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Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.)

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Abstract

Bradyrhizobia and rhizobia are symbiotic bacterial partners forming nitrogen fixing nodules on legumes. These bacteria share characteristics with plant growth promoting rhizobacteria (PGPR). Nodule inducing bacteria, like other PGPR, are capable of colonizing the roots of non-legumes and produce phytohormones, siderophores and HCN. They also exhibit antagonistic effects towards many plant pathogenic fungi. The potential of nodule inducing bacteria to function as PGPR, was examined by using radish as a model plant. Three percent of the 266 strains tested were found to be cyanogens, while a majority (83%) produced siderophores. Fifty eight percent of the strains produced indole 3-acetic acid (IAA) and 54% solubilized phosphorus. Some of the bacterial species examined were found to have a deleterious effect while others were neutral or displayed a stimulatory effect on radishes. *Bradyrizobium japonicum* strain Soy 213 was found to have the highest stimulatory effect (60%), and an arctic strain (N44) was the most deleterious, causing a 44% reduction in radish dry matter yield. A second plant inoculation test, performed in growth cabinets, revealed that only strain Tal 629 of *B. japonicum* significantly increased (15%) the dry matter yield of radish. This indicates that specific bradyrhizobia have the potential to be used as PGPR on non-legumes.

Introduction

Interactions between plants and micro-organisms in the rhizosphere (rhizobacteria) can clearly affect crop yields. Rhizobacteria that benefit plant growth and development are called 'PGPR'. The most studied PGPR belong to gram-negative genera, and the greatest number of strains are members of the fluorescent pseudomonads (Kloepper, 1993). Many reports also suggest that gram-positive bacteria, such as *Bacillus*, are PGPR (Beauchamp, 1993; Kloepper, 1993). Early studies of PGPR were performed only with root crops such as radish (*Raphanus sativus* L.), potato (*Solanum tuberosum* L.) and sugarbeet (*Beta vulgaris* L.) (Kloepper, 1993). Later studies covered a wide range of hosts, including cereals, legumes and trees. PGPR can directly stimulate plant growth by producing phytohormones and by increasing nutrient uptake (Lippmann et al., 1995) or by inducing systemic plant resistance towards pathogenic micro-organisms (Liu et al., 1995a,b). By modifying the microbial balance in the rhizosphere, PGPR can stimulate plant growth indirectly by inhibiting other deleterious microbes or root pathogens (Lemanceau, 1992; Kloepper, 1993). For example fluorescent pseudomonads can influence biological control of root crop diseases,

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by modulating competitiveness, production of antibiotics, siderophores or HCN (O'Sullivan and O'Gara, 1992).

Potential use of rhizobia and bradyrhizobia as PGPR with non-legumes

The beneficial effect of *Rhizobium* and *Bradyrhizobium* on legumes in terms of biological nitrogen-fixation is well known (Werner, 1992). However, additional reports also indicate that these symbiotic bacteria have the potential to be used as PGPR with non-legumes (see below).

Root colonization of non-legumes by rhizobia and bradyrhizobia

Root colonization is an important first step in the interaction of beneficial bacteria with plants (Kloepper and Beauchamp, 1992). To act as PGPR with non-legumes, rhizobia and bradyrhizobia should be able to colonize and survive in the rhizosphere of these plants.

In cereal-legumes crop rotation systems, inoculation of the preceding cereal crop of maize (*Zea mays* L.) with rhizobia and bradyrhizobia, significantly increased nodule volume, the dry weight of shoots, number of pods, and the final grain yield of the following green gram (*Vigna radiata* L.) and groundnut (*Arachis hypogaea* L.) legume crop (Gaur et al., 1980). Rhizobial attachment to asparagus (*Asparagus officinalis* L.), oat (*Avena sativa* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) has been described (Shimshick and Hebert, 1979; Terouchi and Syono, 1990). Moreover, Pena-Cabriales and Alexander (1983) found that strains of rhizobia and bradyrhizobia grew readily in the presence of germinating seeds and developing root systems of soybeans (*Glycine max* (L.) Merr.), kidney beans (*Phaseolus vulgaris* L.), red clover (*Trifolium pratense* L.), cowpeas (*Vigna unguiculata* L.), oats, wheat and corn. Growth of rhizobia in the rhizosphere of non-legumes was appreciable. Recently Wiehe and Höflich (1995) demonstrated that strain R39 of *Rhizobium leguminosarum* bv. trifolii, can multiply and survive under field conditions, in the rhizosphere of non-host legumes (lupine, *Lupinus albus* L.; and pea, *Pisum sativum* L.) and non-legumes (corn, rape, *Brassica napus* L. and wheat). The study of root colonization of corn by strain R39, using monospecific polyclonal antisera, showed that 4 weeks after inoculation this strain was found exclusively in the rhizosphere soil and on the rhizoplane, and only occasionally in the inner root tissues (Schloter et al., 1997). By using bioluminescent phosphate solubilizing strains of *R. leguminosarum* bv. phaseoli, Chabot et al (1996b) also found that 4 weeks after seeding the rhizobial population in maize roots average log 4.1 CFU/g (fresh weight) and that rhizobia were superior root colonizers as compared to the other PGPR tested. *B. japonicum* was also found as an endophyte in the roots of sweet corn and cotton (*Gossypium hirsutum* L.), indicating that this bacteria is capable of colonizing internal plant niches (McInroy and Kloepper, 1995).

