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Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar. *phaseoli*

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Abstract

Rhizobium leguminosarum bv. *phaseoli* strains P31 and R1, *Serratia* sp. strain 22b, *Pseudomonas* sp. strain 24 and *Rhizopus* sp. strain 68 were examined for their plant growth-promoting potential on lettuce and forage maize. All these phosphate solubilizing microorganisms (PSM) were isolated from Québec soils. The plants were grown in field conditions in three sites having high to low amounts of available P. In site 1 (very fertile soil), strains R1 and 22b tended to increase the dry matter yield of lettuce shoots ($p \leq 0.10$). Lettuce inoculated with rhizobia R1 had a 6% higher P concentration ($p \leq 0.10$) than the uninoculated control. In site 2 (poorly fertile soil), the dry matter of lettuce shoots was significantly increased ($p \leq 0.05$) by inoculation with strain P31 and 24 plus 35 kg ha^{-1} P-superphosphate, or with strain 68 plus 70 kg ha^{-1} P-superphosphate. In site 3 (moderately fertile soil), the dry matter of maize shoots was significantly increased ($p \leq 0.05$) by inoculation with strain 24 plus 17.5 kg ha^{-1} P-superphosphate, or with strain P31 plus 35 kg ha^{-1} P-superphosphate. Inoculation with PSM did not affect lettuce P uptake in the less fertile soil in site 2. In site 3 with the moderately fertile soil, maize plants inoculated with strain R1 had 8% higher P concentration than the uninoculated control ($p \leq 0.01$), and 6% with strains P31 and 68 ($p \leq 0.05$). The results clearly demonstrate that rhizobia specifically selected for P solubilization function as plant growth promoting rhizobacteria with the nonlegumes lettuce and maize. The P solubilization effect seems to be the most important mechanism of plant growth promotion in moderately fertile and very fertile soils when P uptake was increased with rhizobia and other PSM.

Introduction

The beneficial effect of inoculating legumes with rhizobia and bradyrhizobia is well known. However, many studies indicate that these nitrogen-fixing soil bacteria have the potential to be used as plant growth promoting rhizobacteria (PGPR) with nonlegumes. In fact, rhizobia can attach to the surface of monocots in the same manner as they attach to dicot hosts (Shimshick and Hebert, 1979; Terouchi and Syõno, 1990). Rhizobia grew readily in the presence of germinating seeds and developing root systems in a similar manner with legumes and nonlegumes (Pena-Cabriales and Alexander, 1983). In maize-legume crop rotation system, the legume - *Rhizobium* symbiosis was improved by inoculating the preceding maize crop with *Rhizobium* (Gaur et al., 1980), suggesting that the growth of rhizobia could be stimulated in the maize rhizosphere. A recent report shows that *Rhizobium leguminosarum* bv. *trifolii* can colonize sites in rice roots and enhance its growth (Yanni et al., 1995). Noel et al. (1996) also observed under gnobiotic conditions, direct growth promotion of the early seedling root of canola and lettuce by *R. leguminosarum*. However, this interesting observation requires further testing in nonsterile field conditions.

Most PGPR strains consist of gram-negative genera, and the greatest number of strains are members of the fluorescent pseudomonads (Kloepper, 1993). Very little is known about plant growth promoting

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activity of rhizobia with nonlegumes. Growth promotion by PGPR can result from one or more mechanisms including: biological control through competition, production of siderophores or antibiotics, and induced disease resistance, and direct growth promotion through phytohormone production and increased nutrient availability through nitrogen fixation or organic and inorganic phosphate (P)-solubilization, (Kloepper, 1993). Like PGPR, rhizobia produce siderophores (Carson et al., 1992), phytohormones (Williams and Signer, 1990), and they are antagonistic to deleterious or phytopathogenic fungi (Ehteshamul-Haque and Ghaffar, 1993). Rhizobia can also solubilize organic and inorganic phosphate (Abd-Alla, 1994; Halder and Chakrabartty, 1993).

The majority of agricultural soils contain large reserves of phosphorus of which a considerable part has accumulated as consequence of regular applications of P fertilizer (Richardson, 1994). The phenomena of fixation and precipitation of P in soil, which is highly dependant on pH, causes a low efficiency of soluble P fertilizers such as superphosphate (Goldstein, 1986). According to Lindsay (1979), superphosphate contains a sufficient amount of calcium to precipitate half of its own P, in the form of dicalcium phosphate (DCP) or dicalcium phosphate dihydrate. In addition, acidic conditions resulting from the precipitation of DCP produces an additional precipitation of P with Al and Fe in acid soils, while additional precipitation is caused by the high availability of Ca in calcareous soils. P-solubilizing microorganisms (PSM) are a soil component which can solubilize precipitated P and other soil P. Several studies have shown that P-solubilization by PSM is an important characteristic of several PGPR strains (Kloepper et al., 1989; Kucey et al., 1989; Subba Rao, 1982). In pot experiments, Salih et al. (1989) demonstrated that P-solubilizing fungi can directly increase P availability in soil fertilized with triple superphosphate or rock phosphate. In addition, greenhouse and field experiments (Chabot et al., 1993) demonstrated that some PSM isolated from Québec soils stimulated the growth of maize and lettuce in field trials. P-solubilization could be important to plant growth because P is an essential element and has low availability to plants due to its chemical properties (Goldstein, 1986): A large number of *Rhizobium* strains are able to solubilize insoluble P compounds (Halder and Chakrabartty, 1993), but this ability is variable among the strains. Microbial P-solubilization in the rhizosphere is not completely understood because it is difficult to do in situ analysis in that complex ecosystem where plant roots and microbes contributions are extremely difficult or impossible to isolate.

