Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions

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Inoculation of canola (*Brassica campestris*) seeds with a nitrogen-fixing strain of *Pseudomonas putida* (GR12-2) drastically increased the root length of seedlings grown in sterile growth pouches. Seed inoculation with inactive bacteria did not affect root lengths. Root elongation capacity was retained by nonnitrogen-fixing mutants of strain GR12-2. On the other hand, two other wild-type pseudomonads that do not fix nitrogen also increased root elongation. The addition of mineral nitrogen to the growth solution at concentrations of 1 m*M* or higher significantly inhibited root elongation of either inoculated or noninoculated seedlings. On the other hand, the addition of phosphate to the growth solution at similar concentrations stimulated root elongation of inoculated and noninoculated seedlings. The combined effects of bacterial inoculation and addition of phosphate on root and shoot elongation and on root and shoot weight were additive. Seed inoculation with *P. putida* GR12-2 increased the uptake of labelled phosphorus (^{32}P) by seedlings grown in growth pouches and also enhanced the shoot elongation of seedlings grown in sterile soil. The capacity of *P. putida* GR12-2 to enhance phosphate uptake and to promote plant growth under gnotobiotic conditions may open the door to a new direction in the development of plant growth promoting inoculants.

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L'inoculation de graines de canola (*Brassica campestris*) avec la souche GR12-2 fixatrice d'azote de *Pseudomonas putida* s'est traduite par une forte augmentation de la longueur de la racine de plantules croissant sous sachets stériles. L'inoculation à l'aide de bactéries inactives n'a pas affecté la longueur des racines. La capacité d'allongement racinaire a été entravée par des mutants non-fixateurs d'azote de la souche GR12-2. Toutefois, deux autres pseudomonas de type sauvage non-fixateurs d'azote ont aussi accrû l'allongement racinaire. L'ajout d'azote minéral à la solution de croissance, à des concentrations de 1 m*M* ou davantage, a inhibé l'allongement racinaire tant chez les plantules inoculées que chez les non-inoculées. Cependant, l'ajout de phosphate à la solution de croissance à des concentrations similaires a stimulé l'allongement racinaire chez les plantules inoculées et chez les non-inoculées. Les effets combinés de l'inoculation bactérienne et de l'addition de phosphate sur l'allongement et sur le poids des racines et des tiges furent additifs. L'inoculation avec *P. putida* GR12-2 a causé une augmentation de l'absorption du phosphore marqué (^{32}P) par les plantules en croissance dans les sachets, ainsi que l'allongement des tiges des plantules croissant en sol stérile. La capacité de *P. putida* GR12-2 de favoriser l'absorption du phosphate et de promouvoir la croissance des plantes sous conditions gnotobiotiques peut être à l'origine d'une orientation nouvelle dans le développement d'inoculants favorisant la croissance végétale.

[Traduit par la revue]

Introduction

While considerable attention has been given to the potential use of fluorescent pseudomonads as biocontrol agents which exclude pathogenic or deleterious microorganisms from the rhizosphere (Howell and Stipanovic 1980; Kloepper and Schroth 1978, 1981; Scher and Baker 1982; Suslow and Schroth 1982*a*, 1982*b*; Weller and Cook 1983; J. O. Becker and R. J. Cook. 1984. Phytopathology, **74**: 806 (Abstr.)), little is known about the direct effect of competitive root-colonizing pseudomonads on the plant in the absence of plant pathogenic or deleterious microorganisms. Recently, cold-tolerant and nitrogen-fixing species of *Pseudomonas* were isolated from the rhizosphere of plants grown in the Canadian High Arctic (Lifshitz et al. 1986). These pseudomonads were found to be highly competitive root colonizers of canola (*Brassica campestris*) grown in field soil. One of these strains, *P. putida* GR12-2, promoted canola yield via seed inoculation in several field trials when no apparent disease occurred (J. W. Kloepper, unpublished). This suggested a direct effect of bacteria-induced plant growth promotion, which is not dependent upon antagonism to plant pathogens.

The aim of this study was to determine if a representative strain of this bacterial group (cold-tolerant and nitrogen-fixing pseudomonads), *P. putida* GR12-2, directly promotes plant growth in the absence of either plant pathogens or deleterious microorganisms.

