World J Microbiol Biotechnol
DOI 10.1007/s11274-007-9591-4

ORIGINAL PAPER

Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.)

M. V. B. Figueiredo · C. R. Martinez · H. A. Burity · C. P. Chanway

Received: 29 July 2007 / Accepted: 21 October 2007

Abstract A greenhouse experiment was performed to evaluate the effects of plant growth-promoting rhizobacteria (PGPR) on nodulation, biological nitrogen fixation (BNF) and growth of the common bean (*Phaseolus vulgaris* L. cv. Tenderlake). Single and dual inoculation treatments of bean with *Rhizobium* and/or PGPR were administered to detect possible changes in the levels of and interactions between the phytohormones IAA and cytokinin. Bean plants cv. Tenderlake were grown in pots containing Fluvic Neosol eutrophic (pH 6.5). Fourteen kilogram aliquots of soil contained in 15-l pots were autoclaved. Bean seeds were surface sterilized and inoculated with *Rhizobium tropici* (CIAT 899-standard strain) alone and in combination with one of the PGPR strains: *Bacillus endophyticus* (DSM 13796), *B. pumilus* (DSM 27), *B. subtilis* (DSM 704), *Paenibacillus lautus* (DSM 13411), *P. macerans* (DSM 24), *P. polymyxa* (DSM 36), *P. polymyxa* (Loutit L.) or *Bacillus* sp.(65E180). The experimental design was randomized block design with three replications. Beans co-inoculated with *Rhizobium tropici* (CIAT899) and *Paenibacillus polymyxa* (DSM 36) had higher leghemoglobin concentrations, nitrogenase activity and N_2 fixation efficiency and thereby formed associations of greater symbiotic efficiency. Inoculation with *Rhizobium* and *P. polymyxa* strain Loutit (L) stimulated nodulation as well as nitrogen fixation. PGPR also stimulated specific-nodulation (number of nodules per gram of root dry weight) increases that translated into higher levels of accumulated nitrogen. The activities of phytohormones depended on their content and interactions with *Rhizobium tropici* and *Paenibacillus* and/or *Bacillus* (PGPR) strains which affect the cytokinin in content in the common bean.

Keywords Symbiosis · PGPR · *Rhizobium* sp · *Paenibacillus polymyxa* · Phytohormones

M. V. B. Figueiredo · H. A. Burity
Laboratório de Biologia de Solo, IPA/EMBRAPA, CEP 50761-000 Recife, PE, Brazil

M. V. B. Figueiredo (✉)
Rua Sá e Souza, 1020/1401, CEP 51030-065 Boa Viagem, Recife, PE, Brazil
e-mail: mbarreto@elogica.com.br

C. R. Martinez
Departamento de Biologia Molecular, Universidade Federal da Paraíba, Joao Pessoa, PB, Brazil

C. P. Chanway
Department of Forest Sciences, UBC, 3041-2424 Main Mall, Vancouver, Canada V6T 1Z4

Introduction

During the past couple of decades, the use of plant growth-promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in various parts of the world. Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been repeatedly reported (Kloepper et al. 1980; Chen et al. 1994; Zhang et al. 1996; Amara and Dahdoh 1997; Chanway 1998; Pan et al. 1999; Bin et al. 2000; Biswas et al. 2000; Asghar et al. 2002; Vessey 2003; Silva et al. 2006). Studies have also shown that the growth-promoting ability of some bacteria may be highly specific to certain plant species, cultivar and genotype (Bashan 1998).

PGPR are thought to stimulate plant growth through any of the following mechanisms: (1) by altering the hormone balance in the host plant; (2) by increasing mineral nutrient

solubilization; and (3) antagonism towards plant pathogens (Glick 1995). The phytohormone, indole-3-acetic acid (IAA), has been reported in *Bacillus* and was associated with a nodulation stimulus on bean (Srinivasan et al. 1996). PGPR that had positive effects on shoot and/or root growth in soybean tested positive for production of IAA or 1-aminocyclopropane-1-carboxylate (ACC) deaminase, however, no positive effects were found on nodulation (Cattelan et al. 1999).