In the present work, the effects of inoculation of forage maize and lettuce with previously tested PSM (Chabot et al., 1993) and with phosphate solubilizing rhizobia was studied in field trials. Three sites having different high to low levels in available P (very fertile to poorly fertile soils) were selected.

Materials and methods

Microorganisms

Strains P31 (Lalande et al., 1986) and R1 (from our lab) of *R. leguminosarum* bv. *phaseoli* were isolated. These strains were the best P-solubilizing rhizobia selected for the present work from approximately 300 strains tested with DCP. *Enterobacter* sp. 22a, *Serratia* sp. 22b, *Pseudomonas* sp. 24, and the fungal isolate *Rhizopus* sp. 68 were also isolated from Québec soils and are phosphate-solubilizing microorganisms with plant growth promoting potential (Chabot et al., 1993). All bacteria were maintained at -70 °C in tryptic soy broth (TSB; Difco) containing 20% glycerol. *Rhizopus* sp. 68 was maintained on tryptic soy agar (TSA; Difco) blocks submerged in sterile water at 4 °C (Kirsop and Snell, 1984).

Determination of in vitro PGPR characteristics

The PGPR characteristics of PSM determined were P-solubilization, siderophore, HCN and IAA production (and IAA analogs). P-solubilization was qualitatively determined in DCP plates (Goldstein, 1986) by the observation of a distinct zone of clarification around the colonies. Organic P solubilization was determined in the same basic media as DCP, but phytic acid (inositol hexaphosphate, Sigma) replaced the DCP precipitate as the P-source. Production of siderophore (Alexander and Zuberer, 1991), HCN (Bakker and Schippers, 1987) and IAA and/or IAA-analogs (Bric et al., 1991) were assessed by previously described methods.

Inoculant preparation and seed inoculation

Bacterial inoculants were prepared by harvesting the cells from a 24 h 10% TSA culture with a sterile cotton swab. The cells were suspended in sterile 0.85% NaCl solution to an optical density at 590 nm (OD_{590}) of 1.6 for rhizobia and 1.4 for other bacteria, giving a min-

imum log CFU mL^{-1} of 8. To obtain a good sporulation, *Rhizopus* sp. 68 was grown on nutrient broth yeast extract (NBY) agar (Shaad, 1980). Plates were then filled with 0.85% NaCl solution and spores were suspended with a loop to an OD_{590} of 2.0 (log 7 CFU mL^{-1}). A 0.85% NaCl solution without cells was used for all uninoculated controls. Lettuce seeds with a commercial clay coating (Paris Island COS, Asgrow seeds Co, Ontario) were inoculated by adding in a Petri plate 12 mL of the bacteria or fungal spores suspensions to 500 seeds. The seeds were dried overnight at room temperature in Petri Plates. Maize seeds (hybrid Funk's 4066) were surface sterilized by washing for 2 min in 70% ethanol and 15 min in 6% sodium hypochlorite followed by several rinses in sterile water. Inoculation was performed in a plastic bag by soaking about 800 seeds for 1 h in a mixture of 10 mL inoculum and 140 mL of a 1% carboxymethylcellulose solution. Seeds were decanted and dried overnight at room temperature in Petri plates. The number of microorganisms per dried seed was estimated before sowing, on samples of 10 seeds, by serial dilution in 0.85% NaCl solution, and by plate count on 10% TSA.

Lettuce in site 1

Prior to the beginning of the field experiment, the 0-15 cm layer of this very fertile soil had a pH of 6.35 and contained 5% organic matter and 618 kg ha^{-1} of available P (Table 1, site 1). According to the guidelines in Québec (AFEQ and CPVQ, 1990), a soil is considered very fertile for the culture of lettuce when it contains more than 400 kg ha^{-1} in available P, and poorly fertile when it contains less than 100 kg ha^{-1}. Inoculated seeds were placed in multicell plates (2.5 cm × 2.5 cm cell) containing Promix™ substrate (peat moss from Tourbières Premier, Rivière du Loup, Québec, Canada) for transplant production. The transplants were grown for 3 weeks in a greenhouse with 16 h daylight at 25 °C and 8 h darkness at 20 °C. Plants were watered daily by using tap water as required to maintain soil at field capacity. A solution of commercial fertilizer 20-10-20 (Peter's soluble fertilizers, WR Grace and Co. of Canada Ltd, Ajax, Ontario) containing 125 μg mL^{-1} N was used every 2 days after emergence of the first real leaf. The randomized block design was used with 6 treatments and 4 replicates. Treatments included inoculation with strains 22a, 22b, P31 and R1 and two uninoculated controls. One of the controls received the equivalent of 13 kg ha^{-1} of P-superphosphate applied in furrow as P-starter, to compare the efficiency of microbial P-solubilization to applied P. Fertilization rates of N and potassium (K) were calculated as recommended for Québec soils (AFEQ and CPVQ, 1990) and the equivalent of 80 kg ha^{-1} $N\text{-}NH_4NO_3$ and 62 kg ha^{-1} K-KCl were applied in furrow before transplantation. Each 3.0 m × 2.0 m plot included 4 rows separated by 75 cm. Each row received a transplant every 20 cm and only the two center rows received the inoculated transplant. Planting was done on July 14 1993, and mature lettuce was harvested on August 27. Six randomized samples were harvested from central rows of each plot, and each sample consisted of two plants.