Materials and methods

Bacteria

Pseudomonas putida 25-71, *P. putida* biovar B 25-33, and *Arthrobacter citreus* 44-9 are growth-promoting rhizobacteria of canola (J. W. Kloepper, unpublished). *Pseudomonas putida* GR12-2, *P. putida* GR19-1, and *P. putida* GR8-8 were isolated from grasses grown in the High Arctic and were previously characterized as Arctic diazotrophic pseudomonads (Lifshitz et al. 1986).

Growth pauch assay

Seed-pack growth pouches (Northrup King Co., Minneapolis, MN, U.S.A. 33440) were filled with 10 mL deionized water (unless otherwise mentioned) and autoclaved for 60 min at 121°C. Seeds of canola (*Brassica campestris* cv Tobin) were surface sterilized by soaking in 1% sodium hypochlorite solution for 10 min followed by thorough rinsing in sterile water and air drying in a laminar flow-hood overnight. Bacterial cultures (20 mL) were grown in Tryptic soybean broth (TSB) in 250-mL flasks shaken at 100 rpm at 25°C for 20 h. Bacterial cells were then washed (twice) in 0.1 *M* $MgSO_4$ buffer and the density of the suspension was adjusted to contain log 7.8 cfu/mL

(unless otherwise mentioned). Surface-sterilized seeds were soaked in bacterial suspensions for 60 min, then aseptically sown in the growth pouches (6 seeds/pouch). The pouches were incubated at 20°C (in the dark) for 6 days. At the end of incubation the pouches were opened and the seedling root length was determined.

The bacterial density on the seeds (after soaking) or on the roots (at the end of the incubation period) was determined by washing seeds or roots in 9 mL of sterile buffer (0.1 *M* $MgSO_4$) with a vortex blender for 15 s. The bacterial suspension was plated on Pseudomonas agar (PAF), using a spiral plater (Spiral Systems Inc., Bethesda, MD, U.S.A. 20814). After 48 h of incubation at 25°C, colonies were counted using a laser counter (model 500A, Spiral Systems). Bacterial cells were then washed (twice) in 0.1 *M* $MgSO_4$ buffer, centrifuged, and the density of the suspension was adjusted to contain log 7.8 cfu/mL (unless otherwise mentioned). Surface-sterilized seeds were soaked in the bacterial suspensions for 60 min then asceptically sown in the growth pouches (6 seeds/pouch). The pouches were incubated at 20°C in the dark for 6 days (unless otherwise mentioned). At the end of incubation the pouches were opened and the seedling root length, root dry weight, shoot length, and shoot dry weight were determined.

Transponson mutagenesis

Transposon Tn5 was introduced into *Pseudomonas* cells by conjugation. *Escherichia coli* cells containing the transposon delivery vector pMKK23:Tn5 (M. Kozlowski and G. Wilkinson, to be published) was used to deliver Tn5 to *Pseudomonas* cells. The vector can efficiently transfer to *Pseudomonas*, but it is unable to replicate in their host. *Escherichia coli* cells containing pMKK23:Tn5 were used as donors in intergeneric matings with rifampicin-resistant *P. putida* GR12-2 recipients. Bacterial matings were conducted on membrane filters. Donors and recipient bacteria (log 8 of each) were mixed and placed a sterile cellulose nitrate filter (Millipore filter; 0.45 μm porosity, 45 mm diameter). The filter, bearing donors and recipients, was placed on the surface of PAF, and incubated for 12–16 h at 25°C. After this time the cells were washed off the filter, resuspended in tryptic soy broth (TSB; Difco) and plated on the selective medium. The transconjugants were selected on PAF supplemented with rifampicin (100 μg/mL). Transconjugant colonies were purified and tested for negative acetylene reduction activity (ARA), using the procedure of Lifshitz et al. (1986). Three ARA-negative (*nif*$^-$) mutants were obtained and were designated as GR12-2/16, GR12-2/25, and GR12-2/764.