Most *Rhizobium* species have been shown to produce IAA, and many studies indicate that changes in endogenous auxin concentration are a prerequisite for nodule organogenesis (Lambrecht et al. 2000). Cytokinin-like compounds were produced and metabolized by *Paenibacillus polymyxa* culture, respectively, through stationary to logarithmic growth-phases (Timmusk et al. 1999). An effective *Bacillus* PGPR with ACC deaminase activity has been reported by Ghosh et al. (2003), but legume nodulation was not evaluated. However, Ferguson and Mathesius (2003) found that the auxin/cytokinin ratio could be important for regulating nodule numbers.

The use of plant growth-promoting rhizobacteria (PGPR) as bio-protectors and yield stimulators will probably be one of the most significant tactics of plant disease management in the world. This is due to the emerging dependence on diminishing synthetic chemical products, and to the growing necessity of sustainable agriculture focussing on environmentally-friendly practices. Indeed, some PGPR effective in plant disease protection have already been commercialized and are in use worldwide. These include: Galtrol-A and Agrocin (*Agrobacterium radiobacter*, strain K84); Quantum-4000 (*Bacillus subtilis*, strain GB03); YIB (*Bacillus* spp.); Nogall (*A. radiobacter*, strain K1026); Dagger G (*Pseudomonas fluorescens*); Kodiak (*B. subtilis*, improved GB03 strain); Kodiak plus (*B. subtilis*, improved GB03 strain + Apron + terraclor); and Blue circle (*P. cepacia* type Wisconsin).

To understand the biology of plants and their microbial ecology, many studies performed with PGPR have focused on evaluating the colonization pattern of vegetative tissues, as well as the effects of bacterial endophytes on plant growth (Chanway 1998; Cattelan 1999).

Rhizobia performance was greatly improved by selecting strains for increased survival in specific soil types, greater compatibility with crop species or cultivars, superior functioning under diverse climates, improved compatibility and competitiveness with other soil microorganisms and higher nitrogen-fixing efficiency (Vessey 2003).

The general aim of this work was to evaluate the response of plant growth-promoting rhizobacteria on nodulation, biological nitrogen fixation and growth of the common bean (*Phaseolus vulgaris* L.). We used different inoculation treatments to demonstrate possible interactions between phytohormones either produced by the bacterium or the host plant and to assess the potential of the rhizobial association with PGPR.

Material and methods

Soil preparation, inoculation and planting

The experiment was conducted in a greenhouse at a temperature range of 26–33°C with 50–70% relative humidity. Pots were filled with soil samples (0–20 cm), of Fluvic Neosol eutrophic (pH 6.5). The soil was air dried, sieved (5.0 mm). Fourteen kilogram aliquots of soil contained in 15-l pots were autoclaved for 30 min at 121°C and 101 KPa, once a day for three consecutive days. Chemical and physical analyses of the soil were conducted at the Pernambuco Enterprise of Agricultural and Livestock Research (Empresa Pernambucana de Pesquisa Agropecuária-IPA) in accordance with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA 1997).

Seeds of the common bean (*Phaseolus vulgaris* L. cv. Tenderlake (Geo. W. Park Seed Co. USA, by Dr. R. Brent Thomas) were surface sterilized in 80% (v/v) ethanol for 30 s and then in 5% (w/v) sodium hypochlorite for 2 min before washing nine times in sterilized distilled water. Seeds were then inoculated and/or co-inoculated using the following treatments: CIAT 899 alone (*Rhizobium tropici*) (standard strain, origin University of Minnesota-COL); DSM 13796 (*Bacillus endophyticus*); DSM 27 (*B. pumilus*); DSM 704 (*B. subtilis*); DSM 13411 (*Paenibacillus lautus*); DSM 24 (*P. macerans)*; DSM 36 (*P. polymyxa)* origin DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany); Loutit (L) (*Paenibacillus polymyxa)* origin Otago, New Zealand); 65E180 (*Bacillus* sp.) origin Instituto de Antibiótico—UFPE-Recife-PE, Brazil; and one control (without inoculation).

