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Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora

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Abstract Inoculants are of great importance in sustainable and/or organic agriculture. In the present study, plant growth of barley (*Hordeum vulgare*) has been studied in sterile soil inoculated with four plant growth-promoting bacteria and mineral fertilizers at three different soil bulk densities and in three harvests of plants. Three bacterial species were isolated from the rhizosphere of barley and wheat. These bacteria fixed N_2, dissolved P and significantly increased growth of barley seedlings. Available phosphate in soil was significantly increased by seed inoculation of *Bacillus* M-13 and *Bacillus* RC01. Total culturable bacteria, fungi and P-solubilizing bacteria count increased with time. Data suggest that seed inoculation of barley with *Bacillus* RC01, *Bacillus* RC02, *Bacillus* RC03 and *Bacillus* M-13 increased root weight by 16.7, 12.5, 8.9 and 12.5% as compared to the control (without bacteria inoculation and mineral fertilizers) and shoot weight by 34.7, 34.7, 28.6 and 32.7%, respectively. Bacterial inoculation gave increases of 20.3–25.7% over the control as compared with 18.9 and 35.1% total biomass weight increases by P and NP application. The concentration of N and P in soil was decreased by increasing soil compaction. In contrast to macronutrients, the concentration of Fe, Cu and Mn was lower in plants grown in the loosest soil. Soil compaction induced a limitation in root and shoot growth that was reflected by a decrease in the microbial population and activity. Our results show that bacterial population was stimulated by the decrease in soil bulk density. The results suggest that the N_2-fixing and P-solubilizing bacterial strains tested have a potential on plant growth activity of barley.

Keywords Plant growth promoting bacteria · Phosphate solubilization · *Bacillus* spp. · Soil compaction

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Introduction

Phosphorus, one of the major nutrients limiting plant growth, is rapidly immobilized after addition to soil as a soluble fertilizer, and thus, it become less available to plant. Seed or soil inoculation with phosphate-solubilizing bacteria (PSB) such as *Bacillus* spp. can solubilize fixed soil P and applied phosphates, resulting in higher crop yields (Yadav and Dadarwal 1997; Puente et al. 2004a,b) and also in increased inorganic P availability to plant by mineralization of organic P (Kumar and Narula 1999; Rodriguez et al. 2004). The presence of a high number of bacteria in the rhizosphere is important since they may convert organic and inorganic substances into available plant nutrients (Badalucco and Kuikman 2001). Solubilization of insoluble compounds is due to the excretion of microbial metabolites such as organic acids (Puente et al. 2004a; Iyamuremye and Dick 1996). Indeed, the production of microbial metabolites including organic acids may result in a decrease in soil pH, which plays a major role in solubilization of some nutrients (Rodriguez and Fraga 1999; Nautiyal et al. 2000).

Plant growth-promoting bacteria (PGPB) are classified into two different groups (Bashan and Holguin 1998). The first group includes bacterial strains that have the capability of synthesizing plant growth-promoting substances (i.e.

phytohormones, vitamins, enzymes and siderophores); inhibiting ethylene synthesis; fixing atmospheric nitrogen; solubilizing inorganic phosphate and iron; mineralizing organic phosphate and improving plant stress tolerance to drought, salinity, metal toxicity, and pesticide load (Glick and Bashan 1997; Lucy et al. 2004). A second group is able to decrease or prevent the deleterious effects of phytopathogenic microorganisms (Bashan and Holguin 1998).

The aim of the study was to evaluate the efficiency of novel P-solubilizing and N_2-fixing bacterial strains isolated from barley and wheat rhizosphere soils so as to asses their possible use as inoculants for increasing productivity of agricultural crops by minimizing the need for chemical fertilizers.

Materials and methods

Bacterial strains

Three bacterial strains were isolated from the rhizosphere field-grown crops and identified as *Bacillus* RC01, *Bacillus* RC02, and *Bacillus* RC03 based on fatty acid methyl ester analysis using MIDI System (Sherlock Microbial Identification System version 4.5, MIDI, Inc., Newark, DE). The bacterial strain *Bacillus* M-13 was originally isolated from pepper plants at Atatürk University, Erzurum, Turkey (Şahin et al. 2004). In the present study, P-solubilizing activities of three new (RC01, RC02 and RC03) and a previously isolated strain (M-13, positive control) were measured according to the qualitative and quantitative methods (Mehta and Nautiyal 2001). The bacterial strains were characterized by morphological, biochemical and physiological tests, including pigment production on nutrient agar medium and the Gram reaction (Forbes et al. 2002). These bacterial strains were also able to grow in N-free basal medium (Table 1).