Soil plate assay

Petri plates (100 × 25 mm) were filled with 75 g of ground, air-dried, and autoclaved soil (clay loam, pH = 7.0 (determined directly from saturated soil)). The soil was moistened with sterile solution of 1 m*M* K_2HPO_4 or deionized water only, and was left overnight in order to obtain a uniform moisture. Ten canola seeds were planted per plate at a depth of 1 cm. Plates were then incubated at 20°C and 100% relative humidity (RH) in the dark for 5 days. At the end of the incubation the shoots were cut at the soil line and their length was determined.

Phosphorus uptake experiment

Seeds were sown in pouches as described above (growth pouch assay) in the presence of 1 m*M* K_2HPO_4 and 5 μCi (1 μCi = 37 GBq) per mL [^{32}P]phosphoric acid (from New England Nuclear, carrier free at 10 mCi per mL). After incubation for 21 days at 12°C, shoots and roots were harvested separately and washed extensively in several changes of 1 m*M* K_2HPO_4 to remove nonspecifically bound ^{32}P. The amount of incorporated ^{32}P was determined as cpm (counts per minute) by direct Cherinkov counting of roots or shoots; extraction or solubilization of tissue proved unnecessary. In each experiment, 20 or more pouches were sown for each treatment (i.e., in the presence or absence of GR12-2 bacteria). Roots and shoots from each pouch were washed and assayed together, but each pouch was treated as a separate replicate.

Analysis of data

Seedling root lengths, dry weights, and incorporated cpm were analyzed using the SAS statistics package for ANOVA (Fisher LSD) for linear regression analysis and for polynamial curve fitting.

TABLE 1. The effect of *Pseudomonas putida* GR12-2 on *Brassica campestris* root elongation in growth pouches

Seed treatment[a]	Root length (mm)[b]
GR12-2	59.2
GR12-2 autoclaved	37.1
GR12-2 + kanamycin[c]	35.5
Kanamycin control	34.6
$MgSO_4$ control	31.4
LSD, $p = 0.05$	6.3
LSD, $p = 0.01$	8.5

[a]Seeds were soaked in a bacterial suspension (log 7.8 cfu/mL in 0.1 *M* $MgSO_4$) for 60 min which gave log 4.9 cfu/seed, prior to suwing in the growth pouch.

[b]Root length was determined after 6 days of incubation at 20°C in the dark.

[c]Kanamycin was suspended in 0.1 *M* $MgSO_4$, 100 μg/mL, then filter-sterilized through 0.2-μm membrane. Bacteria were suspended in kanamycin solution (log 7.8 cfu/mL) prior to the seed treatment.

All the experiments in this study were repeated at least twice, with similar results.

Results

Bacterial effect on canola root elongation in the growth pouch assay

Canola (*B. campestris*) seedlings, which were treated with *P. putida* GR12-2 developed significantly longer roots than untreated (nonbacterized) seedlings in growth pouches (Table 1). Seed treatment with autoclaved (dead) bacteria or bacteria treated with kanamycin (inhibitory to GR12-2) had no effect on root elongation.

Seed soaking in a bacterial suspension containing only log 1.8 cfu/seed resulted in a significant increase in root length (61%, $p = 0.01$), as compared with the nonbacterized control (Fig. 1) (curve was fit by polynomial regression analysis, $r = 0.9370$, $p = 0.001$). An increase in the seed bacterial density from log 1.8 cfu/seed to log 4.9 cfu/seed resulted in an increase in root length up to 154.9% over the nonbacterized control. Further increases in the seed bacterial density above log 6.0 cfu/seed resulted in a decrease in root length of 12.5%, as compared with log 4.9 cfu/seed treatment.

The bacterial populations on the canola roots at the end of the experiment reached log 3 – log 3.5 cfu/cm root, on all seedlings treated with log 1.9 cfu/mL or above. No bacteria were detected from noninoculated seedlings. The bacterial populations which were determined by washing the roots in buffer were similar to the populations of the same treatment which were determined by grinding (with a pestle and mortar) and suspending the whole root tissue in buffer. This probably indicates that the bacterial root colonization was external only.

Three *nif*$^-$ mutants (negative acetylene reduction activity) of strain GR12-2 significantly enhanced root elongation ($p = 0.01$), similar to the wild-type strain (Table 2). In comparison, among six wild-type canola PGPR, two nitrogen-fixing pseudomonads (GR8-8 and GR12-2) were the most effective, and increased the mean root length by 64.8 and 62.2%, respectively, as compared with the untreated control. A weaker but significant effect ($p = 0.05$) was also shown by *P. putida* 25-71 and by *P. putida* 25-33, which are not capable of fixing atmospheric nitrogen.

