of substrate were added to 0.5 mL of centrifuged PVK culture medium incubated at 37°C during 90 min. The reaction was stopped by cooling at 2°C for 15 min. Then 0.5 mL of 0.5 M $CaCl_2$ and 2 mL of 0.5 M NaOH were added and the mixture centrifuged at 4000 rpm during 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai and Bremmer, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

Immobilization of microorganism by spray drying

To prepare the inoculum, *Serratia* T3 strain was inoculated into 1000 mL of trypticase soy broth and incubated at 30°C during 24 h on a rotary shaker (160 rpm). After fermentation culture medium was mixed with 200 g of maltodextrin (provided by Prinal S.A., Santiago, Chile) and was spray dried in a pilot scale apparatus (Niro Atomizer, Soeborg, Denmark; Fig. 1). Spray drying conditions were: outlet air temperature 80-90°C, inlet air temperature 145°C. Powder was collected in a single cyclone separator.

Fig. 1. Picture of spray-drying device used in this experiment (Niro Atomizer, Denmark).

Fig. 1. Fotografia del sistema de secado por aspersión usado en este experimento (Niro Atomizer, Dinamarca).

Microorganism survival

To calculate the survivors after spray drying, 1 g of sample was rehydrated mixed with 9 mL of sterile physiological solution (0.85% NaCl). Samples were homogenized in a vortex mixer and maintained at room temperature during 15 min and then serially diluted. Bacteria were enumerated on plate count agar.

Plant–formulated rhizobacteria assay

The experiment was carried out in a plant growth room in order to evaluate the effects of rhizobacteria on plant growth and P uptake of wheat. Three-day-old wheat seedlings were used in all the experimented. Seed were disinfected in 2% sodium hypochlorite during 30 min and rinsing 3 times with sterile distilled water. Disinfected seeds were transferred to Petri dishes with 20% water-agar and incubated during 3 days at 30°C in dark conditions. Then, 3-day-old seedlings were planted individually in each polyethylene pot. The P in quartz sand was removed during 14 h with HCl (3 M). Then the quartz sand was rinsed with tap water and dried at 35°C for 5 days.

P fertilization

P amendments were applied to each pot using 100 mL of Hoagland nutrient solution (Hoagland and Arnon, 1950) per week with different regimes of soluble P and insoluble phosphate rock. Six different P regimes were evaluated: (1) Solution without soluble P and phosphate rock; (2) Solution without soluble P: 7.5 mL of $Ca(NO_3)_2 * 4H_20$ (1 M), 3 mL of $MgSO_4 * 7H_20$ (1 M), 10 mL of K_2SO_4 (0.5 M), 1 mL of iron chelate (0.1%) and 1 mL of trace elements ($MnCl_2 * 4H_20$ 1.8 g L^{-1}; H_3BO_3 3.0 g L^{-1}; $ZnSO_4 * 7H_20$ 0.3 g L^{-1}; $CuSO_4 * 5H_20$ 0.1 g L^{-1} and H_2Mo0_4 0.1 g L^{-1}); (3) Solution 0.25 mg L^{-1} soluble P: 5 mL $Ca(NO_3)_2 * 4H_20$ (1 M), 5 mL of KNO_3 (1 M), 0.08 mL of KH_2PO_4 (1 M), 4 mL of $MgSO_4 * 7H_20$ (1 M), 1 mL of iron chelate (0,1%) and 1 mL of trace elements; (4) Solution 0.5 mg L^{-1} soluble P: similar to solution 3 with 0.15 mL of KH_2PO_4 (1 M) and 0.5 mL of KCl (0.68 M); (5) Solution 1 mg L^{-1} soluble P: similar that solution 3 with 0.3 mL of KH_2PO_4 (1 M) and 1 mL of KCl (0.68 M); (6) Solution 3 mg L^{-1} soluble P: similar that solution 3 with 1 mL of KH_2PO_4 (1 M) and 3 mL of KCl (0.68 M). The treatments 2-6 were supplemented with phosphate rock powder (17-19% P_2O_5) (Bifox, Compañía Minera de Fosfatos Naturales Bifox Ltda, Santiago, Chile). Pots were fertilized with 10 mg kg^{-1} of insoluble rock powder phosphate, except pots without P.