Isolation and identification of bacteria

Bacillus RC01 and *Bacillus* RC03 were initially isolated from the rhizosphere of wheat grown in alkaline soil. The strain *Bacillus* RC02 was isolated from the rhizosphere of barley grown in sandy loam. Plants were removed from a field site with non-rhizosphere soil, brought immediately to the laboratory in polythene bags and air-dried. The non-rhizosphere sandy-loam soil was removed by gentle shaking, whereas the soil adhering strongly to the root was referred to as rhizosphere soil. Ten grams soil from each sample was aseptically weighed, transferred to an Erlenmeyer flask with 100 ml sterile water and shaken for 30 min at 150 rpm. Immediately after shaking, a series of tenfold dilutions of the suspension was made for each sample by pipetting 1-ml aliquots into 9 ml sterile water. The final dilution was 10^5-fold; 0.1 ml of each dilution of the series was placed onto a Petri dish with nutrient agar (NA) (Difco). Three replicate dishes were made for each dilution. Dishes were placed in an incubator at 28°C for 7 days (aerobically). Rhizobacteria isolates were selected to represent distinct types based on differences in colony morphology, including colony form, elevation and pigment production. Isolates were re-streaked on NA and checked for purity. Isolated bacterial strains were identified based on whole-cell fatty acids methyl esters (FAMEs) analysis (De Freitas et al. 1997) performed according to the method described by the MIDI System manufacturer's manual. The identified bacterial strains at the genus level were maintained in nutrient broth (NB) with 15% glycerol at −86°C for further tests.

Seed inoculation

For this experiment, pure cultures were grown in NB at 28°C and diluted to a final concentration of 10^9 colony-forming units (CFU) ml^{-1} in sterile distilled water containing 0.025% Tween 20. Barley seeds were sterilized in 70% ethanol for 2 min and 1.2% sodium hypochlorite for 10 min and rinsed ten times in sterile tap water. Seeds were then treated with the bacterial suspensions at the concentration of 10^8 CFU ml^{-1} for 30 min under sterilized conditions.

Microbial populations

Determinations of viable microbial bacteria and fungi counts were carried out 15, 30 and 45 days after sowing. The rhizosphere soil, which was adhering to the root, was separated by gentle tapping, air-dried at room temperature (25°C) and analysed for the content of culturable bacteria, fungi and P-solubilizing bacteria by the CFU according to the pour plate method (Salle 1973) in asparagine–mannitol

Table 1 Source and biochemical characteristics of the bacterial strains tested

Bacterial strain	Sources	Biochemical characteristics								
		Gram stain	P solubilization	Catalase	Oxidase	Pigment	NO_3^- reduction	Starch hydrolysis	Growth at 36°C	Growth in N-free basal medium
B. RC01	Wheat	+	+	+	−	−	+	+	+	+
B. RC02	Barley	+	+	+	−	−	+	+	+	+
B. RC03	Wheat	+	+	+	−	−	+	+	+	+
B. M-13	Pepper	+	+	+	−	−	+	+	+	+

agar, dextrose–rose bengal agar (Martin 1950) and sucrose–tricalcium phosphate agar (Pikovskaya 1948) media, respectively. Ten grams soil from each sample was aseptically weighed, transferred to an Erlenmeyer flask with 100 ml sterile water and shaken for 30 min at about 150 rpm. Immediately after shaking, each suspension was tenfold diluted by pipetting 1-ml aliquots into 9 ml sterile water. Thus, a 10^8-fold final dilution was obtained; 0.1 ml of each dilution of the series was placed onto a Petri dish with NA. Three replicate dishes were made for each dilution. The agar plates were incubated at 30°C for 7 days (aerobically). After the incubation period, the CFU of the bacteria developed on the respective agar plates were counted by the standard method. The averaged CFU per gram of oven-dried soil was calculated for each soil sample.