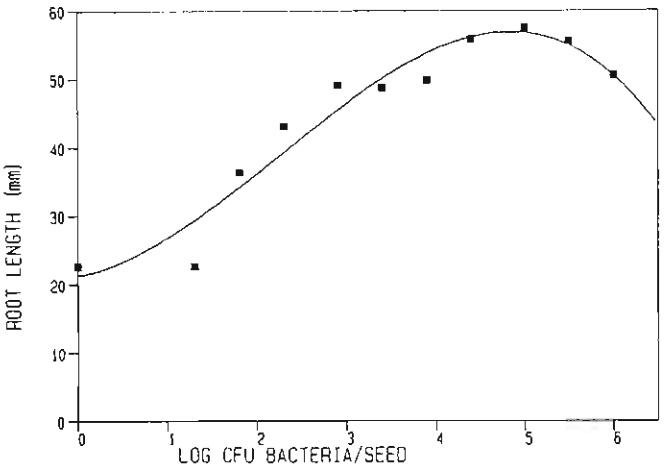

FIG. 1. The relationships between the inoculum density of *Pseudomonas putida* GR12-2 on the seed of canola (*Brassica campestris*) and the seedlings root length after 6 days of growth in sterile pouches.

TABLE 2. Comparative effect of different bacterial strains on *Brassica campestris* root elongation in growth pouches

	Root length (mm)[b]
Pseudomonas putida	
GR8-8	61.5
GR12-2	60.5
GR12-2/16 (mutant)	59.0
GR12-2/25 (mutant)	57.5
GR12-2/764 (mutant)	56.9
25-71	48.9
25-33 biovar B	45.4
GR19-1	44.0
Arthrobacter citreus 44-9	37.7
$MgSO_4$ control	37.3
LSD, $p = 0.05$	6.8
LSD, $p = 0.01$	9.0

NOTES: Seeds were soaked in a bacterial suspension (log 7.8 cfu/mL in 0.1 *M* $MgSO_4$) for 60 min, which gave log 4.9 cfu/seed, prior to sowing in the growth pouch.

[b]Root length was determined after 6 days of incubation at 20°C in the dark.

The effect of external minerals on interaction between P. putida *GR12-2 and canola seedlings*

The root length of canola seedlings either bacterized or nonbacterized was reduced with increasing concentration of NH_4NO_3 above 0.63 m*M* in the growth pouches (Table 3). Root elongation of nonbacterized seedling was also depressed in the growth pouches with the addition of 1 m*M* KNO_3 (Table 4).

On the other hand, the root length of either inoculated or noninoculated seedlings was increased in growth pouches supplemented with 1 m*M* potassium phosphate buffer at pH levels between 5 and 8, by comparison with the water control (Table 5). To determine if the effect of potassium phosphate buffer on root elongation was due to the supply of potassium or due to the supply of phosphate, solutions of the following minerals were assayed in the growth pouch system at a concentration of 1 m*M*: Na_2HPO_4, NaH_2PO_4, KCl, K_2SO_4, NaCl, Na_2SO_4. All the solutions containing phosphate (KH_2PO_4, K_2HPO_4, Na_2HPO_4, NaH_2PO_4) significantly ($p = 0.05$) increased root elongation over the water control (by 19.0, 17.1, 15.9, and 12.9%, respectively). On the other hand, no effect was detected with solutions of KCL, K_2SO_4, NaCl, and Na_2SO_4.

TABLE 3. The effect of the external concentration of NH_4NO_3 and bacterial inoculation in growth pouches on canola root length

NH_4NO_3 (m*M*)[a]	Bacterial inoculation[b]	Root length (mm)[c]
0	+	70.0
0	−	42.8
0.13	+	73.5
0.13	−	47.2
0.31	+	75.5
0.31	−	48.2
0.63	+	64.1
0.63	−	45.2
1.25	+	56.2
1.25	−	33.5
2.50	+	44.1
2.50	−	37.6
6.25	+	41.6
6.25	−	30.0
12.5	+	31.8
12.5	−	28.3
LSD, $p = 0.05$	+	7.7
LSD, $p = 0.01$	−	10.2

[a]The growth pouch contained 10 mL of NH_4NO_3 at various concentrations.