Reaction of non-legumes to the presence of bradyrhizobia and rhizobia

Many data indicate that non-legumes react to the presence of bradyrhizobia and rhizobia in the rhizosphere. Root hair curling induced by these symbiotic bacteria was observed on maize, rice and oat plants (Plazinsky et al., 1985; Terouchi and Syono, 1990). Studies also show that Nod-factors (lipochitooligosaccharides) produced by *Bradyrhizobium* and *Rhizobium*, can be perceived by tomato (*Lycopersicon esculentum* Mill.), as indicated by the induction of alkalinization in tomato cell cultures (Staehelin et al., 1994). Nodule-like structures or hypertrophies formed by rhizobia have also been observed on oilseed rape (*Brassica napus*), *Arabidopsis thaliana*, rice and other non-legumes (Al-Mallah et al., 1990; Ridge et al., 1992; Trinick and Hadobas, 1995). Finally, some isolates of *Bradyrhizobium* sp. can form nitrogen fixing nodules with *Parasponia* a nonlegume belonging to the family Ulmaceae (Trinick, 1973; Werner, 1992).

Other PGPR characteristics of bradyrhizobia and rhizobia

Phytohormone production

PGPR can directly affect plant growth through the production of phytohormones (Lippmann et al., 1995). Nodulating and non-nodulating strains of *Rhizobium leguminosarum* produce indole-3-acetic acid (Wang et al., 1982). Noel et al. (1996) observed under gnotobiotic conditions, a direct growth promotion of the early seedling root of canola (*Brassica campestris*) and lettuce (*Lactuca sativa*) by *R. leguminosarum*. This direct growth-promotive effect appears to involve the plant growth regulators indole-3-acetic acid and cytokinin.

Siderophore production

Microbial siderophores play an important role in the biocontrol of some soil-borne plant diseases and in plant iron nutrition (Loper and Buyer, 1991). Bradyrhizobia and rhizobia produce different type of siderophores (Guerinot, 1991). To overcome iron starvation, *B. japonicum* can utilize its own siderophores and those produced by other organisms (Plessner et al., 1993). This ability may confer upon nodule bacteria a selective advantage in the rhizosphere. In assays using an iron-inefficient variety of peanuts, Jadhav et al. (1994) found that the catechol siderophore of a peanut *Rhizobium* isolate, increased plant growth and chlorophyll content compared with plants grown with iron alone. This observation might explain in part the rhizobia enhanced mineral uptake in peanut tissues (Howell, 1987).

Effect on phosphorus availability

Because of the relative immobility of phosphate and its very low concentration in soil solutions, substantial amounts of phosphate fertilizers are applied to agricultural soils. This results in an accumulation of large quantities of total phosphorus in the soil, of which 20–80% is in organic form (Richardson, 1994). Availability of this phosphorus depends largely on microbial activity. Inoculation of plants with phosphate solubilizing micro-organisms frequently stimulates plant growth by increasing phosphorus uptake (Chabot et al., 1993; Kucey et al., 1989). A large number of strains of *Rhizobium* and *Bradyrhizobium* are able to solubilize inorganic phosphate (Halder and Chakrabartty, 1993). For example, Abd-Alla (1994) has shown that strain TAL 1236 of *R. leguminosarum* bv. viciae contributed significantly to the release of phosphorus from organic compounds through the action of acid and alkaline phosphatase. In a field study, Chabot et al (1996a) have observed that phosphate solubilization by strains of *R. leguminosarum* bv. phaseoli was the most important mechanism of maize and lettuce growth promotion, in moderately fertile and very fertile soils.