Soil sampling and laboratory analysis techniques

The soil was sampled from the Ap horizon, dried indoors until it could be crumbled to pass through 4 mm for pots experiment and 2 mm sieves for analyses of physicochemical properties. The soil is classified as Ustorthents according to USDA soil taxonomy (Soil Survey Staff 1999). The loam soil had an organic matter content of 1.8%, pH 6.9, available P content of 13.7 mg kg^{-1} (Olsen and Sommers 1982), NH_4^+-N and NO_3^--N contents of 8.3 and 7.6 mg kg^{-1}, respectively, cation exchange capacity and exchangeable K and Ca+Mg of 24.9, 1.6 and 17.8 cmol kg^{-1}, respectively. Soil samples were sterilized by autoclaving at 121°C at 1.1 atm pressure in a Pyrex beaker for 2 h and then cooled for 24 h.

Soil compression

Approximately 2.5 kg of loose sterile soil at 15% water content was placed in PVC cylindrical pots, and the bulk density was 1.1 Mg m^{-3} in the non-compacted pots. The compacted pot was prepared by packing soil in a Proctor hammer (5 cm i.d., 30 cm drop height, 2.5 kg weight) in three layers by giving a sufficient number of blows using a flat-bottom hammer to reach the target bulk density (approximately 1.25 and 1.4 Mg m^{-3}). Soil bulk densities were determined by the core method (Blake and Hartge 1986).

Pot experiment

Pots were sterilized with 20% sodium hypochlorite solution, filled with 2.5 kg soil and seeded. The following treatments with three replicates were investigated: (1) control (without bacteria inoculation and mineral fertilizers), (2) N fertilizer (40 mg N kg^{-1} soil), (3) P fertilizer (20 mg P kg^{-1} soil), (4) NP fertilizer (40 mg N kg^{-1} soil+20 mg P kg^{-1} soil), (5) *Bacillus* RC01, (6) *Bacillus* RC02, (7) *Bacillus* RC03, (8) *Bacillus* M-13. There were eight treatments, three soil compaction (1.1, 1.25 and 1.40 Mg m^{-3}), three harvest times (15, 30 and 45 days) and three replicates totalling 216 pots. Pots were arranged on a bench in the greenhouse according to a randomized complete block design with three blocks (i.e. each block contained all 8 treatments×3 soil compaction×3 harvests). Barley seeds were placed at the same depth (approximately 2.5 cm below the soil surface) in all pots.

The seedlings were grown in a greenhouse under a day–night cycle of 15–9 h natural light (ranged from 900 to 1,200 μmol/m^{-2} s^{-1}), 25–16°C and 55% relative humidity. Plants were thinned at the earliest to maintain the desired number of uniform plants (five seedlings per pot) in each pot. The pots were watered to 60% water-holding capacity and were maintained at this moisture content by watering to weight every 2–3 days. Sterile water was slowly added over the top soil in each pot. The treated plants were uprooted after 15, 30 and 45 days of sowing, with minimal damage to the root system, and were washed gently under running tap water to remove the adhering soil particles. At harvest, the root system was separated from the shoots, which were oven-dried for 3 days at 70°C. Total root length was measured as described by Farrell et al. (1993). Macronutrient (N, P, K, Ca and Mg) and micronutrient (Fe, Mn, Zn and Cu) of barley seedlings were determined according to the Association of Official Analytical Chemists (AOAC 1990). An analysis of variance (ANOVA) and Duncan's multiple range test (at $P<0.05$) were performed to analyse statistical differences and to discriminate between means.