[b]Seed of *Brassica campestris* cv. Tobin were soaked in bacterial suspension (*Pseudomonas putida* GR12-2, log 7.8 cfu/mL in 0.1 *M* $MgSO_4$) for 60 min which gave log 4.9 cfu/seed prior to sowing in the growth pouches. The untreated seeds were soaked in 0.1 *M* $MgSO_4$.

[c]Root length was determined after 6 days of incubation at 20°C in the dark.

TABLE 4. The effect of the external concentration of KNO_3 and bacterial inoculation in growth pouches on canola root length

KNO_3 (m*M*)[a]	Bacterial inoculation[b]	Root length (mm)[c]
0	+	67.0
0	−	47.5
0.1	+	68.3
0.1	−	44.4
1	+	61.2
1	−	29.0
LSD, $p = 0.05$		8.9
LSD, $p = 0.01$		11.8

[a]The growth pouch contained 10 mL of KNO_3 at various concentrations.

[b]Seeds of *Brassica campestris* cv. Tobin were soaked in bacterial suspension (*Pseudomonas putida* GR12-2, log 7.8 cfu/mL in 0.1 *M* $MgSO_4$) for 60 min which gave log 4.9 cfu/seed prior to sowing in the growth pouches. The untreated seeds were soaked in 0.1 *M* $MgSO_4$.

[c]Root length was determined after 6 days of incubation at 20°C in the dark.

The combined effects of bacterial inoculation and the concentration of K_2HPO_4 on canola seedling growth were determined for root length, shoot length, root dry weight, and shoot dry weight (Table 6). Generally bacteria increased the seedling development in all of these criteria. In the presence of bacteria, root length was significantly increased ($p = 0.01$) at all concentrations of K_2HPO_4, ranging from 42.6 to 102.2% increase over noninoculated seedlings in K_2HPO_4. Shoot length was increased significantly ($p = 0.01$) over noninoculated seedlings ranging from 22.0 to 40.9%, at similar concentrations of K_2HPO_4. The dry weight of roots of inoculated seedlings tended to be greater than noninoculated seedlings, ranging from

TABLE 5. The effect of the pH (potassium phosphate buffer) and bacterial inoculation on canola root length in growth pouches

Buffer pH[a]	Bacterial inoculation[b]	Root length (mm)[c]
5	+	63.7
5	−	40.3
6	+	54.5
6	−	40.7
7	+	62.8
7	−	45.4
8	+	57.3
8	−	43.8
H_2O control	+	49.4
	−	30.0
LSD, $p = 0.05$		10.2
LSD, $p = 0.01$		13.5

[a]The growth pouch contained 10 mL of 1 m*M* potassium phosphate buffer of adjusted pH.

[b]Seeds of *Brassica campestris* cv. Tobin were soaked in bacterial suspension (*Pseudomonas putida* GR12-2, log 7.8 cfu/mL in 0.1 *M* $MgSO_4$) for 60 min which gave log 4.9 cfu/seed prior to sowing in the growth pouch. The untreated seeds were soaked in 0.1 *M* $MgSO_4$.

[c]Root length was determined after 6 days of incubation at 20°C in the dark.

2.8 to 20.2% increase, at similar concentrations of K_2HPO_4. However, these differences were not significant ($p = 0.05$) under the experimental conditions used. Similarly, the dry weight of shoots tended to be greater than that of noninoculated seedlings, with increases ranging from 1.7 to 27.0%, at similar concentrations of K_2HPO_4. These differences were also not significant ($p = 0.05$) under the experimental conditions used. However, the overall average of root dry weight of inoculated seedlings was 10.8% greater than noninoculated roots, which was significant at $p = 0.05$. Similarly, the overall average of shoot dry weight of inoculated seedlings was 11.3% greater than the overall average of noninoculated average of noninoculated shoots, which was significant at $p = 0.05$.