Lettuce in site 2

Prior to the beginning of the experiment, the 0-15 cm layer of this poorly fertile soil (52 kg ha^{-1} P) had a pH of 6.09 and contained 4.11% organic matter (Table 1, site 2). To compare the microbial solubilization efficiency at different levels of added P (0, 50 and 100% the recommended rate) a split-plot design with the 3 P-fertilization rates (0, 35 and 70 kg ha^{-1} P-superphosphate) as main plots, 5 inoculation treatments as subplots, and 4 replications was used. Inoculation treatments included bacterial strain 24, P31 and R1, *Rhizopus* sp. 68 and the uninoculated control. Lettuce transplants were produced and transferred to the field as described in the previous section. According to recommendation for Québec soils (AFEQ and CPVQ, 1990), the equivalent of 80kg ha^{-1} $N\text{-}NH_4NO_3$ and 83kg ha^{-1} K-KCl was also applied to furrows before planting. Planting was done on June 14 1993, and mature lettuce was harvested on July 29. Harvesting and sampling were performed as described before.

Maize in site 3

This experiment was similar to that described for lettuce in site 2. Comparatively to other soils, this one cultivated with forage maize, was moderate in available P (122 kg ha^{-1}) according to the guidelines in Québec (AFEQ and CPVQ, 1990). Prior to the beginning of the experiment, the 0-15 cm layer of site 3 had a pH of 6.80 and contained 3.13% organic matter (Table 1). The 3 P-rates applied were 0, 17.5 and 35 kg ha^{-1} P-superphosphate. An equivalent of 180 kg ha^{-1} $N\text{-}NH_4NO_3$ and 71 kg ha^{-1} K-KCl was applied to each plot before seeding. Each 3.0 m × 2.0 m plot included 4 rows separated by 75 cm. Each row received two seeds every 15 cm, and after emergence by selective

Table 1. Some characteristics of the soils used in field trials

Site[a]	Texture	pH	Organic matter (%)	Mehlich-III available elements (kg ha^{-1})			
				P	K	Ca	Mg
1	Silty clay loam	6.35	4.99	618	487	4968	255
2	Loam	6.09	4.11	52	298	5488	371
3	Loam	6.80	3.13	122	373	5705	213

[a] All field trials were located at Ile d'Orléans, Québec. Soil analysis were performed on the 0-15 cm layer.

thinning, healthy plants were spaced 15 cm from one another. Only the two central rows received inoculated seeds. Seeding was done on May 19 1993, and the 60th day, a plant height measurement was done from soil surface to the tip of the highest leaf. Plants were harvested on August 17 after the ear formation stage, and sampling was performed in the two central rows as described before.

Soil and plant analysis

All soil samples were air-dried and sieved (2 mm). Soil pH was measured in water (1:1,30 min equilibrium). Available elements were extracted and determined using the Mehlich 3 procedure (Mehlich, 1984). Soil organic matter was assessed by the modified Walkley and Black method (McKeague, 1978). The plant shoot samples were dried at 70 °C, weighed, ground and digested in 15 mL $HClO_4$ and 5 mL HNO_3. The spectrophotometric vanado-molybdate method was used to measure P (Tandon et al., 1968), and other mineral elements (K, Ca, Mg, Mn, Cu, and Zn) were determined by atomic absorption spectrophotometry (Gaines and Mitchell, 1979). The P in shoots was expressed as P concentration in the tissues (mg P g^{-1}) and as total P uptake per plant (mg $plant^{-1}$) which is the product of P concentration and dry matter yield.

Statistical analysis

All data were tested for homogeneity of the variance by using Hartley's test (Kirk, 1982). Analysis of variance was done with the GLM (General Linear Models) procedure of SAS (Littel et al., 1991). Treatments means were compared by single-degree orthogonal contrasts (Steel and Torrie, 1980) between each treatment and the uninoculated control. The heterogeneity of slopes comparing all regressions coefficients of inoculation treatments at increasing levels of P-fertilization was tested with the GLM procedure of SAS (Littel et al., 1991).