Inoculation assay

The pots were prepared using 800 g quartz sand. Four germinated Pandora-INIA spring wheat cultivar were planted in each polyethylene pot (8 cm diameter, 13 cm height). For the inoculation treatments, 1 g of *Serratia* sp. powder was used. Control plants were non-inoculated. The growth period of wheat was of 60 days in a growth chamber at 25°C with 16 h light and 8 h darkness. 200 mL of sterilized water was added per week in quartz sand as necessary to maintain soil moisture levels near field capacity. Growth promotion effects of bacterial treatments were assessed by measuring shoot and root dry weight, plant height and P uptake of plants. The dry weights were determined by using an oven at 70°C for 48 h. The P contents in the wheat plant were measured by molybdate-blue method (Murphy and Riley, 1962).

Statistical analysis

The experiment design contained three replicates, where the factor evaluated was the solubilization of three different inorganic phosphates in liquid mediums (control: without phosphate; $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$). The experiment on wheat was conducted in a growth chamber. Plant growth data were analyzed by one-way ANOVA and post-hoc mean separation was performed by LSD test at $P \leq 0.05$ using the software package SPSS (2011) (version 19.0 for Windows; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

P solubilization in liquid media

Serratia sp. was isolated from the rhizosphere of wheat plants (*Triticum* sp.) and it was evaluated to solubilize three different inorganic phosphates. This isolate was able to decrease the initial pH at least one unit after 3, 5 and 7 days of incubation at 25°C (Table 1). In this study, the highest amount of P solubilization was measured for $Ca_3(PO_4)_2$ with a decrease in pH up to 4.1, followed by $FePO_4$ with a maximum decrease in pH to 3.6. The minimum amount of soluble P was observed with $AlPO_4$ and pH of the medium drop to 4.1. Although, the highest level of P solubilization were not accompanied by a maximum drop in pH. Nevertheless, it is well documented the strong correlation between P solubilization and low pH (3-4) (Rodriguez and Fraga, 1999).

The relationship observed between pH and soluble P concentration suggested that acidification of the medium could facilitate the phosphate solubilization (pH specific 3.6-4.8; see Tables 1 and 2). Thus, the P solubilizing activity is determined by the microbial activity to produce organic acids, which via their carboxylic group chelate the cation bounds to phosphate converting

Table 1. Changes on pH mediated by *Serratia* sp. in the liquid mediums containing $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$ at 3, 5 and 7 days after incubation.

Tabla 1. Cambios en pH producidos por *Serratia* sp. en medios liquidos que contienen $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$ a los 3, 5 y 7 días después de la incubación.

	pH			
	0	3	5	7
$Ca_3(PO_4)_2$	5.8 ± 0.01	4.1 ± 0.01	4.1 ± 0.04	4.8 ± 0.01
$FePO_4$	5.7 ± 0.01	3.7 ± 0.01	3.6 ± 0.01	4.1 ± 0.02
$AlPO_4$	5.9 ± 0.01	4.3 ± 0.01	4.1 ± 0.01	4.2 ± 0.10
Control	5.8 ± 0.01	5.8 ± 0.01	5.8 ± 0.02	5.8 ± 0.02

Mean ± standard error (n = 3).

Table 2. Soluble phosphate production by *Serratia* sp. in Pikovskaya medium containing $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$ at 0, 3, 5 and 7 days after incubation.

Tabla 2. Producción de fosfato soluble por *Serratia* sp. en medio Pikovskaya conteniendo $Ca_3(PO^4)_2$, $FePO_4$ y $AlPO_4$ a 0, 3, 5 y 7 días después de la incubación.