Results

Table 2 showed that sampling time and bulk density affected soil properties. In general, soil pH and organic matter values were reduced when increasing bulk density and time. Maximum available P in soil was observed in the P and NP fertilizer treatments followed by the previously isolated strain P-solubilizing *Bacillus* M-13 and new strain *Bacillus* RC01. *Bacillus* M-13 and *Bacillus* RC01 inoculations increased available P, respectively, by 16.9 and 8.8% compared with control pots. Inoculation with other bacterial strain did not show significant differences in available P with respect to the control, and it was not affected by bulk density. Bacterial inoculations significantly affected the contents of both NO_3^--N and total mineral N content of soil. Among the bacterial inoculants, the maximum NO_3^--N content of soil was seen in *Bacillus* RC01, followed by RC03, RC02 and M-13. There was no significant difference in the concentration of extractable NH_4^+-N among the various treatments, except for the N and NP fertilizer treatments. Concentration of NO_3^--N was greater than that of NH_4^+-N. Maximum concentration of NO_3^--N and NH_4^+-N was observed on the 15th and 30th day, respectively, and then declined. Soil NO_3^-N and NH_4^+N contents were reduced by soil compaction (Table 2).

In general, time and bulk density significantly affected all the tested parameters in barley. Root length and/or dry weight increased in all treatments with time, whereas they

Table 2 The effect of soil compaction, PGPB inoculation and fertilizer application on same soil properties

	pH[a,b]	Organic matter (%)	Available P (mg kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	Total mineral N (mg kg^{-1})
Treatments (*n*=27)						
Control	6.86±0.04 a	1.76±0.02 a	13.4±0.4 a	7.4±0.6 a	8.1±0.3 a	15.5±0.6 a
N fertilizer	6.88±0.08 a	1.76±0.03 a	13.4±0.3 a	21.8±1.2 de	35.0±2.8 b	56.8±3.3 e
NP fertilizer	6.87±0.07 a	1 76±0.02 a	24.3±0.7 d	22.1±1.1 e	35.7±2.2 b	57.9±2.6 e
P fertilizer	6.87±0.04 a	1.75±0.02 a	24.5±0.7 d	7.3±0.5 a	8.6±0.6 a	15.9±0.8 a
B. RC01	6.85±0.05 a	1.76±0.03 a	14.6±0.4 b	20.8±1.2 cd	8.9±0.7 a	29.6±1.6 d
B. RC02	6.87±0.04 a	1.76±0.03 a	13.9±0.4 a	19.7±1.0 c	8.1±0.6 a	27.7±1.0 c
B. RC03	6.86±0.06 a	1.76±0.02 a	13.9±0.4 a	20.3±1.2 c	8.6±0.8 a	28.9±1.5 d
B. M-13	6.83±0.05 a	1.74±0.03 a	17.9±0.6 c	12.4±0.6 b	8.5±0.7 a	20.9±0.9 b
Bulk density (Mg m^{-3}) (*n*=72)						
1.10	6.89±0.03 b	1.79±0.02 c	17.2±1.1 a	17.0±1.6 b	16.2±2.9 c	33.2±4.0 c
1.25	6.87±0.03 b	1.76±0.01 b	16.8±1.1 a	16.2±1.5 a	15.1±2.9 b	31.3±3.9 b
1.40	6.84±0.04 a	1.73±0.01 a	16.9±1.1 a	16.1±1.4 a	14.3±2.8 a	30.4±3.7 a
Harvest time (days) (*n*=72)						
15	6.97±0.02 c	1.78±0.02 c	17.4±1.1 b	17.8±1.6 c	16.3±3.1 b	34.0±4.1 c
30	6.92±0.01 b	1.76±0.01 b	16.9±1.1 a	16.2±1.5 b	16.2±3.1 b	32.4±4.1 b
45	6.71±0.02 a	1.73±0.01 a	16.7±1.1 a	15.4±1.4 a	13.1±2.3 a	28.5±3.2 a

[a]Values are means±SE
[b]Averages of the same column values (each section separately) followed by same letter did not differ significantly from Duncan's multiple range tests at 5% significance

were decreased with soil compaction (Table 3). Of the bacterial inoculation, root length was only affected by *Bacillus* RC01 compared to the control. Mineral fertilizer and bacterial inoculation significantly increased root and shoot dry weight of barley plants. The results showed that there was numerical but no statistical difference between bacterial inoculation and P fertilizer in terms of root and shoot dry weight. As bulk density decreased and harvest time increased, bacterial count and seedling growth increased. Soil compaction caused low root and shoot dry weight, but the effect depended on the treatment. Of the treatments, the maximum barley root dry weight was observed in the NP fertilizer treatment, followed by the *Bacillus* RC01 and N fertilizer treatments.