The amount of phosphate in the growth solution clearly affected seedling growth in growth pouches. Root length and dry weight of either inoculated or uninoculated seedlings was best when K_2HPO_4 was supplied at concentrations between 0.1 to 1 m*M*. The shoot length of inoculated seedlings increased significantly ($p = 0.01$) when K_2HPO_4 was supplied at concentrations equal to or greater than 0.05 m*M*. Shoot dry weight of either inoculated or uninoculated seedlings was generally increased with increasing concentrations of K_2HPO_4 in the growth solution. However, this effect was not statistically significant under the experimental conditions ($p = 0.05$ linear regression analysis).

Linear regression analysis indicated significant correlations ($p = 0.01$) between root length and root weight ($r = 0.26100$), between root length and shoot length ($r = 0.48711$), and between root length and shoot weight ($r = 0.19087$).

The effect of P. putida *GR12-2 on phosphorus uptake by the plant*

Phosphorus uptake by canola seedlings was determined by using ^{32}P-labelled phosphate in the growth pouch solution (Table 7). Bacterial inoculation resulted in clear increases of ^{32}P levels in roots (100.4% over noninoculated seedlings, significant at $p = 0.05$) and in the levels of ^{32}P in shoots (123.4% increase over uninoculated seedlings, significant $p = 0.01$). Linear regression analysis indicated significant correlations ($p = 0.01$) between root length and the level of ^{32}P in roots ($r = 0.2226$) and between root length and the level of ^{32}P in shoots ($r = 03638$).

TABLE 6. The effect of the external concentration of K_2HPO_4 and bacterial inoculation in growth pouches on canola root length, shoot length, root dry weight, and shoot dry weight

K_2HPO_4 (m*M*)[a]	Bacterial inoculation[b]	Root length (mm)[c]	Shoot length (mm)[c]	Root dry weight (mg)[c]	Shoot dry weight (mg)[c]
0	+	70.4	36.4	0.272	1.667
0	−	48.0	28.8	0.254	1.313
0.01	+	75.6	35.8	0.260	1.626
0.01	−	46.4	29.1	0.253	1.469
0.05	+	79.3	44.8	0.295	1.781
0.05	−	49.8	31.8	0.274	1.519
0.1	+	80.7	45.9	0.321	1.981
0.1	−	55.2	33.6	0.267	1.791
0.5	+	86.8	49.3	0.314	2.016
0.5	−	58.4	33.5	0.294	1.772
1	+	86.0	44.9	0.291	1.981
1	−	60.3	36.8	0.256	1.891
10	+	72.4	46.2	0.263	2.033
10	−	35.8	35.8	0.226	2.000
LSD, $p = 0.05$		8.8	3.5	0.063	0.379
LSD, $p = 0.01$		11.6	4.6	0.084	0.500

[a]The growth pouch contained 10 mL of K_2HPO_4 at various concentrations.

[b]Seeds of *Brassica campestris* cv. Tobin were soaked in bacterial suspension (*Pseudomonas putida* GR12-2, log 7.8 cfu/mL in 0.1 *M* $MgSO_4$) for 60 min which gave log 4.9 cfu/seed prior to sowing in the growth pouch. The untreated seeds were soaked in 0.1 *M* $MgSO_4$.

[c]Root and shoot length and dry weight were determined after 6 days of incubation at 20°C in the dark.

TABLE 7. The effect of the bacterial inoculation on root length, shoot length, ^{32}P uptake by root, and ^{32}P uptake by shoots of canola seedlings in growth pouches

Bacterial inoculation[a]	Root length (mm)[b]	Shoot length (mm)	^{32}P (cpm) in: roots[c]	^{32}P (cpm) in: shoots
+	64.3	31.6	980.4	648.2
−	53.4	24.5	489.2	290.2
LSD, $p = 0.05$	9.7	5.9	455.7	234.1
LSD, $p = 0.01$	NS	NS	NS	314.7

[a]Seeds of *Brassica campestris* cv. Tobin were soaked in bacterial suspension (*Pseudomonas putida* GR12-2, log 7.8 cfu/mL in 0.1 *M* $MgSO_4$) for 60 min which gave log 4.9 cfu/seed prior to sowing in the growth pouch. The untreated seeds were soaked in 0.1 *M* $MgSO_4$.

[b]Root length, shoot length, ^{32}P in root, and ^{32}P in shoot were determined after 20 days of incubation at 12°C in the dark.