	P solubilization (mg L^{-1})			
	0	3	5	7
$Ca_3(PO_4)_2$	0 ± 0	158.7 ± 1.16	160.3 ± 0.90	175.6 ± 2.87
$FePO_4$	0 ± 0	13.4 ± 0.21	15.5 ± 0.72	17.5 ± 3.02
$AlPO_4$	0 ± 0	4.2 ± 0.19	6.6 ± 0.83	6.5 ± 0.33
Control	0 ± 0	2.6 ± 0.06	2.0 ± 0.03	1.1 ± 0.03

Mean ± standard error (n = 3).

them into the soluble forms (Yu et al., 2012). In the present study, the greatest increase on P solubilization in response to *Serratia* sp. inoculation was observed in the liquid medium contained $Ca_3(PO_4)_2$. Using these phosphate minerals the P solubilization was tenfold higher compared to $FePO_4$ and twenty-five fold higher than $AlPO_4$, at the 7th day of incubation (Table 2). This observation was previously reported by Yu et al. (2012). In that way, would seem reasonable that specific isolation methods should be developed to characterize phosphate-solubilizing bacteria that are relevant in acid soils.

It is accepted that solubilization of insoluble P compound is due to the excretion of microbial metabolites such as organic acids. In addition to acid production, other mechanisms can cause phosphate solubilization (Nautiyal et al., 2000). PSB are normal inhabitants in the rhizosphere and secretion of phosphatases are common method of facilitating the conversion of insoluble forms of P to plant available forms (Rodriguez et al., 2006). In this regard, we found a higher acid and alkaline phosphatase activity in *Serratia* sp. in comparison to non-inoculated (control). Therefore, we noticed a clear connection between the decrease in pH values, increase enzyme activity (Fig. 2) and P available on PVK liquid medium.

Immobilization of *Serratia* sp. by spray-drying

In our work, the initial cell concentration in culture medium was 2.8 x 10^9 CFU g^{-1} and at the end of process it was determined a cell concentration around 2.8 x 10^6 CFU g^{-1} using an inlet temperature of 145ºC. For that reason, the spray-drying technology is not considered as a good cell immobilization technique due to a high mortality resulting from simultaneous dehydration and high temperature inactivation of microorganisms like non-spore forming bacteria (Picot and Lacroix, 2003). For instance, Amiet-Charpentier et al. (1998) did not find viable *Pseudomonas* at the end of the drying process, when the inlet temperature was at 80°C. On the other hand, when the inlet temperature was at 60°C the cells survival was estimated around 10^7 CFU g^{-1}. Some reports indicated that the lowest air temperature was associated with the highest survival rate for the microorganisms during drying process (Mauriello et al., 1999; Gardiner et al., 2000; Golowczyc et al.,

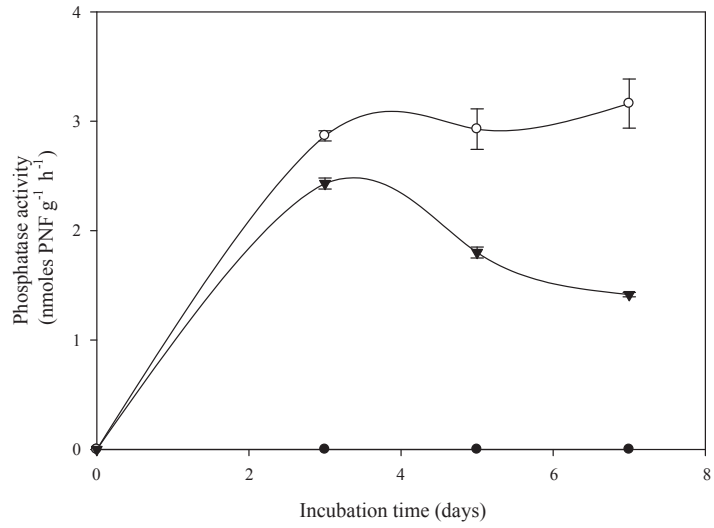

Fig. 2. Phosphatase activity of *Serratia* sp. during 3, 5 and 7 days in Pikovskaya culture medium containing $Ca_3(PO_4)_2$; acid phosphatase (○), alkaline phosphatase (▼) and control (●).