Synergistic effect of rhizobia on vesicular-arbuscular mycorrhizal (VAM) fungi

In general rhizobia and VAM fungi when used together, synergisticaly stimulate plant growth and phosphorus accumulation (Valdés et al., 1993). For example, Xie et al. (1995) have demonstrated that strains of *Bradyrhizobium japonicum*, and the acetylated Nod-factors of *Rhizobium* sp. NGR234, considerably enhance colonization of nodulating and non-nodulating soybean roots by the mycorrhizal fungus *Glomus mosseae*.

Antagonistic activity of rhizobia against plant pathogenic micro-organisms

Rhizobia can inhibit the growth of some phytopathogenic fungal isolates, and they have the potential to be used as biological control agents. In fact, Antoun et al. (1978) studied the antagonism of 49 strains of *R. meliloti* towards *Fusarium oxysporum*, and found that all strains regardless of their symbiotic effectiveness, inhibited the fungal growth in pure cultures. None of the tested strains produced antifungal antiobiotics and the observed fungal growth inhibitions ranged from 5 to 50%. By inoculating the seeds of bean with strains of *R. leguminosarum* bv. phaseoli, antagonistic to *Fusarium solani* f.sp. *phaseoli*, a significant reduction in root rot was observed in plants grown in pasteurized soil artificially infested with the fungal pathogen (Buonassisi et al., 1986). Rhizobia isolated from root nodules of *Acacia pulchella*, significantly reduced *Phytophthora cinnamoni* zoospore survival in both non-sterile suppressive and conducive soil extracts, suggesting that rhizobia, in concert with other soil micro-organisms can actively suppress this pathogen *in vitro* (Malajczuk et al., 1984). Finally Ehteshamul-Haque and Ghaffar (1993) observed in the field, that antagonistic rhizobia and bradyrhizobia used as seed dressing or as soil drench reduced infection of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp., in both leguminous (soybean and mungbean, *Vigna radiata* (L.) Wilczeck var. *radiata*) and non-leguminous (sunflower, *Helianthus annuus* L. and okra, *Abelmoschus esculentus* (L.) Moench) plants.

Direct evidence of plant growth promotion of non-legumes by rhizobia

Höflich et al. (1994) obtained significant shoot dry matter yield increases (7–8%) by inoculating maize, spring wheat and spring barley (*Hordeum vulgare* L.) with strain R39 of *R. leguminosarum* bv. trifolii, in field experiments. Yanni et al. (1995) also observed that certain effective wild type strains of *R. leguminosarum* bv. trifolii establish natural plant-bacterial associations that have the potential to promote rice growth under both field and laboratory conditions. In field inoculation trials of maize and lettuce, Chabot et al. (1996a) also reported significant increases in

Table 1. Strains of rhizobia and bradyrhizobia used in this study

Bacteria	Total number tested	Strains	Source[a]
Bradyrhizobium japonicum	18	USDA 110	2
		CIAT 3545, 4777, 4911	3
		TAL 101, 102, 153, 155, 629, 1905, 409, 1783, 1904, 1906	4
		ARC 502, 517	5
		Soy 213, 217	7
Rhizobium leguminosarum bv. trifolii	22	CIAT 2153, 3835	3
		TAL 143, 151, 190, 303, 376, 1818, 1819, 1821, 1823, 1825, 1826, 1828	4
		ARC 100, 102	5
		ANU 794, 843, T1, UNZ29, Tr8, NA30	6
Rhizobium leguminosarum bv. phaseoli	30	1402, 4405, 4906, 6002, 6701, Hrw 11, 12, 41, 46 RGB2	1 Lalande et al. (1990)
		P 6, 31, 35, 66, 67, 73, 74, 75, 78-1, 79, 80, 91, 103, 105, 114, 118, 121.	1 Lalande et al. (1986)
		CIAT 632, 899, 7115	3
Rhizobium leguminosarum bv. viciae	82	P8, 10, 12, 17, 18, 19, 21, 22, 26, 28, 33, 38, 40, 43, 45, 47, 48, 50, 51, 52, 53, 55, 56, 58, 59, 60, 61, 63, 65, 69, 70, 71, 72/1, 72/2, 94, 95, 120. P127 to P146,128C53K, 128C56G, 248, 2356, 2357, 3264, L4sp,	1
		USDA 2369	2
		TAL 53, 167, 227, 634, 638, 640, 1399, 1400, 1402	4
		ARC 201P, 202F, 202L, 202P, 204F, 205L, ICARDA 441	5
		300	6
Rhizobium meliloti	62	A1 to A6, D1 to D3, I1 to I3, V1 to V7, S1 to S5, S7, 8, 10, 11, 13, 14, 16, S18 to S22, 3Doa20a, 3Doa8, 23A, 54032, 54033, E1, E2, R1, AlfalfaD	1 Antoun et al. (1984)
		CIAT 44, 3051, 3882	3
		TAL 380, 700/1, 700/2, 804, 807, 1372/1, 1372/2, 1373, 1479, 1874, 1878	4
		ARC 1, 2, 3	5