Total culturable bacteria varied from 0.9 to 10.5× 10^6 CFU g^{-1} of soil depending on the treatment. The bacterial and fungal population at day 45 was significantly greater than at days 15 or 30 in all treatments (Table 4). The highest number of total culturable bacteria and PSB was observed in pots inoculated with P-solubilizing *Bacillus* M-13. The counts of PSB ranged between 1.2×10^4 and 6.7×10^6 CFU g^{-1} dry weight soil. The treatments, either that based on fertilizer application or those with PSB inoculations, were affected by both bacterial and fungal pop-

Table 3 Effect of soil compaction, mineral fertilizer and inoculation of barley with PGPB on root length, root and shoot weight in non-sterile soil

	Root length[a,b] (cm)	Root dry weight (g)	Shoot dry weight (g)	Total plant dry weigth (g)
Treatments (*n*=27)				
Control	16.6±1.1 ab	0.24±0.04 a	0.49±0.14 a	0.74±0.17 a
N fertilizer	19.8±2.6 d	0.28±0.06 cd	0.64±0.21 bc	0.93±0.26 b
NP fertilizer	19.0±1.4 d	0.30±0.06 d	0.70±0.22 c	1.00±0.27 c
P fertilizer	16.9±1.1 ab	0.26±0.05 ab	0.62±0.19 b	0.88±0.23 b
B. RC01	18.0±1.0 c	0.28±0.06 bc	0.66±0.21 bc	0.93±0.26 b
B. RC02	16.4±1.5 a	0.27±0.05 bc	0.66±0.20 bc	0.92±0.23 b
B. RC03	17.0±1.1 bc	0.26±0.04 bc	0.63±0.20 b	0.89±0.24 b
B. M-13	17.6±1.4 bc	0.27±0.04 bc	0.65±0.21 bc	0.93±0.25 b
Bulk density (Mg m^{-3}) (*n*=72)				
1.10	19.3±0.7 c	0.31±0.03 c	0.67±0.12 b	0.99±0.03 c
1.25	18.1±0.6 b	0.29±0.03 b	0.65±0.12 b	0.94±0.03 b
1.40	15.7±1.1 a	0.21±0.02 a	0.58±0.11 a	0.78±0.06 a
Harvest time (days) (*n*=72)				
15	16.2±0.7 a	0.15±0.01 a	0.21±0.03 a	0.36±0.03 a
30	16.6±0.7 a	0.26±0.01 b	0.39±0.02 b	0.65±0.03 b
45	20.2±1.0 b	0.40±0.02 c	1.29±0.05 c	1.70±0.06 c

[a]Values are means±SE
[b]Averages of the same column values (each section separately) followed by same letter did not differ significantly from Duncan's multiple range tests at 5% significance

ulation. On the other hand, total number of bacteria and fungi decreased with increasing bulk density in all treatment groups as well as in the control (Table 4). The number of days between sowing and seedling harvest time was positively and significantly correlated ($p<0.01$) with total culturable bacteria ($r=0.53$), fungi ($r=0.48$) and PSB ($r=0.19$) counts, but they were negatively and significantly correlated ($p<0.01$) with soil compaction ($r=-0.63$, $r=-0.63$ and $r=-0.26$, respectively).

Both inoculations with PGPB and fertilizer application increased the N and P contents of barley seedlings compared to the untreated control. Inoculation with the P-solubilizing bacteria *Bacillus* M-13 also significantly enhanced Mn, Zn and Cu contents of barley seedling. Both NP and P applications increased P content up to 40%, whereas there were no significant differences in K, Ca and Fe concentrations between inoculation and fertilizer treatments. Bulk density did not affect K, Mg and Zn contents of barley, whereas high bulk density decreased N, P and Ca contents. The Fe, Mn and Cu contents of barley were higher in compacted than in loose soil (Table 5).