Results

Some in vitro PGPR characteristics of PSM

Rhizopus sp. 68 had the highest P solubilization activity on DCP plates (Table 2). *R. leguminosarum* bv. *phaseoli* strains P31 and Rl showed solubilization activity similar to that of *Pseudomonas* sp. 24. Whereas, the other bacteria exhibited lower activity. All strains used in this work were able to solubilize organic P as phytic acid as observed by the presence of a large zone of clarification around the colonies. All microorganisms studied also produced siderophores and IAA and/or IAA analogs, except *Rhizopus* sp. 68 which was IAA negative. *R. leguminosarum* bv. *phaseoli* P3l was the only HCN producer (Table 2).

Effect of PSM inoculation on lettuce in site 1

In this very fertile soil, inoculation of lettuce with *Serratia* sp. 22b or with *R. leguminosarum* bv. *phaseoli* R1 tended ($p \leq 0.10$) to increase dry matter yield of lettuce shoots by 7 and 6% respectively, as compared to the uninoculated control (Table 3). Addition in furrow of a starting fertilization of 13 kg ha^{-1} P-superphosphate caused 17% increase in lettuce dry matter yield ($p \leq 0.05$). The addition of starting P-fertilization significantly increased ($p \leq 0.05$) P-concentration of lettuce by 8% and P uptake by 27% as compared to the uninoculated control (Table 3). Inoculation with rhizobia R1 increased lettuce P-concentration by 6%, however this increase is significant only at $p \leq 0.10$. Inoculation of lettuce seeds with PSM did not show any significant influence on the mineral content of the plant tissues (Figure 1). However, inoculated plants and the

Table 2. Phosphate solubilization and production of siderophores, HCN and IAA by different P-solubilizing microorganisms (PSM)

Microorganism	Strain	P-solubilization[a] (mm)	Siderophores[b] (mm)	HCN[c]	IAA[d]
Enterobacter sp.	22a	3-4	1-2	-	+
Serratia sp.	22b	2-3	3-5	-	+
Pseudomas sp.	24	4-5	3-5	-	+
R. leguminosarum	P31	4-5	3-5	++	+++
bv. *phaseoli*	R1	4-5	2-3	-	++
Rhizopus sp.	68	>10	>10	-	-

[a] Radius of the clarification zone around colonies on DCP agar.
[b] Radius of the orange halo around colonies on CAS agar.
[c] -, negative reaction; ++, intense orange-brown coloration.
[d] IAA or IAA analogs: -, negative reaction; + to +++, intensity of the pink to red reaction.

control receiving starting P-fertilization tend to accumulate more Cu in their tissues than the uninoculated control.

Effect of PSM inoculation and P-fertilization on lettuce in site 2

In this less fertile soil, a significant interaction was found between PSM inoculation and fertilization treatments, on lettuce dry matter production (Table 4). This indicates that lettuce reacted differently to inoculation according to the rate of P-superphosphate applied. Interpreting interaction (Steel and Torrie, 1980) indicated that increasing P-fertilization resulted in a significant increase of the dry matter yield of shoots (Figure 2A). In comparison to the uninoculated control, the dry matter yield was significantly ($p \leq 0.05$) increased by strains P31 and 24 at the intermediate P-superphosphate rate, and by the isolate 68 at 70 kg ha^{-1} P-superphosphate (Figure 2A). No interaction was found between the effects of PSM inoculation and P-fertilization on P concentration and total uptake by lettuce (Figure 2B and 2C). In general, P-superphosphate addition significantly increased ($p \leq 0.01$) P concentration and uptake by lettuce, while PSM inoculation had no significant effect (Table 4). As with lettuce in site 1, seeds inoculation with PSM did not significantly influence plant mineral composition (Figure 3), but a general trend of increasing mineral uptake was observed. Also, testing for heterogeneity of slope was not significant, showing that in general inoculated and uninoculated control treatments reacted each in a similar manner to increasing rates of P-fertilization for all measured variables (dry matter, P concentration and uptake, K, Ca, Mg, Fe, Mn, Cu and Zn content).

Effect of PSM inoculation and P-fertilization on maize in site 3

In this moderately fertile soil, no significant interaction was observed between PSM inoculation and P-superphosphate fertilization on the height of 60-days old maize plants (Figure 4). This indicates that maize generally had the same reaction to inoculation under the different rates of P used. However, the independent effect of P-fertilization and PSM inoculation effects were significant ($p \leq 0.01$), and contrasts indicated that maize inoculated with strains P31 ($p \leq 0.01$) and 24 ($p \leq 0.05$) was generally taller than uninoculated control plants (Figure 4). At harvesting, a significant interaction was found between PSM inoculation and P-fertilization effects on dry matter yield of shoots (Table 4, Figure 2D), showing that maize reacted differently to inoculation according to the rate of P applied. Interpreting interaction (Steel and Torrie, 1980) indicated that addition of increasing amount of P, resulted in a significant increase of the dry matter production (Table 4). However in general, maximum maize dry matter yield was reached when 17.5 kg ha^{-1} P-superphosphate were used (Figure 2D). The dry matter yield was significantly ($p \leq 0.05$) increased by strain 24 when 17.5 or 35 kg ha^{-1} P-superphosphate were added, and by strain P31 at 35 kg ha^{-1} P-superphosphate rate (Figure 2D). No significant interaction was observed between PSM inoculation and P-fertilization treatments on maize P concentration and total uptake (Figure 2E and F), indicating that maize had the same response to inoculation at increasing P-fertilization levels. P-fertilization significantly decreased P concentration of maize plants by the effect of dilution by increased dry matter (Figure 2E), but the effect of PSM inoculation was a general