Five bean seeds were sown in each pot and inoculated with 5-ml pot^{-1} of liquid culture containing *Rhizobium* sp. (10^9 cfu ml^{-1}) and *Bacillus* sp. and/or *Paenibacillus polymyxa* (10^8 cfu ml^{-1}). After emergence three plants were left per pot. Hoagland and Arnon (1950) solution with a low level of N (1 mM NO_3^- was temporarily available for 3 weeks) was applied weekly at a rate of 2 ml kg of soil^{-1}.

Plants were harvested 40 days after germination (DAG) and the following data were collected: leghemoglobin (LHb) concentration in nodules was assayed spectrophotometrically (540 nm) using a Drabkin solution as the "blank" (Wilson and Reisenauer 1963); leaf soluble protein concentration was determined according to Bradford method (it was assessed with bovine serum albumin as the

standard); approximately 2 g leaves of bean were harvested and used for hormone analysis and the extraction and purification of ABA (abscisic acid), IAA (indoleacetic acid), GA_3 (gibberellic acid) and zeatin were determined according to Yurekli et al. (2001), Izumi et al. (1988) and Nefedieva (2003) with modifications. Briefly, extracts were dissolved in a small volume of methanol and used for HPLC analysis. Samples (20 µl) were injected into a reversed-phase LC18 column, connected to an HPLC pump (Cecil 1100, Cambridge, UK), and the column was eluted with a linear gradient using 20–80% methanol in 1% (w/v) aqueous acetic acid at a flow rate of 1.2 ml min^{-1} for ABA; using 20–75% methanol in 0.4% acetic acid at a flow rate of 1.0 ml min^{-1} for GA_3 and IAA; and using 10% acetonitrile at a flow rate of 2.0 ml min^{-1} for cytokinins (CKs).

Other measurements included shoot, nodule and root dry weights (65°C for 72 h), number of nodules and shoot/root ratio. Total nitrogenase activity was assayed in agreement with Hunt et al. (1987), intact root systems were isolated by capping the gas-exchange pots and sealing them with Terostat IX. The sealed pots were connected to a gas-exchange system according to Vessey (1992). Total N was determined using a Tecator 1030 auto-analyzer following the Kjeldahl method (Bremner 1965).

Statistical design and analysis

Treatments were arranged in a randomized block design with three replications. Analysis of variance (ANOVA) was performed using Statsoft Inc. (Tulsa) statistical software. Treatment effects were first analysed by F tests ($P < 0.05$). Comparison of treatment means was performed using Tukey's HSD.

Treatments evaluated were G_1: co-inoculation of *Rhizobium tropici* CIAT 899 with each PGPR strain separately i.e., *Bacillus* or *Paenibacillus* strains DSM 13796, DSM 27, DSM 704, DSM 13411, DSM 24, DSM 36, Loutit (L) or 65E180. G_2 treatments consisted of inoculation with each PGPR strain alone. Controls were inoculation with *R. tropici* CIAT 899 alone as well as no inoculation with any bacteria.

Results and discussion

ANOVA indicated significant ($P < 0.01$) main effects for both G_1 and G_2 (Tables 1 and 2). However, G_1 had a significant positive effect on most plant growth variables, but G_2 only increased root dry matter (RDM) ($P < 0.01$).

The common bean had lower RDM (0.67 g pot^{-1}) when co-inoculated with *Rhizobium tropici* (CIAT 899) + Loutit (L) strains compared to inoculation with *Rhizobium tropici* (CIAT 899 alone −1.25 g pot^{-1}) and plants co-inoculated with CIAT 899 + DSM 36 (1.30 g pot^{-1}) (Table 3). Other treatment means fell within the range of these treatments, and had no significant effect on RDW. However, co-inoculation with CIAT 899 + Loutit strains affected shoot / root ratio (SDM / RDM) significantly compared to all other treatments.