Fig. 2. Actividad fosfatasa de *Serratia* sp. durante 3, 5 y 7 días en medio de cultivo Pikovskaya conteniendo $Ca_3(PO_4)_2$; fosfatasa acida (○), fosfatasa alcalina (▼) y control (●).

2010). Nevertheless, the results are more promising using spore-forming bacteria, because can withstand an even higher temperature. According to our experience in spore-forming bacteria like *Bacillus pumilus* strain C26, which was multiplied in an alternative culture medium based on molasses, lupine protein extract and maltodextrin. The outcome using spray drying was 10^9 CFU g^{-1} at time zero and after one year of storage at room temperature the cells concentration in the powder was estimated at 10^8 CFU g^{-1} (Data not published).

Plant growth and P uptake

After use of HCl to remove de P content on quartz sand, the final amount of nutrients was 12.6 mg kg^{-1} for nitrogen, 0.2 mg kg^{-1} for phosphorus, 4.7 mg kg^{-1} for potassium, and pH value of 5.5. Growth parameters were measured to assess the growth promotion of immobilized *Serratia* sp. Table 3 shows a statistically significant improvement ($p < 0.05$) in shoot and root biomass mediated by all the levels of P fertilization compared to the control plants (without soluble and rock phosphate). The inoculation of immobilized bacteria did not significantly increase biomass and plant height. The high P fertilization levels (1.0 and 3.0 mg L^{-1} of soluble P) significantly increased the P total in plants. The effect on P uptake was also positive to the treatment inoculated with immobilized rhizobacteria, since P absorption was significantly ($p < 0.05$) higher in plants inoculated with 0.25, 0.5 and 3.0 mg L^{-1} of soluble P.

The results of this experiment are not in agreement with those found by other authors, who reported that the use of immobilized rhizobacteria had a pronounced beneficial effect on plant biomass (Vessey, 2003; Rekha et al., 2007). In this experiment, wheat plants did not respond to inoculated treatments with respect to control. These results showed a low microorganisms activity in relation to biomass production. However, it was observed that the immobilized *Serratia* sp. had a beneficial effect on P uptake, indicating that *Serratia* sp. would be of minor importance in plant growth promotion by supplying roots with soluble P in soils. Higher P uptake may be attributable to the mobilization of nutrients from soil because of the secretion of organic acids mediated by rhizobacteria (Basak and Biswas, 2010). Rhizobacteria are rhizosphere competent bacteria that colonize plant roots; they are able to colonize all the ecological niches found on the rhizosphere (Antoun and Kloepper, 2001) and consequently, can explore a wider range for nutrients mobilization. In this sense, P nutrient content can be taken as a representative parameter of rhizobacteria immobilized effectiveness (Vassileva et al., 1999; 2001; 2010). In addition it has been reported (Schoebitz et al., 2014) that immobilized rhizobacteria could help plants to compensate deficiencies in phosphorous and potassium.

Table 3. Biomass production and total P uptake of wheat plant inoculated with immobilized *Serratia* sp. by spray drying.

Tabla 3. Producción de biomasa y absorción de P en plantas de trigo inoculadas con *Serratia* sp. inmobilizadas en secado por aspersión.