Table 1. Contd.

Bradyrhizobium sp. (Lupinus)	5	TAL 1306	4
		Lup 025, 026, 245-38, wu-425	7
Arctic rhizobia	47	N1 TO N47	1 Prévost et al. (1987)

[a] 1 = Agriculture and Agri-food Canada, Ste-Foy, Quebec; 2 = Beltsville Agricultural Research Centre, USDA Beltsville, Maryland; 3 = Centro Internacional de Agricultura Tropical, Cali, Columbia; 4 = Niftal Project and Mircen, University of Hawaii; 5 = Soils & Water Research Centre, Ministry of Agriculture, Egypt; 6 = Research School of Biological Sciences The Australia National University, Camberra, Australia; 7 = Urbana Laboratories, St. Joseph, Missouri.

shoot dry matter yield and total P-content by using phosphate solubilizing strains of *R. leguminosarum* bv. phaseoli.

In the present work, we present results of two inoculation experiments with radish (*Raphanus sativus* L.) that corroborate previous observations indicating that *Bradyrhizobium* and *Rhizobium* spp. can act as PGPR with non-legumes.

Materials and methods

Bacteria

Table 1 shows the source of the 266 strains of *Bradyrhizobium* and *Rhizobium* used in this study. The strains were purified and maintained on yeast extract mannitol agar (Vincent, 1970), and stored at −80 °C in yeast extract mannitol broth containing 20% glycerol.

Some in vitro *characteristics of* Bradyrhizobium *and* Rhizobium

Siderophore production by *Bradyrhizobium* and *Rhizobium* was determined as described by Alexander and Zuberer (1991). Production of indoleacetic acid (IAA) and/or IAA analogs was analysed using the method of Bric et al. (1991), as described further by de Britto Alvarez et al. (1995). Cyanide production was analysed according to Bakker and Schippers (1987) and phosphate solubilization was measured on dicalcium phosphate agar plates (Goldstein, 1986). Plates were incubated at 25 °C or 28 °C for arctic or other *Rhizobium*, respectively (see Table 1), and observed daily for up to 7 days.

Effect of Bradyrhizobium *and* Rhizobium *on the growth of radish*

The inoculum for each strain was prepared by resuspending the cells from 2–3 days old yeast extract mannitol agar cultures, in sterile saline (0.85% NaCl) solution containing 1% methyl cellulose. Radish seeds cv. Pocker (25) were stirred in 1 mL inoculum containing about 10^8 cfu/mL and kept in the refrigerator for 12–24 h until used. Un-inoculated control plants were treated in a similar way in saline without bacteria. Seeds were planted in a mixture of 20% perlite and 80% non-sterile field soil. The loam soil used, collected on the experimental field plots at Laval University campus (Ste-Foy, Quebec), had the following properties: pH, 5.2; organic matter, 4.2%; P, 620 kg/ha; K, 630 kg/ha; Mg, 150 kg/ha and Ca, 2300 kg/ha. Soil properties were determined as previously described (de Britto Alvarez et al., 1995). Each pot ($18 \times 12.5 \times 4$ cm) received 8 inoculated seeds and after emergence seedlings were thinned to 4 per pot.

A first screening was performed in the greenhouse under natural temperature (average day 21 ± 3 °C and night 11 ± 1 °C) and light conditions (140 μEm^{-2} s^{-1}). Radish was planted on May 10 and harvested on June 23 and 24, 1994. The experimental design used was a randomized complete block design with two replicates, and 267 treatments (266 bacteria and an un-inoculated control). At harvest, whole plants were collected, washed and dried to a constant weight at 80 °C.