[c]The growth pouch contained 10 mL of 1 m*M* K_2HPO_4 labelled with ^{32}P at 5 μCi/mL.

TABLE 8. The effect of added K_2HPO_4 and bacterial inoculation on canola shoot length in autoclaved soil[a]

Soil moisture (%, v/w)	Added K_2HPO_4 (1 m*M*)	Bacterial inoculation[b]	Shoot length (mm)[c]
15	−	+	31.0
15	−	−	21.0
15	+	+	41.1
15	+	−	32.7
20	−	+	49.7
20	−	−	31.9
20	+	+	47.5
20	+	−	38.3
25	−	+	47.1
25	−	−	35.4
25	+	+	45.3
25	+	−	41.2
LSD, $p = 0.05$			9.8
LSD, $p = 0.01$			12.5

[a]Petri plates (100 × 25 mm) were filled with 75 g air-dried, ground, and autoclaved soil. Soils were moistened with sterile deionized water or sterile solution of 1 m*M* K_2HPO_4, and were left overnight in order to obtain uniform moisture distribution.

[b]Seeds of *Brassica campestris* cv. Tobin were soaked in bacterial suspension (*Pseudomonas putida* GR12-2, log 7.8 cfu/mL in 0.1 *M* $MgSO_4$) for 60 min which gave log 4.9 cfu/seed prior to sowing in the soil plates. The untreated seeds were soaked in 0.1 *M* $MgSO_4$.

[c]Shoot length was determined after 5 days of incubation at 20°C and 100% RH in the dark.

The effect of P. putida *GR12-2 on the growth of canola seedlings in sterile soil*

Bacterial enhancement of canola growth was also evident when seedlings were grown in autoclaved soil. Five-day-old seedlings, inoculated with bacteria, developed longer shoots than noninoculated seedlings. Their shoot length was increased by 47.6, 40.4, and 33.1%, at 15, 20,and 25% soil moisture (v/w, air-dried soil), respectively (Table 8). These effects were all significant at $p = 0.05$. In soil moistened with 1 m*M* K_2HPO_4, uninoculated seeds also developed longer shoots (significant at $p = 0.05$) at 15% soil moisture.

Discussion

In recent years much attention has been given to plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978). This group of bacteria consists primarily of fluorescent pseudomonads, which are aggressive root colonizers (i.e., capable of moving from seeds to roots) and capable of promoting plant yield under field conditions (Burr et al. 1978; Kloepper and Schroth 1978, 1981; Suslow and Schroth 1982*b*). The mechanism of growth promotion by PGPR is generally believed to be of antagonistic interactions, i.e., antibiosis and eompetition, which result in the exclusion of pathogenic and deleterious microorganisms from the rhizosphere. Our study demonstrated, for the first time to the best of our knowledge, a significant plant growth promotion under gnotobiotic conditions via seed inoculation with a pseudomonad PGPR (*P. putida GR12-2*).

Pseudomonas putida GR12-2 enhanced root elongation of canola (*Brassica campestris*) grown under gnotobiotic conditions in growth pouches. This effect was not caused by bacterial nitrogen fixation for the following reasons: (*i*) the addition of mineral nitrogen (NH_4NO_3, KNO_3) decreased, rather than increased, root elongation; (*ii*) mutants which did not fix nitrogen (*nif*$^-$) retained the ability to enhance root elongation (not significantly different from wild type); (*iii*) other pseudomonads (wild types) which do not fix nitrogen were also active in enhancing root elongation.

The addition of phosphate ions ($H_2PO^-_4$, HPO^{2-}_4) to the growth solution increased root elongation, while other ions (K^+, Na^+, Cl^-, and SO^{2-}_4) showed no effect at similar concentrations (1 m*M*). The combined effects of K_2HPO_4 and *P. putida* GR12-2 were found to be additive (Table 6); hence, root and shoot length were greater for plants receiving combined treatments of K_2HPO_4 and bacteria than for plants receiving single treatments of either bacteria or K_2HPO_4 alone. The bacterial effect on root elongation consistently promoted a marked increase in the uptake of labelled phosphorus (^{32}P) by the roots and its translocation to the shoots (Table 7). The bacteria-induced increase in phosphorus uptake by the plant could be caused by two possible mechanisms. (*i*) The bacteria first stimulated root elongation via a "hormonelike factor," which then increased the nutrient and water absorptive capacity of the plant. (*ii*) The bacteria first increased the phosphorus uptake capacity of the plant, which stimulated root elongation, which then further increased the nutrient and water absorptive capacity of the plant.