Discussion

The soil NO_3^--N content and bacterial and fungal counts at 15, 30 and 45 days were influenced significantly ($p<0.05$) by the application of chemical fertilizers, PGPB inocula-

Table 4 The effect of soil compaction, harvest time, PGPB inoculation and fertilizer application on contents of total bacteria, total fungi and P-solubilizing bacteria

Treatments	Harvest time (day)	Total bacteria ($\times10^6$)	Total fungi ($\times10^6$)	P-solubilizing bacteria ($\times10^6$)	Bulk density (Mg m^{-3})	Total bacteria ($\times10^6$)	Total fungi ($\times10^6$)	P-solubilizing bacteria ($\times10^6$)
		Total number of CFU g^{-1} dry soil				Total number of CFU g^{-1} dry soil		
Control	15	1.3	1.0	0.0	1.10	2.8	1.7	0.0
	30	2.0	1.3	0.0	1.25	2.0	1.4	0.0
	45	2.8	1.8	0.0	1.40	1.3	0.9	0.0
	Average	2.0 a	1.4 a	0.02 a	Average	2.0 a	1.4 a	0.02 a
N fertilizer	15	2.3	1.7	0.0	1.10	4.9	2.9	0.1
	30	3.4	2.0	0.0	1.25	3.3	2.3	0.0
	45	4.7	2.8	0.1	1.40	2.1	1.5	0.0
	Average	3.5 b	2.2 b	0.04 a	Average	3.5 b	2.2 b	0.04 a
NP fertilizer	15	2.9	2.2	0.0	1.10	6.3	3.6	0.1
	30	4.1	2.6	0.1	1.25	4.1	3.0	0.1
	45	6.0	3.5	0.1	1.40	2.5	1.8	0.0
	Average	4.3 g	2.8 f	0.06 a	Average	4.3 g	2.8 f	0.06 a
P fertilizer	15	2.6	1.9	0.0	1.10	5.5	3.1	0.1
	30	3.5	2.3	0.0	1.25	3.6	2.6	0.1
	45	5.4	3.0	0.1	1.40	2.3	1.5	0.0
	Average	3.8 d	2.4 c	0.05 a	Average	3.8 d	2.4 c	0.05 a
B. RC01	15	2.6	2.1	1.3	1.10	5.0	3.5	2.2
	30	3.6	2.6	1.7	1.25	3.3	2.6	1.3
	45	4.7	3.3	1.9	1.40	2.7	1.9	1.4
	Average	3.7 c	2.7 e	1.60 b	Average	3.7 c	2.7 e	1.60 b
B. RC02	15	2.8	2.3	1.5	1.10	6.0	3.6	3.1
	30	3.9	2.8	1.9	1.25	3.9	3.0	1.9
	45	5.9	3.3	3.1	1.40	2.6	1.8	1.4
	Average	4.2 f	2.8 f	2.1 d	Average	4.2 f	2.8 f	2.1 d
B. RC03	15	3.0	2.2	1.7	1.10	6.0	3.3	3.2
	30	3.6	2.3	1.6	1.25	3.3	2.8	1.3
	45	5.5	3.3	2.7	1.40	2.8	1.8	1.5
	Average	4.0 e	2.6 d	2.0 c	Average	4.0 e	2.6 d	2.0 c
B. M-13	15	3.5	2.4	2.1	1.10	7.1	3.2	4.3
	30	4.5	2.3	2.5	1.25	4.5	3.1	2.5
	45	6.6	3.7	3.8	1.40	2.9	2.1	1.6
	Average	4.9 h	2.8 f	2.8 e	Average	4.9 h	2.8 f	2.8 e
Treatment average	15	2.6 a	2.0 a	0.8 a	1.10	5.5 c	3.1 c	1.6 c
	30	3.6 b	2.3 b	1.0 b	1.25	3.5 b	2.6 b	0.9 b
	45	5.2 c	3.1 c	1.5 c	1.40	2.4 a	1.6 a	0.7 a

Averages of the same column followed by same letter did not differ significantly from Duncan's multiple range tests at 5% significance

Table 5 The effect of soil compaction, PGPB and fertilizer application on nutrient concentration