A second plant assay was performed in a growth cabinet with the following bacterial strains which indicated a good plant growth promoting potential after the first greenhouse trial: *B. japonicum*, Tal 629 and Soy 213; *Rhizobium* sp. (arctic), N18 and N40; *R. leguminosarum* bv. phaseoli, H78-1 and 1402; *R. legu-*

Table 2. Percentages of *Bradyrhizobium* and *Rhizobium* strains exhibiting some *in vitro* characteristics

	Number of strains tested	Cyanogens[a] (%)	Siderophore producers[b] (%)	IAA producers[c] (%)	P-solubilizers[d] (%)
B. japonicum	18	0	67	33	5
R.leguminosarum bv. trifolii	22	9	86	45	4
R. leguminosarum bv. phaseoli.	30	13	93	50	67
R.leguminosarum bv. viciae	82	2	91	51	71
R. meliloti	62	0	95	56	84
Bradyrhizobium sp. (Lupinus)	5	0	100	60	80
Arctic rhizobia	47	0	49	96	2
Total	266	3	83	58	54

[a] A change of color from yellow to orange-brown of filter papers impragnated with 0.5% picric acid–2% sodium carbonate indicated the production of cyanide (Bakker and Schippers, 1987).
[b] A bacteria forming an orange halo on chrome azurol S agar plates or growing on TSA (10%) agar plates containing 50 mg liter^{-1} of 8-hydroxyquinoline was considered as positive siderophore producer (Alexander and Zuberer 1991).
[c] IAA producing bacteria were separated from organisms producing other indoles (yellow to yellow-brown pigment) by their characteristic pink to red color produced after exposure to Salkowski reagent for 0.5–3 h (de Britto Alvarez, 1995).
[d] The bacterial colonies forming clarification halos on dicalcium phosphate agar plates (Goldstein, 1986) were considered phosphate solubilizers.

minosarum bv. trifolii, arc 100; *R. leguminosarum* bv. viciae, P43 and P55; *Bradyrhizobium* sp. (Lupinus), wu425 and *R. meliloti* TAL 700 and S20 (see Table 4). The observed increases in radish dry matter yield in the first trial compared to the un-inoculated control, ranged fron 22% with strain 1402 to 60% with strain Soy 213. The identity of the 12 strains used in this test was confirmed by their ability to form nodules on their respective legume plant hosts. Radish seed inoculation, planting, and plant harvest were performed as described in the first assay. This time plants from each pot were harvested and weighed individually. The growth cabinet was adjusted to 14 h light period (400 μEm^{-2} s^{-1}) at 15 °C and 10 h darkness at 12 °C. Radish was planted on August 8 and harvested on September 16, 1994. The experimental design was a randomized complete block design with 7 replicates and 13 treatments (12 bacteria and an un-inoculated control). Data were subjected to the Bartlett test of homogeneity of variance. An analysis of variance with a sampling error was performed and treatment means were compared by using the least significant difference test (Steel and Torrie, 1980).

Results and discussion

In vitro *characteristics of* Bradyrhizobium *and* Rhizobium

Some PGPR and other characteristics of the strains of *Bradyrhizobium* and *Rhizobium* used in this study are presented in Table 2. Only eight (3%) of the 266 strains tested produced hydrogen cyanide (HCN), and cyanogens were only found among biovars of *Rhizobium leguminosarum*. The low incidence of cyanogens in rhizobia is comparable to the observations made with other PGPR. In fact, de Britto Alvarez et al. (1995) observed that less than 1% of the 709 isolates obtained from the rhizosphere of tomato were cyanogens. In a study involving 32 strains of opine-utilizing rhizobacteria, only four strains produced HCN (Beauchamp et al., 1991). Production of HCN by pseudomonads is associated with biological control of the black root rot of tobacco, but other workers observed that it can have a detrimental effect on plant growth (O'Sullivan and O'Gara, 1992). In this work, two HCN producing strains (P31 and P145) were

Table 3. Effect of inoculation with different strains of *Bradyrhizobium* and *Rhizobium* on growth of the radish cultivar Pocker. First greenhouse trial