Treatments	Shoot	Root	Plant length	Total P
	g dw[3]	g dw	cm	mg g plant^{-1}
Quartz sand only (-P)	0.27 ± 0.05 c	0.17 ± 0.04 c	15.5 ± 1.71 e	0.18 ± 0,02 c
(-P) + *Serratia* sp.	0.27 ± 0.07 c	0.19 ± 0.05 c	15.7 ± 0.92 e	0.19 ± 0.03 c
(+RP)[1]	0.37 ± 0.04 b	0.24 ± 0.05 b	16.4 ± 1.57 d	0.19 ± 0.02 c
(+RP) + *Serratia* sp.	0.50 ± 0.06 b	0.26 ± 0.07 b	18.9 ± 1.32 d	0.23 ± 0.01 c
(+RP) + 0.25 mL L^{-1} SP[2]	0.52 ± 0.04 b	0.31 ± 0.04 b	21.1 ± 2.25 b	0.19 ± 0.01 c
(+RP) + 0.25 mL L^{-1} SP+ *Serratia* sp.	0.57 ± 0.03 b	0.39 ± 0.06 b	21.8 ± 1.14 b	0.25 ± 0.03 b
(+RP) + 0.5 mL L^{-1} SP	0.49 ± 0.03 b	0.31 ± 0.08 b	16.2 ± 1.44 ed	0.19 ± 0.01 c
(+RP) + 0.5 mL L^{-1} SP+ *Serratia* sp.	0.49 ± 0.03 b	0.49 ± 0.03 b	16.7 ± 1.04 ed	0.23 ± 0.02 b
(+RP) + 1.0 mL L^{-1} SP	0.47 ± 0.10 b	0.41 ± 0.06 b	19.3 ± 1.06 c	0.24 ± 0.04 b
(+RP) + 1.0 mL L^{-1} SP+ *Serratia* sp.	0.52 ± 0.05 b	0.41 ± 0.03 b	19.8 ± 2.16 c	0.26 ± 0.03 b
(+RP) + 3.0 mL L^{-1} SP	0.70 ± 0.30 a	0.54 ± 0.06 a	25.4 ± 3.33 a	0.25 ± 0.02 b
(+RP) + 3.0 mL L^{-1} SP+ *Serratia* sp.	0.71 ± 0.05 a	0.57 ± 0.07 a	26.2 ± 2.01 a	0.32 ± 0.03 a

[1] RP = rock phosphate; [2] SP = soluble phosphate; [3] g dw = grams dry weight. Values are means of three replicates. Significant difference according to the LSD test at $P < 0.05$ levels were indicated by different letters.

CONCLUSIONS

This study concludes that *Serratia* sp. was effective in dissolving inorganic phosphate. However, plants biomass did not respond to inoculated treatments. It was observed that immobilized *Serratia* sp. had a beneficial effect on P uptake; this may indicate that *Serratia* sp. would be of minor importance in promoting plant biomass. In that way, introduction of microbial inoculant have demonstrated that can improve plant P uptake and thereby increase the efficiency of applied chemical fertilizers.

ACKNOWLEDGEMENTS

This study is part of the project funded by Fund for the Promotion of Scientific and Technological Development (FONDEF D08I 1039), National Commission for Scientific and Technological Research of Chile (CONICYT).

LITERATURE CITED

Adesemoye, A.O., and J.W. Kloepper. 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85:1-12.

Amiet-Charpentier, C., P. Gadille, and J.P. Benoit. 1999. Rhizobacteria microencapsulation: properties of microparticles obtained by spray-drying. J. Microencapsul. 16:215-229.

Amiet-Charpentier, C., P. Gadille, B. Digat, and J.P. Benoit. 1998. Microencapsulation of rhizobacteria by spray-drying: formulation and survival studies. J. Microencapsul. 15:639-659.

Antoun, H., and J.W. Kloepper. 2001. Plant growth-promoting rhizobacteria (PGPR). p.1477-1480. In: S. Brenner and J.H. Miller (eds.) Encyclopedia of Genetics. Academic Press, New York, USA.

Basak, B.B., and D.R. Biswas. 2010. Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. Biol Fertil Soils 46:641-648.

Bashan, Y. 1986. Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. Appl. Environ. Microbiol. 51:1089-1098.

Bashan, Y., and L.E. Gonzalez. 1999. Long-term survival of the plant-growth-promoting bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* in dry alginate inoculant. Appl. Microbiol. Biotechnol. 51:262-266.