Co-inoculation of the common bean with CIAT 899 + DMS 36 enhanced plant nitrogen accumulation (Nac) compared to all other treatments. However, this combination did not differ statistically from CIAT 899 + Loutit and CIAT 899 alone. Co-inoculation with CIAT 899 + 65(E)180, CIAT899 + DSM 36 as well as CIAT 899 + Loutit resulted in greater nitrogen fixation efficiency (EFN_2) than plants inoculated with *Rhizobium* alone. Bean co-inoculated with CIAT899 + DSM 36 and CIAT899 + Loutit caused the greatest nitrogenase activity (N_2ase) leghemoglobin content (LHb) of all treatments (Table 4). Co-inoculation with CIAT899 + Loutit (L) inhibited RDW and increased shoot/root ratio compared to the other PGPR strains. However, the inhibition of root growth did not affect N_2 fixation indicated by N_2ase and EF N_2 activities.

Plants inoculated with DSM 27 (*B. pumilus*) had 70% greater RDM (1.46 g pot^{-1}) than the non-inoculated plants or control (0.86 g pot^{-1}) (Fig. 1). In addition the content of abscisic acid (ABA) in bean co-inoculated with CIAT899 + DSM 27 (175 µg ml^{-1}) increased in comparison to other treatments (Table 4).

The inoculation with other PGPR induced an intermediate RDM efficiency without any significant difference in the efficiency inducted in both treatments mentioned previously. For those plants cultivated in a N-limited environment and inoculation with *Bacillus* and/or *Paenibacillus* alone have shown a reduction in the root growth for soybean and green grass (Camacho et al. 2001), however, the increase in the root growth was reported in canola *Brassica campestris* (Ghosh et al. 2003). Date of the experiment (present study) indicated that the promoting nature of the root growth in the common bean was established in most PGPRs tested. The lack of pathogens and the limitation of nutrient (except N) led us to infer the direct mechanism of action for those PGPRs. The low level of N (1 mM NO_3^-) was temporarily available (for 3 weeks) for the common bean. The probable N_2 fixation activity of PGPR, as a whole, it is expected to have small effects or be non-detectable in the growth of plants (Vessey 2003). However, both limited sources of nitrogen allow the maintenance of the common bean plants.

The regulation of root system development in plants depends on auxin activity, which can increase or decrease radicle cell size, depending on the concentration as well as

Table 1 Effects of co-inoculation with *Rhizobium tropici* CIAT 899 and PGPR (G_1) as well as inoculation with PGPR alone (G_2) on shoot dry matter (SDM) root dry matter (RDM) and SDM/RDM ratio, nitrogen accumulation (Nac), nitrogenase activity (N_2ase), N_2 fixation efficiency (N_2FE) and nodulation (individual nodule (IN); specific nodulation (SN) in the common bean (*Phaseolus vulgaris* L.)

Treatments's cluster	SDM (g pot^{-1})	RDM (g pot^{-1})	SDM/RDM (g g^{-1})	IN (μg nodule^{-1})	SN (n nodule. gRDM^{-1})	Nac (mg N pot^{-1})	N_2ase (mmolH_2 g^{-1} h^{-1})	N_2FE (g Ng^{1}NDM)
CIAT 899 + PGPR (G_1)	6.62 a	1.00 b	6.68 a	1.00 a	6.68 a	263.40 a	183.2 a	0.80 a
PGPR (G_2)	0.50 b	1.18 a	0.420 b	NA	NA	0.49 b	NA	0.92 b
% CV	20.6	16.2	22.4	30.06	16.04	19.4	18.89	20.30

Values presented are treatment means (27 replicates) and the coefficient of variance

In each column the means followed by the same letters do not differ statistically ($P < 0.01$) by F test. CV (coefficient of variance); NA—not applicable

Table 2 Effects of co-inoculation with *Rhizobium tropici* CIAT 899 and PGPR (G_1) as well as inoculation with PGPR alone (G_2) on leaf soluble protein (PT), leghemoglobin (LHb), zeatin (Z), abscisic acid (ABA), indoleacetic acid (IAA) and gibberellic acid (GA_3) content and nodulation (nodule numbers (NN), nodule dry matter (NDM), in the common bean (*Phaseolus vulgaris* L.)