Many bacteria are capable of producing various phytohormones *in vitro* and may affect plant root development upon inoculation (Brown 1974; Brown and Burlington 1968; Barea and Brown 1974; Kapustka et al. 1985; Kapulnik et al. 1985*a* 1985*b*: Patriquin et al. 1983; Tien et al. 1979). These findings suggest a possible common mechanism of direct growth promotion by various rhizobacteria, resulting from an increased supply of phytohormones provided by the bacteria, or from the induction of the roots to produce hormones.

Many bacteria isolated from the rhizosphere (rhizobacteria) are capable of increasing the availability of phosphorus to plants either by mineralization of organic phosphate (solubilization by action of phosphatase) or by solubilization of insoluble inorganic phosphates by production of acids. Such bacteria, often termed "phosphobacteria," attracted considerable attention for their potential use as inoculants (Wani 1980). Intensive investigations showed successful yield increases in the field (Cooper 1959). However, later studies showed inconsistent and conflicting

results; thus, it is the common belief now that reported plant growth promotions induced by phosphobacteria were more likely caused by mechanisms other than phosphate solubilization (Brown 1974).

Our study demonstrated bacteria-induced increases in phosphorus uptake by plants when phosphorus was available to the plant in the growth solution. As opposed to the considerable interest in bacteria-induced phosphate solubilization, to date relatively little attention has been given to the effect of rhizobacteria on phosphorus uptake by plants. Barber et al. (1976) showed that mixed populations of rhizosphere microorganisms enhanced the uptake of phosphorus by young barley seedlings, while causing a decrease in the uptake of phosphorus by older plants; however, the increased phosphorus uptake was not correlated with an increase in dry weight. Their data indicated that the microorganisms generally reduced dry weight of older plants, i.e., older plants which grew in sterile conditions obtained greater dry weights than plants which grew in the presence of microorganisms. It seems likely that the observed differential phosphate uptake between young and old plants occurred because of an interference caused by deleterious or pathogenic microorganisms. Such microorganisms, which are common in soil, would be expected to inhibit root development, thus reducing the nutrient uptake capacity of the plants. Since Barber et al. (1976) used undefined mixtures of microorganisms in their studies, it is not possible to relate or compare their "microbial factor" to the mechanism by which *P. putida* GR12-2 increased phosphorus uptake by plant roots.

The best known example of mierobial enhancement of phosphorus uptake by plants is the symbiotic relationship between plants and mycorrhizal fungi (Hackskaglo 1941). These fungi were shown to increase the nutrient absorptive capacity of plants, particularly in phosphorus-deficient soils or where phosphorus is not readily available to plants. Under such conditions, mycorrhizal fungi have been shown to substantially enhance seedling growth. *Pseudomonas putida* GR12-2, which affects phosphate uptake by plants and increases growth rates, should have potential similar to mycorrhizae as a microbial inoculant. In addition, *P. putida* GR12-2 should have distinct advantages to mycorrhizal inoculants because (*i*) unlike mycorrhizae, the enhancement of phosphate uptake following treatment with GR12-2 is additive to phosphate fertilizing, thus its potential use in agriculture will not be limited only to phosphate-deficient soils; (*ii*) these bacteria are capable of moving from seeds to roots and increasing their biomass in the rhizosphere (Lifshitz et al. 1986) and thus may have a broader use as seed treatments than mycorrhizae which must be used as soil amendments.

The bacteria-induced plant growth promotion, even in the absence of plant pathogenic or deleterious microorganisms, may provide a substantial benefit upon inoculation to field crops. An understanding of the mechanisms by which *P. putida* GR12-2 increases plant growth may lead to improved methods for selecting new and more efficient wild-type PGPR or for improving the efficiency of previously selected PGPR via genetic engineering. Accordingly, we are continuing studies with GR12-2 in an attempt to elucidate the growth promotion mechanism.

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