Table 3. Effect of PSM inoculation on dry matter production, P concentration and total P uptake of lettuce shoots grown on site 1

Treatments	% of non-inoculated control[a]		
	Dry matter	[P]	Total P
Enterobacter sp. (22a)	100	99	99
Serratia sp. (22b)	107**	103	109
R. leguminosarum bv. *phaseoli* (P31)	104	101	106
R. leguminosarum bv. *phaseoli* (R1)	106**	106**	113
C+PS[b]	117*	108*	127*
C.V.	12.6	5.9	13.4

[a] The average values for the uninoculated control (100%) are: dry matter, 29.4 g plant^{-1}; P-concentration ([P]), 4.08 mg g^{-1} dry matter: and total P, 120.1 mg plant^{-1}.
[b] Uninoculated control + 13 kg ha^{-1} P-superphosphate applied in furrow.
*,**Significantly different from the control at $p \leq 0.05$ and $p \leq 0.10$ respectively.

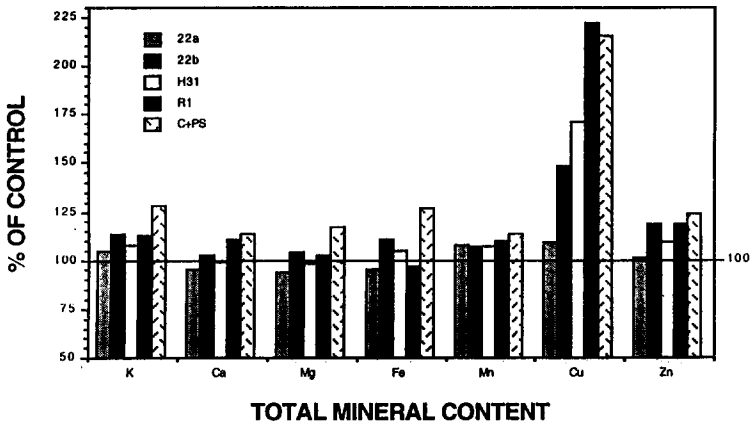

Figure 1. Mineral composition of lettuce in site 1 inoculated with PSM or receiving in-furrow 13 kg ha^{-1} P-superphosphate (C+PS). PSM used were *Enterobacter* sp. (22a), *Serratia* sp. (22b), and *R. leguminosarum* bv. *phaseoli* (P31 and R1). The average values for the uninoculated control (100%) are in mg plant^{-1}: K, 1335; Ca, 244; Mg, 117.4; Fe, 72,6; Mn, 3.03; Zn, 3.12; and Cu, 0.75.

significant increase of P concentration as compared to the uninoculated control (Table 4 and Figure 2E). On average, the concentration of P in maize was 8% higher in plants inoculated with rhizobia R1 ($p \leq 0.01$) and 6% higher in plants inoculated with rhizobia P31 or *Rhizopus* sp. 68 ($p \leq 0.05$). As observed with lettuce grown in sites 1 and 2, inoculation of maize seeds with PSM did not have a significant influence on the mineral composition of plant tissues (Figure 3), but PSM inoculation generally did tend to increase mineral uptake. However, Cu in maize tissues was not in sufficient concentration to be detected by the method used. The test for heterogeneity of slopes was not significant, indicating that as observed with lettuce, in general inoculated and uninoculated control maize plants reacted each in a similar manner to increasing rates of P-fertilization for all measured variables (dry matter, P concentration and uptake, K, Ca, Mg, Fe, Mn, Cu and Zn content).

Discussion

The results presented here indicate that PSM can act as plant growth-promoting microorganisms. Of partic-

Table 4. Summary of the analyses of variance for lettuce shoots in site 2 and maize shoots in site 3

Source of variation	df	Mean squares					
		Lettuce			Maize		
		Dry matter matter	[P][a]	Total P	Dry matter matter	[P]	Total P
Treatments							
P-rates	2	5467*	1.43*	13905*	48625*	0.587*	9152*
PSM	4	102.1**	0.134	349.9	271.1	0.0463**	1384
Interaction							
P-rates × PSM	8	103.7*	0.0765	283.4	1101*	0.00911	772.3
Contrasts							
R1 vs C[b]	1	12.08	0.336	366.8	7.47	0.157*	4085
P31 vs C	1	237.4*	0.0104	247.3	450.8	0.0925**	4059
24 vs C	1	15.34	0.299	145.3	392.4	0.0360	2548
68 vs C	1	31.83	0.118	19.24	13.38	0.0925**	1724
Error		33.94	0.0635	240.9	453	0.0153	1578
C.V.		21.2	8.6	18.6	14.14	5.6	12.0

[a] P concentration in plant dry matter.
[b] Uninoculated control.
*,**Significantly different from control at $p \leq 0.01$ and $p \leq 0.05$, respectively.

ular interest is the finding that P-solubilizing rhizobia can enhance growth of nonlegumes. This increases the potential applicability of rhizobial inoculants in agriculture. The low variability of the field results permitted the detection of significant increases or trends with differences between means as low as 6%. This low variability was obtained because the 1993 cropping season was especially beneficial in the Québec city area, giving high and uniform yield. In 1992, a less beneficial cropping season, the PSM inoculation with lettuce and maize gave higher increases (up to 20%) but the variation was also higher (Chabot et al., 1993). It is also of special interest that PSM can promote the growth in beneficial as in a less beneficial season.