	Macronutrient (% of dry matter)				Micronutrient (mg kg^{-1} of dry matter)				
	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Treatments (*n*=27)									
Control	3.06 a	0.25 a	3.41 a	0.63 a	0.28 a	98.8 a	73.4 ab	66.2 a	15.1 a
N fertilizer	3.91 d	0.26 a	3.47 a	0.62 a	0.28 a	98.7 a	72.1 a	67.2 ab	16.2 a–c
NP fertilizer	3.91 d	0.35 d	3.41 a	0.62 a	0.28 ab	101.1 a	74.3 a–c	67.1 ab	16.4 a–c
P fertilizer	3.15 b	0.35 d	3.38 a	0.65 a	0.29 ab	100.4 a	78.5 c	68.4 ab	16.2 a–c
B. RC01	3.23 bc	0.30 b	3.37 a	0.64 a	0.29 ab	98.9 a	72.1 a	68.6 ab	17.0 bc
B. RC02	3.22 bc	0.30 b	3.38 a	0.63 a	0.29 ab	96.9 a	74.4 a–c	71.9 ab	15.9 ab
B. RC03	3.15 b	0.33 c	3.37 a	0.63 a	0.30 b	100.2 a	77.5 bc	70.0 ab	17.3 c
B. M-13	3.25 c	0.31 b	3.39 a	0.63 a	0.29 ab	98.2 a	77.5 bc	73.0 b	17.5 c
Bulk density (Mg m^{-3}) (*n*=72)									
1.10	3.40 b	0.32 b	3.40 a	0.65 c	0.29 a	96.5 a	71.3 a	68.7 a	15.8 a
1.25	3.35 ab	0.30 a	3.41 a	0.63 b	0.28 a	98.6 a	75.4 c	69.4 a	16.8 b
1.40	3.33 a	0.30 a	3.38 a	0.61 a	0.29 a	102.3 b	78.2 b	69.1 a	16.7 b
Harvest time (days) (*n*=72)									
15	3.59 c	0.32 b	3.31 a	0.65 b	0.29 b	97.7 b	65.0 a	68.6 a	15.1 a
30	3.32 b	0.31 a	3.45 b	0.65 b	0.30 c	109.7 c	76.9 b	68.9 a	18.7 b
45	3.16 a	0.29 a	3.42 b	0.60 a	0.27 a	90.1 a	82.9 c	69.7 a	15.5 a

Averages of the same column values (each section separately) followed by same letter did not differ significantly from Duncan's multiple range tests at 5% significance

tion and/or soil bulk density. Seed inoculation resulted in an increased content of soil NO_3^--N as compared to the control. Available amount of P by *Bacillus* M-13 and newly isolated *Bacillus* RC01 was higher than that of the control and the other strains tested. Bacterial inoculation did not influence soil pH. Similarly, Deubel et al. (2000) could not find a clear connection between the decrease in pH value and bacterial P mobilization. Generally, it is believed that solubilization of insoluble compound including P from rock phosphate is due to the excretion of microbial metabolites such as organic acids (Gyaneshwar et al. 1998; Carrillo et al. 2002; Rodriguez et al. 2004). However, in addition to acid production, other mechanisms can cause phosphate solubilization (Nautiyal et al. 2000).

All bacterial inoculations caused significant differences on the tested plant parameters, such as root and shoot dry weight, with respect to the control, although differences between various bacterial strains were insignificant. Interestingly, only strain *Bacillus* RC01 had a prolonged effect on the root length, whereas PGPB enhanced the early seedling vigour, and the root and shoot dry weights of plants inoculated with the PGPB were equal to those of plants receiving N and P fertilizers. Our data showed that a higher nutrient uptake by inoculated roots significantly improved seedling growth. This result confirmed that the PGPB inoculations may effectively increase the surface area of roots (Bashan et al. 2004) and the root weight (Bertrand et al. 2001). Similar findings were reported by Puente et al. (2004b), who showed that the separate inoculation of cactus seedling with *Bacillus pumilus* and *Bacillus subtilis* changed several plant growth parameters.