Treatments's cluster	PT (mg prot gFM^{-1})	LHb (mg ^{1}NDM)	Z Eq[a] (μg ml^{-1})	IAA Eq. (μg ml^{-1})	ABAEq. (μg ml^{-1})	GA_3 Eq. (μg ml^{-1})	NN (nodule pot^{-1})	NMD (mg pot^{-1})
CIAT 899 + PGPR (G_1)	11.0 a	24.10 a	155.20 a	162.75 a	179.52 a	153.97 a	54.18 a	0.320 a
PGPR (G_2)	NA	NA	92.2 b	87.9 b	65.8 b	74.7 b	NA	NA
% CV	9.59	8.45	10.54	13.93	12.82	11.37	11.85	17.99

Values presented are treatment means (27 replicates) and the coefficient of variance

In each column the means followed by the same letters do not differ statistically ($P < 0.01$) by F test. CV (coefficient of variance). NA—not applicable

[a] Equivalent

Table 3 Effects of co-inoculation with *Rhizobium tropici* CIAT 899 and different PGPR (G_1) on shoot dry matter (SDM) root dry matter (RDM) and SDM/RDM ratio, nitrogen accumulation (Nac), nitrogenase activity (N_2ase) and N_2 fixation efficiency (N_2FE) in the common bean (*Phaseolus vulgaris* [L.])

Treatments (G_1)	SDM[a] (g pot^{-1})	RDM (g pot^{-1})	SDM/RDM (g g^{-1})	Nac (mg N pot^{-1})	N_2 ase (mmol H_2 g^{-1} h^{-1})	N_2FE (mg N g^{-1}NDM)
CIAT 899 alone	7.24	1.25 ab	5.68 c	297.2 a	198 ab	0.80 ab
CIAT 899 + DMS 13796	6.72	1.12 ab	6.09 bc	256.2 ab	194 ab	0.85 ab
CIAT 899 + 65 (E)180	6.82	1.01 ab	6.76 bc	273.4 ab	153 ab	0.88 a
CIAT 899 + Loutit (L)	6.21	0.67 b	9.28 a	308.2 a	208 a	0.95 a
CIAT 899 + DMS 27	6.09	0.97 ab	6.25 bc	224.2 b	178 abc	0.63 b
CIAT 899 + DMS 13411	5.35	1.10 ab	4.85 c	221.7 b	154 bc	0.77 ab
CIAT 899 + DMS 704	6.06	0.96 ab	6.33 bc	248.9 ab	182 abc	0.75 ab
CIAT 899 + DMS 24	6.39	0.80 ab	7.99 ab	244.9 ab	171 abc	0.76 ab
CIAT 899 + DMS 36	7.33	1.30 a	5.64 c	316.5 a	211 a	0.92 a
% CV	20.6	16.2	22.4	19.4	18.89	20.30

Values presented are treatment means (3 replicates) and the coefficient of variance

In each column the means followed by the same letters do not differ statistically ($P < 0.05$) from each other, according to Tukey's HSD

[a] Letters missing for the column because F-test (G_1); NS (not significant to SDM)

its interaction with other phytohormones such as the cytokinins (Evans 1984). In legume root nodules, IAA activates the enzyme H^+-ATPase, which is fundamental for enery production in the nodule (Rosendahl and Jochimsen 1995). Phytohormone production, particularly that of Indoleacetic Acid (IAA) has been repeatedly observed in rhizobacteria as well as in symbiotic bacteria such as the genus *Bradyrhizobium* (Boddey and Hungria 1994).

G_1 treatments (co-inoculated treatments with CIAT899 + PGPRs) generally reduced RDM compared to plants inoculated with CIAT899 alone (Table 3). The lone exception was co-inoculation with *Paenibacillus* strain

Table 4 Effect of co-inoculation with *Rhizobium tropici* CIAT 899 and different PGPR (G_1) on leaf soluble protein (PT), leghemoglobin (LHb), zeatin (Z), abscisic acid (ABA), indoleacetic acid (IAA) and gibberellic acid (GA_3) content in the common bean (*Phaseolus vulgaris* [L.])