In site 1, the dry matter increases of lettuce and the observed trends of increasing P uptake by *Serratia* sp. 22b and *R. leguminosarum* bv. *phaseoli* R1, as expected, were less important than the stimulation caused by the addition of 13 kg ha^{-1} P in-furrow, since a part of the P-superphosphate can be rapidly used by plants. However, these results also suggest that PSM inoculation might replace a part of the effect of added superphosphate by the solubilization of soil P, since no superphosphate was added in inoculated plots. This growth promotion observed with lettuce in this very fertile soil was probably mainly due to P solubilization by PSM, and especially rhizobial strain R1, but other mechanisms as described above cannot be excluded.

The results in site 2 confirm the possible involvement of other mechanisms since PSM did not increase P-uptake of lettuce when yield was increased. In fact only increasing P-superphosphate rate significantly enhanced lettuce P concentration and total uptake in this less fertile soil. Noel et al. (1996) suspected that IAA production by *R. leguminosarum* plays a role in the growth promotion of nonlegumes such as lettuce. All of our bacterial strains were IAA producers, and especially the rhizobial strains. The absence of an effect with PSM inoculation on P uptake by lettuce can be attributed in part to microbial alteration of one or more of the critical factors influencing the concentration of P in the soil solution and the absorption of P by plants (Richardson, 1994). However, the major source of phosphatase activity which mineralize organic phosphorus in soils is generally considered to be of microbial origin, but phosphatase activity might be decreased by high phosphate concentrations (Richardson, 1994). Also, inorganic P solubilization might be repressed by the level of soluble P (Goldstein, 1986). The presence of high amounts of soluble P in site 1 or when P-superphosphate was applied in sites 2 and 3, might reduce PSM efficiency. The absence of response to PSM inoculation in absence of P-fertilization in site 2 can be explained by a possible occurrence of competition between plant and microorganisms in this low P environment (52 kg ha^{-1} P). In the case of maize growing in a moderately fertile soil (site 3), the plants