At the end of the pot experiment (7 weeks after inoculation), total P-solubilizing bacteria differed significantly between control and the PGPB-inoculated pots bearing *Bacillus* M-13 and RC02, the most effective viable population. In addition, all the PGPB strains affected the abundance of bacteria, fungi and PSB in soil (Table 4). It has been shown that the PSB application can increase the PSB population and the amount of plant available P in the rhizosphere soil (Sundara et al. 2002). It has been reported that the growth and metabolic activity of the PGPB population in soil declined probably due to nutrient exhaustion (Rojas et al. 2001; Welbaum et al. 2004). Rojas et al. (2001) demonstrated a synergism between N_2-fixing bacteria and P-solubilizing bacterium. Bacterial and fungal counts of sterile soil were strongly affected by both fertilizer and PGPB inoculation. Indeed, the PBS strain can significantly affect the abundance of bacteria (Shishido and Chanway 1998) and fungal population in soil (Vivas et al. 2003). However, soil commonly reacts as a "biological buffer", and thus, any change in the composition and abundance of microbial population can be temporary (Bashan 1998).

The soil microbial biomass appears to increase with both mineral fertilization and bacterial inoculation, and the microbial community structure (total culturable bacteria and fungi and PSB) changed consistently according to the treatments applied. The beneficial effect of inoculation on microbial population may be direct, due to an increased supply of available P and N, or indirect, through changes in the growth rate and metabolic activities of crop. Microbial growth in the rhizosphere may be limited by available N and P. The nutrient competition between plant and bacteria population in control pots (without bacteria and fertilizer application) probably limited both bacteria and plant growth. Nutritional deficiencies can take place at the ac-

climatization period of micropropacted sugarcane plants; it is possible that such deficiencies may have influenced the composition of soil microflora (Oliveira et al. 2002).

Inoculation of barley with the *Bacillus* RC01 and M-13 significantly increased concentration of P in plants and in soil. Also, bacteria inoculation increased the N content in soil and plant. The higher total N and P uptake of barley indicated that *Bacillus* RC01 and M-13 were able to fix N and solubilize P, with consequent promotion of plant growth. Increased P uptake by plants and plant growth as the result of the PSB inoculation have been already reported (Pal 1998; Rodriguez and Fraga 1999; Puente et al. 2004b). Probably, phosphate-solubilizing and N_2-fixing bacteria improved the N and P nutrition of plants and stimulated of plant growth. *Azospirillum brasilense* and *Azospirillum irakense* strains stimulated overall plant growth, including root development and grain yield of spring wheat and maize, but both rhizobacteria did not change the N concentration in plants or grains (Dobbelaere et al. 2002). By contrast, plants inoculated with the PGPB generally have a higher N content than the uninoculated plants (Puente et al. 2004b).

In the case of *Bacillus* RC02 and RC03 inoculants, stimulation of barley growth probably occurred through the release of plant growth substances by these bacteria since an increase in the available P content of soil was not observed. The production of hormones has been suggested to be one of the mechanisms by which PGPB including *Bacillus* species stimulate plant growth (Amer and Utkheda 2000; Steenhoudt and Vanderleyden 2000). However, only a few experiments with the PSB have been concluded, and relative results are quite diverse, varying according to plant or bacterial species. *Bacillus megaterium* and *Paenibacillus polymyxa* were able to enhance growth and yield but not the P uptake of canola, indicating that P solubilization is not the main mechanism responsible for positive plant response (De Freitas et al. 1997).

Our results have also showed that root length and root and shoot weight of plants were reduced by soil compaction due to increased resistance to root penetration and decreased the effects of fertilizer and bacterial strains. Root growth and soil inorganic N (NH_4^+-N+NO_3^--N) content generally decreased with increasing soil bulk density. The most pronounced effect of soil compaction was the reduction of root growth, and this result confirmed previous reports (Oussible et al. 1992; Groleau-Renaud et al. 1998). The limited root growth caused by soil compaction was accompanied by a decrease in the microbial population and by changes in the composition of microbial communities of rhizosphere soil. Soil compaction can also affect microbial activity (Jordan et al. 2003).

In summary, this study showed that the each isolated bacterial strains had a potential as a PGPB, fixed N_2, dissolved P and increased growth of barley seedlings. Inoculation study carried out under sterile soil conditions with barley and the selected rhizobacteria indicated that all inoculant strains increased total bacteria and the PSB population, root and shoot dry weight, and N and P uptake by plants. This study also showed that root length and root and shoot weight of plants were decreased by soil compaction. The results suggest that the bacterial strains tested in this study have a potential to be formulated and used as inoculants in sustainable and organic agriculture.

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