Ben Farhat, M., A. Farhat, W. Bejar, R. Kammoun, K. Bouchaala, A. Fourati, H. Antoun, S. Bejar, and H. Chouayekh. 2009. Characterization of the mineral phosphate solubilizing activity of *Serratia marcescens* CTM 50650 isolated from the phosphate mine of Gafsa. Arch. Microbiol. 191:815-824.

Brady, N.C. 1990. The nature and properties of soils. 10th ed. Mac Millan Publishing Com-

pany, New York, USA

Daniels, C., C. Michan, and J.L. Ramos. 2009. New molecular tools for enhancing methane production, explaining thermodynamically limited lifestyles and other important biotechnological issues. Microb. Biotechnol. 2:533–536.

Gardiner, G.E., E. O'Sullivan, J. Kelly, M.A. Auty, G.F. Fitzgerald, J.K. Collins, R.P. Ross, and C. Stanton. 2000. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Appl Environ Microbiol 66:2605-2612.

Goldstein, A. 2000. Bioprocessing of rock phosphate ore: Essential technical considerations for the development of a successful commercial technology. IFA Technical Conference. 1-4 October 2000. International Fertilizer Industry Association, Louisiana, New Orleans, USA.

Goldstein, A.H. 2007. Future trends in research on microbial phosphate solubilization: one hundred years of insolubility. p. 91-96. In E. Velazquez and C. Rodriguez-Barrueco (eds.). First International Meeting on Microbial Phosphate Solubilization. 16-19 July 2002. Springer, Dordrecht, The Netherlands.

Golowczyc, M.A., J. Silva, A.G. Abraham, G.L. De Antoni, and P. Teixeira. 2010. Preservation of probiotic strains isolated from kefir by spray drying. Lett. Appl. Microbiol. 50:7-12.

Hoagland, D.R., and D. Arnon. 1950. The water culture method of growing plants without soil. California Agricultural Experiment Station. No. 347 p. 39.

Jorquera, M.A., M.T. Hernandez, Z. Rengel, P. Marschner, and M. Mora. 2008. Isolation of culturable phosphobacteria with both phytatemineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biol. Fertil. Soils 44:1025-1034.

Khan, M., A. Zaidi, and P. Wani. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture. Agron. Sustain. Dev. 27:29-43.

Mauriello, G., M. Aponte, R. Andolfi, G. Moschetti, and F. Villani. 1999. Spray-drying of bacteriocin-producing lactic acid bacteria. J. Food Prot. 62:773-777.

Muñoz-Celaya, A.L., M. Ortiz-García, E.J. Vernon-Carterc, J. Jauregui-Rincónb, E. Galindoa, and L. Serrano-Carreón. 2012. Spray-drying microencapsulation of *Trichoderma harzianum* conidias in carbohydrate polymers matrices. Carbohydrate Polymers 88:1141-1148.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36.

Naseby, D.C., and J.M. Lynch. 1997. Rhizosphere soil enzymes as indicators of perturbation caused by a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biol. Biochem. 29:1353-1362.

Nautiyal, C.S., S. Bhadauria, P. Kumar, H. Lal, R. Mondal, and D. Verma. 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiol. Lett. 182:291-296.

Omar, S.A. 1998. The role of rock-phosphate-solubilizing fungi and vesicular–arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J. Microbiol. Biotechnol. 14:211-218.

Picot, A., and C. Lacroix. 2003. Production of multiphase water-insoluble microcapsules for cell microencapsulation using an emulsification/spray-drying technology. J. Food Sci. 68:2693–2700.

Rekha, P.D., W.A. Lai, A.B. Arun, and C.C. Young. 2007. Effect of free and encapsulated *Pseudomonas putida* CC-FR2-4 and *Bacillus subtilis* CC-pg104 on plant growth under gnotobiotic conditions. Bioresour. Technol. 98:447-451.

Rodriguez, H., and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17:319-339.

Rodriguez, H., R. Fraga, T. Gonzalez, and Y. Bashan. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15-21.

Sashidhar, B., and A.R. Podile. 2010. Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. J. Appl. Microbiol. 109:1-12.