Treatments (G_1)	PT (mg prot g FM^{-1})	LHb (mg g^{-1}SDM)	Z Eq[a] (μg ml^{-1})	ABA Eq. (μg ml^{-1})	IAA Eq. (μg ml^{-1})	GA_3 Eq. (μg ml^{-1})
CIAT 899 alone	13.25 a	26.48 a	145 b	160 ab	187 a	145 ab
CIAT 899 + DMS 13796	10.57 ab	24.12 ab	162 a	158 ab	180 a	153 a
CIAT 899 + 65 (E)180	12.30 a	25.03 ab	155 a	155 ab	181 a	160 a
CIAT 899 + Loutit (L)	11.74 ab	27.10 a	152 a	150 ab	178 a	159 a
CIAT 899 + DMS 27	10.63 ab	23.02 ab	143 b	175 a	172 a	148 ab
CIAT 899 + DMS 13411	13.10 a	19.20 b	154 a	153 ab	175 a	156 a
CIAT 899 + DMS 704	12.43 a	22.11 ab	163 a	143 b	170 ab	150 a
CIAT 899 + DMS 24	11.32 ab	23.82 ab	150 ab	157 ab	179 a	154 a
CIAT 899 + DMS 36	13.14 a	27.98 a	157 a	156 ab	182 a	158 a
% CV	9.59	8.45	10.54	13.93	12.82	11.37

Values presented are treatment means (3 replicates) and the coefficient of variance

In each column the means followed by the same letters do not differ statistically ($P < 0.05$) from each other, according to Tukey's HSD

[a] Equivalent

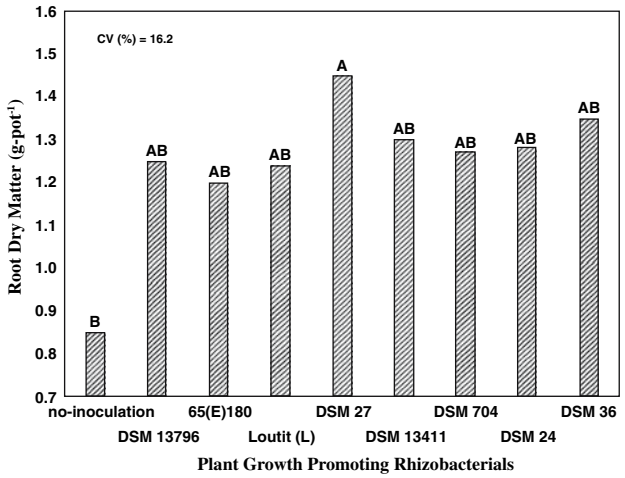

Fig. 1 Production of root dry matter in plants (40 days old) of common bean (*Phaseolus vulgaris* L.) cv. Tenderlake under effects of different plant growth-promoting rhizobacteria: 65(E)180 (*Bacillus* sp.); DSM 13796 (*B. endophyticus*); DSM 27 (*B. pumilus*); DSM 704 (*B. subtilis*); DSM 13411 (*Paenibacillus lautus*); DSM 24 (*P. macerans*); DSM 36 (*P. polymyxa*); Loutit (L) (*P. polymyxa*). Means followed by the same letters do not differ statistically ($P < 0.05$) from each other, according to Tukey's HSD

DMS 36, which did not differ significantly from CIAT899-inoculated plants. In addition, bean roots inoculated with PGPR as well as co-inoculated with CIAT899 + PGPR tended to be larger and smaller, respectively, than controls (Table 3). Such effects could involve phytohormones For example, *Rhizobium* alone (CIAT 899) may have favored an increase in the level of auxin(s) or a lower cytokinin/auxin ratio (0.77 μg ml^{-1} data not shown) resulting in enhanced root development as suggested by Lambrech et al. (2000). Alternatively, ethylene production may have been amplified by increased production of cytokinin, resulting in shorter roots or lower as observed by Lorteau et al. (2001).