	Number of strains producing yields equivalent to that of the un-inoculated control (100%)[a]				
	<80%	80–100%	100–120%	120–140%	>140%
B.japonicum	3	5	5	2	3 Tal 629[b] Soy 213
R. leguminosarum bv. trifolii	0	10	8	3	1 ARC100
R. leguminosarum bv. phaseoli	5	8	10	6	1 P78-1
R. leguminosarum bv. viciae	8	33	25	13	3 P8
R. meliloti	2	12	27	16	5 Tal 700
Bradyrhizobium sp. (Lupinus)	0	3	1	0	1 Wu 425
Arctic rhizobia	12	12	11	7	5 N18 N29 N37
Total	30	83	87	47	19
%	11.3	31.2	32.7	17.7	7.1

[a]The mean dry matter yield obtained with the un-inoculated control was 670 mg/plant. Least significant difference ($p \leq 0.05$) = 332 mg.
[b]The 10 indicated strains significantly ($p \leq 0.05$) stimulated plant growth (more than 50% increase in dry matter yield).

found to be deleterious, and caused 15–23% inhibition of dry matter yield of radish cultivated in the greenhouse, while 4 strains (P79, ANU 794, P79 and P127) showed moderate stimulation (5–13%) and two (P66 and P78-1) exhibited high PGPR activities (31–50% increase). The amount of HCN produced was not measured, which may be a determinant factor in the PGPR behaviour of a strain (Alström and Burns, 1989). Most bradyrhizobia and rhizobia produce siderophores and about 58% of the isolates produced IAA (Table 2). The occurrence of these two PGPR characteristics is generally higher in nodule bacteria than in other PGPR. In studying the effect of different composts on the incidence of PGPR in the rhizosphere of tomato (*Lycopersicon esculentum* Mill.), it was found that between 23 and 38% of the isolates produce siderophores and 24–42% produce IAA (de Britto Alvarez et al., 1995). In opine-utilizing rhizobacteria, 84% of the isolates produced a fluorescent pigment (Beauchamp et al., 1991). In two Quebec agricultural soils Chabot et al. (1993) observed, that P-solubilizing micro-organisms formed 26–46% of the total soil microflora. Only 6–16% of the rhizobacteria of tomato solubilized P (de Britto Alvarez et al., 1995). In this work, we have observed two distinct groups; one formed by *B. japonicum*, *R. leguminosarum* bv. trifolii and arctic rhizobia in which the frequency of P-solubilizing bacteria is very low (2–5% of the strains); the second by *R. leguminosarum* bv. phaseoli, *R. leguminosarum* bv. viciae, *R. meliloti* and *Bradyrhizobium* sp. (Lupinus), with a high incidence (67–84%) of P-solubilizing bacteria (Table 2). It is interesting to note that arctic rhizobia, isolated from indigenous plants growing in non-agricultural soils (Prévost et al., 1987), have the highest frequency of IAA producing bacteria (96% of the isolates) and the lowest frequency of siderophore producing and P-solubilizing organisms (Table 2). The importance of these traits in relation to the competitive ability of these arctic strains, in soil and in the rhizosphere of legumes and non-legumes, deserve to be investigated.

Effect of inoculation with Bradyrhizobium *and* Rhizobium *on radish yield*

Amongst the 266 strains tested bacteria having deleterious, neutral or PGPR effects, as indicated by the

Table 4. Dry matter yield of the radish cultivar Pocker inoculated with selected PGPR strains of *Bradyrhizobium* and *Rhizobium*. Growth cabinet assay

Strain	Source (country of origin)	Yield (g/plant)[a]	% of un-inoculated control
B. japonicum TAL 629[b]	Niftal (USA)	1.464	115.1
B. japonicum Soy 213	Urbana Lab. (USA)	1.424	111.9
Bradyrhizobium sp. (Lupinus) WU-425	Urbana Lab. (Australia)	1.249	98.2
R. leguminosarum bv. phaseoli P78-1	Lalande et al. (1986) (Canada)	1.182	92.9
R. leguminosarum bv. trifolii ARC100	Agriculture Research Centre (Egypt)	1.177	92.5
R. leguminosarum bv. viciae P43	Agriculture Canada (Canada)	1.140	89.6
R. leguminosarum bv. phaseoli 1402	Lalande et al. (1990) (Rwanda)	1.094	86.0
R. meliloti TAL 700	Niftal (Tunisia)	1.072	84.3
R. leguminosarum bv. viciae P55	Agriculture Canada (Canada)	1.048	82.4
Arctic rhizobia N40	Prévost et al. (1987) (Canada)	1.046	82.2
R. meliloti S20	Bordeleau et al. (1977) (Canada)	1.005	79.0
Arctic rhizobia N18	Prévost et al. (1987) (Canada)	1.004	78.9
Un-inoculated control		1,272 lsd($p \leq 0.05$) = 0.178	100