Schoebitz, M., C., Ceballos and L. Ciampi. 2013. Effect of immobilized phosphate solubilizing bacteria on wheat growth and phosphate uptake. J Soil Science and Plant Nutrition. 13:1-10.

Schoebitz, M., Mengual, C., Roldan, A., 2014. Combined effects of clay immobilized *Azospirillum brasilense* and *Pantoea dispersa* and organic olive residue on plant performance and soil properties in the revegetation of a semiarid area. Sci Total Environ 466-467, 67-73.

Schoebitz, M., M.D. López, and A. Roldán. 2013. Bioencapsulation of microbial inoculants for better soil-plant fertilization. A review. Agron. Sustain. Dev. 33:751-765. DOI

10.1007/s13593-013-0142-0.

Schoebitz, M., H. Simonin, and D. Poncelet. 2012. Starch filler and osmoprotectants improve the survival of rhizobacteria in dried alginate beads. J. Microencapsul. 29:532-538.

Tabatabai, M.A., and J.M. Bremmer. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1:301-307.

Tripura, C.B., P. Sudhakar Reddy, M.K. Reddy, B. Sashidhar, and A.R. Podile. 2007. Glucose dehydrogenase of a rhizobacterial strain of *Enterobacter asburiae* involved in mineral phosphate solubilization shares properties and sequence homology with other members of Enterobacteriaceae. Indian J. Microbiol. 47:126–131.

Trivedi, P., and A. Pandey. 2008. Recovery of plant growth-promoting rhizobacteria from sodium alginate beads after 3 years following storage at 4 degrees C. J. Ind. Microbiol. Biotechnol. 35:205-209.

Turan, M., N. Ataoglu, and F. Sahin. 2006. Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. J. Sustain. Agric. 28:99–108.

Van Elsas, J., J. Trevors, D. Jain, A. Wolters,C. Heijnen, and L. van Overbeek. 1992. Survival of, and root colonization by, alginate-encapsulated *Pseudomonas-fluorescens* cells following introduction into soil. Biol. Fert. Soils 14:14-22.

Vassilev, N., M. Vassileva, M. Fenice, and F. Federici. 2001. Immobilized cell technology applied in solubilization of insoluble inorganic (rock) phosphates and P plant acquisition. Bioresour. Technol. 79:263-271.

Vassileva, M., R. Azcon, J.M. Barea, and N. Vassilev. 1998. Application of an encapsulated filamentous fungus in solubilization of inorganic phosphate. J. Biotechnol. 63:67-72.

Vassileva, M., R. Azcon, J.M. Barea, and N. Vassilev. 1999. Effect of encapsulated cells of *Enterobacter* sp. on plant growth and phosphate uptake. Bioresour. Technol. 67: 229-232.

Vassileva, M., M. Serrano, V. Bravo, E. Jurado, I. Nikolaeva, V. Martos, and N. Vassilev. 2010. Multifunctional properties of phosphate-solubilizing microorganisms grown on agro-industrial wastes in fermentation and soil conditions. Appl. Microbiol. Biotechnol. 85:1287-1299.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255:571-586.

Watanabe, Y., X. Fang,Y. Minemoto, S. Adachi, and R. Matsuno. 2002. Suppressive effect of saturated acyl L-ascorbate on the oxidation of linoleic acid encapsulated with maltodextrin or gum arabic by spray-drying. J. Agric. Food Chem. 50:3984-3987.

Wu, Z., L. Guo, S. Qin, and C. Li. 2012. Encapsulation of *R. planticola* Rs-2 from alginate-starch-bentonite and its controlled release and swelling behavior under simulated soil conditions. J. Ind. Microbiol. Biotechnol. 39:317-327.

Yu, X., X. Liu, T.H. Zhu, G.H. Liu, and C. Mao. 2012. Co-inoculation with phosphate-solubilizing and nitrogen-fixing bacteria on solubilization of rock phosphate and their effect on growth promotion and nutrient uptake by walnut. European Journal of Soil Biology 50:112-117.