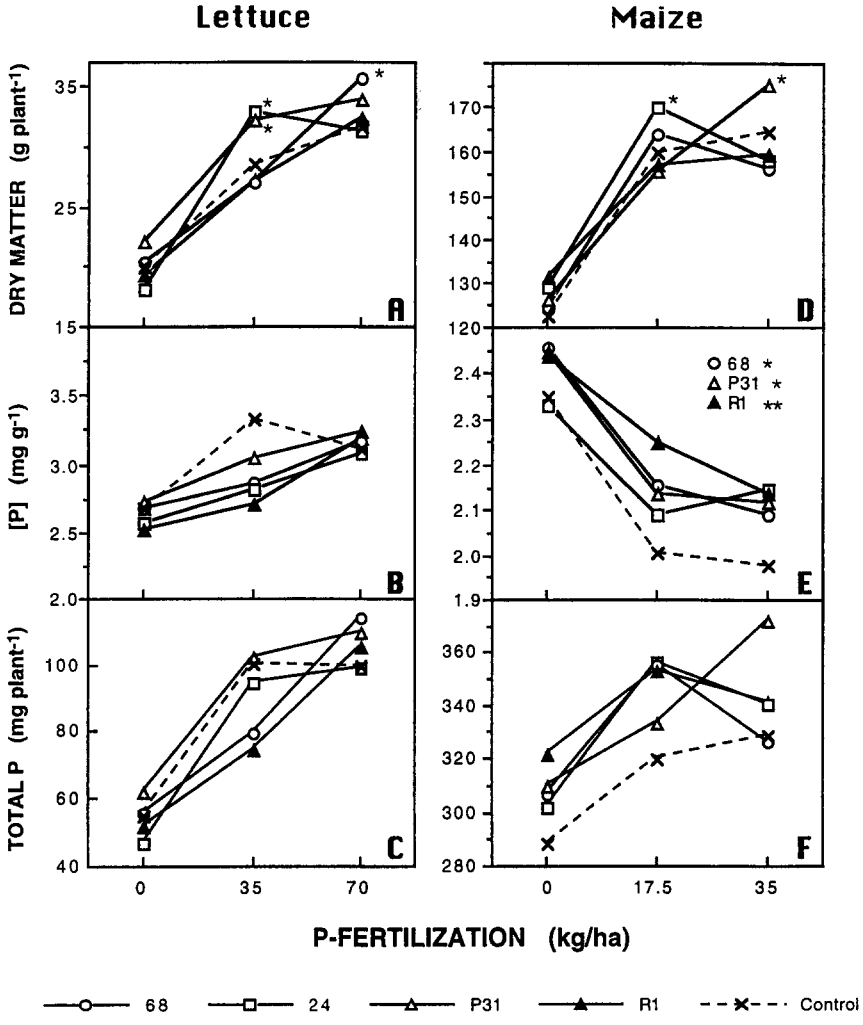

Figure 2. Effect of P-fertilization and PSM inoculation on dry matter production, P concentration ([P]), and total P uptake by lettuce in site 2 and maize in site 3. PSM used were *Rhizopus* sp. (68), *Pseudomonas* sp. (24), and *R. leguminosarum* bv. *phaseoli* (P31 and R1). A significant interaction was found between inoculation and fertilization effects in Figure **A** and **D**. Interpreting interaction showed some significant effects of PSM according to P-rates (* = $p \leq 0.05$). No significant interaction was found between PSM inoculation and P-fertilization effects in Figure **B**, **C**, **E**, and **F**. ** ($p \leq 0.01$) and * ($p \leq 0.05$) indicate general significant effects of PSM only in Figure E (see Table 4).

inoculated with rhizobia P31 or R1 and with *Rhizopus* sp. 68 had a concentration of P significantly higher than that of the uninoculated control at all levels of P applied. However, the level of available P in site 3 (122 kg ha^{-1}) was more than twice the one found in site 2 where the lettuce was grown. The higher total P uptake of maize inoculated with PSM, indicated that P solubilization was probably a major mechanism of growth promotion in this moderately fertile soil.

The inoculation of maize with *Pseudomonas* 24 and rhizobia P31, generally increased plant height after 60 days of growth under field conditions, and was associated with dry matter yield increases with strain 24 only at half the recommended P-fertilization (17.5 kg ha^{-1}) and with rhizobia P31 only at the full recommended rate. A lower growth promotion effect on maize harvested at the same stage, as compared to plant height at 60 days, was previously observed in the very fertile soil (Chabot et al., 1993). The highest PSM effects on lettuce grown in site 2 were observed with rhizobia P31 and strain 24 when half the recommended rate of P (35 kg ha^{-1}) was added, and with *Rhizopus* sp. 68 at the highest rate of P. These observations illustrated the complexity of the interactions occurring in the rhizosphere between plant, soil and microorganisms, and the importance of the soil nutrient level on

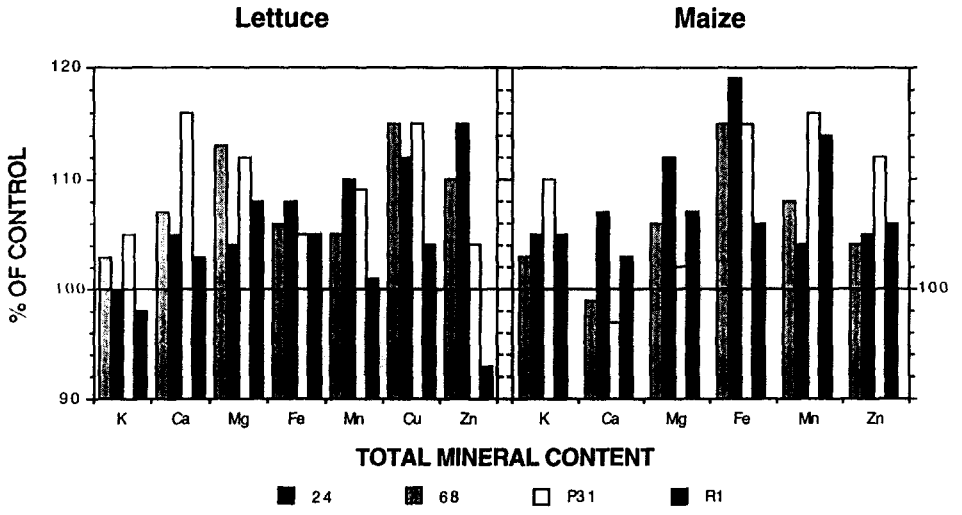

Figure 3. Mineral composition of lettuce in site 2 and maize in site 3 inoculated with PSM. PSM used were *Rhizopus* sp. (68), *Pseudomonas* sp. (24), and *R. leguminosarum* bv. *phaseoli* (P31 and RI). The average values for the uninoculated controls (100%) are in mg plant^{-1} for lettuce: K, 1419; Ca, 256.7; Mg, 123.5; Fe, 167.4; Mn, 2.46; Zn, 6.42; and Cu, 0.80; and for maize: K, 2671; Ca, 448.3; Mg, 240.7; Fe, 16.68; Mn, 2.96; and Zn, 2.61. The Cu in maize was to low to be detected.

the microbial effect in the rhizosphere (Curl and Truelove, 1986). According to plants and soil conditions, the results suggest that different mechanisms of action could be at work and are not necessarily operative at the same time. However, P-solubilization is undoubtedly an important phenotype of PSM since all microorganisms of this work, which were selected exclusively for this characteristic increased P uptake of maize in site 3. In addition, some of them and particularly rhizobia strain R1, tended to increase the P uptake of lettuce in the very fertile soil. In general, regardless of P availability in soil, lettuce and maize inoculated with PSM or receiving P in-furrow, tended to contain greater quantities of nutrients than uninoculated plants. It was reported that rhizobial strains significantly influenced mineral composition of peanut nodules and seeds (Howel, 1987). Under field conditions, wheat plants inoculated with *P. bilaji* also contained more Cu and Fe than uninoculated treatments (Kucey, 1988). Increased nutrient uptake in plants is another sign of solubilizing activities.