[a] Average of 7 replications with 4 sub-samples each.
[b] Other names: USDA 704, Allen 527.

radish dry matter yield, were obtained (Table 3). The maximum stimulatory effect (60% increase as compared to the un-inoculated control), was obtained with strain Soy 213 of *B. japonicum*. Although important, this stimulation is smaller than the maximum increase of 570% in fresh matter yield obtained by Kloepper and Schroth (1978) with other PGPR. On average, about 25% of all the strains of rhizobia and bradyrhizobia tested in this study stimulated radish growth (20% or more increase) in the first greenhouse study (Table 3). This number is comparable to the number of other PGPR isolates stimulating radish growth (21% in

the greenhouse) as reported by (Kloepper and Schroth, 1978), but it is surprisingly larger than the number of isolates (3%) stimulating the growth of sugarbeets (Suslow and Schroth, 1982).

The arctic strain N44 exhibited an important deleterious effect on radish, a −44% reduction in dry matter yield. About 11% of the strains tested caused a 20% or more decrease in radish dry matter yield (Table 3). There are several possible mechanisms that may explain the deleterious effect of rhizobacteria (Alström, 1991). The most probable concern over production of substances that are beneficial to plant growth at low concentration, like IAA and related compounds or HCN. No relationship was observed between the *in vitro* characteristics of a strain and its effect on plant growth. In fact the PGPR strain Soy 213 of *B. japonicum* did not produce HCN, siderophores or IAA and did not solubilize P. The deleterious arctic strain N44 produced siderophore and IAA. Other mechanisms of action like induced resistance, competition or antagonism towards other deleterious micro-organisms might be implicated in the PGPR activity of strain Soy 213.

A second plant inoculation test was performed in growth cabinets, with 12 strains chosen among the best PGPR strains selected from the first greenhouse trial (Table 4). Under these controlled conditions, only strain Tal 629 of *B. japonicum* significantly increased the dry matter yield of radish. This strain produces siderophores, but was negative for all others *in vitro* test performed. The observed increase of 15% is comparable to increases obtained with PGPR of maize (Lalande et al., 1989) in the greenhouse, and with PGPR of lettuce under field conditions (Chabot et al., 1996a). Strain soy 213 that was the best in the first greenhouse assay, tended to increase yield (Table 4); however, the 12% increase observed is not statistically significant. Five strains – Tal 700, P 55, N40, S20 and N18 – caused a significant decrease in yield varying from 16 to 21% (Table 4). The variability of the results is one of the major problems associated with PGPR inoculation assays, and it is mainly due to the complexity of interactions involved in the rhizosphere between the plant, the introduced PGPR and other rhizosphere (neutral of deleterious) microflora. Environmental factors can also influence some of the PGPR traits of the bacteria (O'Sullivan and O'Gara, 1992).

This work indicates that *Bradyrhizobium* and *Rhizobium* have excellent potential to be used as PGPR with non-legumes. Our results corroborate other observations of plant growth promotion in the field by inoculation of non-legumes with some strains of rhizobia (Chabot et al., 1996a; Höflich et al., 1994; Yanni et al., 1995).

Some of the advantages of using bradyrhizobia and rhizobia as PGPR with non-legumes can be summarized as follows:

- the availability of the inoculation and inoculum production technologies,
- the availability of tools to identify strains in soil and in nodules,
- the genetics of these bacteria have been intensively studied making all the necessary genetic tools available (ex. Glazerbrook and Walker, 1991),
- these bacteria can be considered environmentally friendly, since they have been used for many years with legumes without causing harm to the environment, or to users.

Future directions

Future research to elucidate this new association between bradyrhizobia, rhizobia and non-legumes should focus on:

- the study of plant-bacterial signal-exchange,
- the effect of the bacteria and Nod-factors on the vesicular- arbuscular mycorrhizal symbiosis,
- the potential use of these bacteria as biological control agents, and the selection of strains inducing resistance in plants,
- the choice of strains beneficial to both legumes and non-legumes, particularly in the crop-rotation systems,
- the selection of strains deleterious to weeds and beneficial to other legume or nonlegume crops,
- the understanding of mechanisms affecting colonization and their interaction with abiotic factors.

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