In this work strains P31 and R1 of *Rhizobium leguminosarum* bv. *phaseoli*, were the best phosphate solubilizers among 300 strains of rhizobia and bradyrhizobia tested on DCP plates. The solubilization activity of P31 and R1 was comparable or slightly higher than that observed with *Enterobacter* sp. 22a, *Serratia* sp. 22b, or *Pseudomonas* sp. 24 previously selected (Chabot et al., 1993). On the other hand, *Rhizopus* sp. 68 had

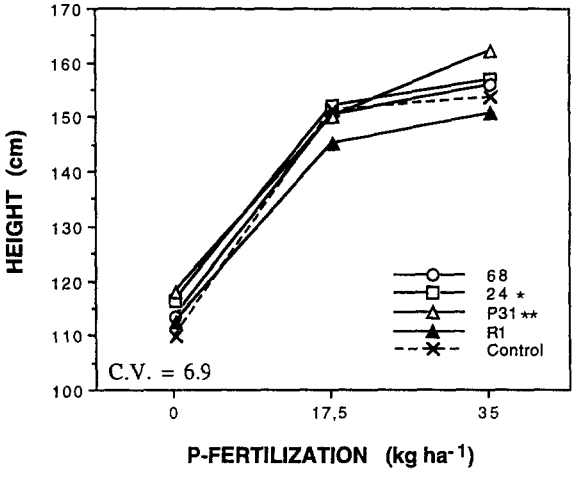

Figure 4. Effect of P-fertilization and PSM inoculation on the height of 60-days old maize in site 3. PSM used were *Rhizopus* sp. (68), *Pseudomonas* sp. (24), and *R. leguminosarum* bv. *phaseoli* (P31 and R1). Plant height was measured from soil surface to the tip of the highest leaf. No significant interaction was found between inoculation and fertilization effects. ** ($p \leq 0.01$) and * ($p \leq 0.05$) indicate general significant effect of PSM.

the highest P solubilization activity, which corroborate a previous report indicating that fungi are superior to bacteria in solubilizing freshly precipitated calcium phosphates (Kucey, 1983). The phosphate solubilizing fungus *Penicillium bilaji* stimulated grain yield in the absence of P-fertilization or when low amount of P fer-

tilizer (4.4 kg ha^{-1}) was added, and incremental yield increases declined with further P addition (Gleddie et al., 1991). On the contrary, *Rhizopus* sp. 68 stimulated lettuce dry matter yield in site 2 only when the highest rate of P was applied, suggesting that the observed stimulation does not result from P-solubilization in this case. It is also interesting to note that *Rhizopus* sp. 68 was the most effective siderophore producer among the PSM tested. However, this fungus also increased P uptake of maize in site 3, showing once again the complexity of plant-microorganism interactions.

Root colonization is an important trait of rhizobacteria and can be strain specific. There is much evidence in the literature indicating that some rhizobia can colonize the roots of nonlegumes (Gaur et al., 1980; Pena-Cabriales and Alexander, 1983; Shimshick and Hebert, 1979; Terouchi and Syõno, 1990). We are presently studying the colonization of the roots of lettuce and maize by rhizobia P31 and R1 and our preliminary results (not shown) indicate that these two strains are very good colonizers.

In the present work, lettuce and maize seeds were inoculated with different carriers because lettuce seeds were obtained with a commercial clay coating. This clay coating is used by lettuce producers to help handling and mechanical sowing because of the very small size of the seeds. It also allowed microorganisms to adhere and survive on the seeds as obtained with 1% carboxymethylcellulose in maize trials.

Rhizobia are well known for their beneficial effect resulting from the symbiotic N_2-fixation with legumes. In this work, we have shown that 2 strains of *R. leguminosarum* bv. *phaseoli* solubilizing soil P can stimulate the growth of lettuce and maize under field conditions, in a fashion similar to other PSM. The advantages of using rhizobia as PGPR, are the availability of the technology of inoculum production and seed inoculation, the excellent understanding of the genetics of these bacteria and since they have been used with legumes for a very long time without any major ecological problem, they can be considered as environmentally friendly bacteria. PGPR activities of rhizobia with nonlegumes, is a characteristic of these bacteria that should be considered in crop rotation practices involving legumes. As PGPR activity can be specific to plant genotypes and can interact with natural mycorrhizal fungi, further work is required to consider these important factors in determining the PGPR activity of rhizobia with nonlegumes.

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