Of the nodulation traits measured, only specific nodulation (SN) (the number of nodules per gram of root) increased significantly ($P < 0.05$) due to co-inoculation with PGPR (Table 5). The best combination was CIAT899 + Loutit (L) which increased SN 119% over CIAT 899 inoculation alone. A positive linear relationship was observed when SN and Nac/RDM underwent correlation analyses ($y = 12.51 + 1.82x$; $r = 0.642$*) reinforcing the hypothesis that PGPR enhance nitrogen fixation through stimulatory effects on SN. Thus the nodulation stimulated in bean mediated by PGPR in our work differs from that reported by Cooper and Long (1994) who found that PGPR stimulated root growth, but not nodulation in soybean and pea, indirectly enhancing nitrogen fixation.

The difference between our study and that of Cooper and Long (1994) may be related to variable cytokinin production by *Paenibacillus*. For the initial phase of the common bean development, the low levels of cytokinin [associated with *P. polymyxa* strain Loutit (L)] could have stimulated the rhizobial growth, the number of infections in roots, as well as the development of nodules. As bean development proceeds, the increase of cytokinin production associated with *Paenibacillus* causes a delay in the radicular growth, possibly by the inhibiting level of the ethylene produced.

Conclusion

Co-inoculation of the common bean with *Rhizobium tropici* strain CIAT 899 and *Paenibacillus polymyxa* strain

Table 5 Effect of co-inoculation with *Rhizobium tropici* CIAT 899 and different PGPR (G_1) on nodulation (nodule numbers (NN), nodule dry matter (NDM), individual nodule (IN) and specific nodulation (SN) in the common bean (*Phaseolus vulgaris* [L.])

Treatments (G_1)	Nodulation			
	NN (nodule pot^{-1})	NDM[a] (mg pot^{-1})	IN (μg nodule^{-1})	SN (nodule g RDM^{-1})
CIAT 899 alone	40.3 a	0.370	9.40 a	32.55 b
CIAT 899 + DMS 13796	33.0 a	0.283	9.25 a	30.32 b
CIAT 899 + 65 (E)180	52.0 a	0.310	6.41 a	52.23 ab
CIAT 899 + Loutit (L)	54.7 a	0.322	5.46 a	71.41 a
CIAT 899 + DMS 27	43.0 a	0.353	8.87 a	80.59 a
CIAT 899 + DMS 13411	61.0 a	0.287	4.77 a	56.25 ab
CIAT 899 + DMS 704	64.3 a	0.339	5.53 a	63.85 ab
CIAT 899 + DMS 24	55.3 a	0.310	5.08 a	68.56 ab
CIAT 899 + DMS36	75.4 a	0.350	10.17 a	57.82 ab
% CV	11.85	17.99	30.06	16.04

Values presented are treatment means (3 replicates) and the coefficient of variance

In each column the means followed by the same letters do not differ statistically ($P < 0.05$) from each other, according to Tukey's HSD. Nodule-number's dates had been transformed by $(x + 1)^{0.5}$ to variance analysis. CV (coefficient of variance)

[a] Letters missing for the column because *F*-test (G_1) NS (not significant to NDM)

DSM 36 resulted in higher nodule LHb concentration, N_2ase, and N_2 fixation efficiency, and thereby formed associations of greater symbiotic efficiency. The PGPR benefits on specific-nodulation were evident on accumulated plant nitrogen. The *Rhizobium tropici* the common bean symbiosis with Loutit (L)-helper bacteria stimulated nodulation which enhanced overall nitrogen fixation. The activities of phytohormones depended on their content and interactions with *Rhizobium tropici* and *Paenibacillus* and/or *Bacillus* strains (PGPR), which affect the cytokinin content in the common bean.

Acknowledgments The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of Brazil and the University of British Columbia (UBC) of Canada for financial support.

References

Amara MAT, Dahdoh MSA (1997) Effect of inoculation with plant growth-promoting rhizobacteria (PGPR) on yield and uptake of nutrients by wheat grown on sandy soil. Egypt J Soil 37:467–484

Asghar HN, Zahir ZA, Arshad M, Khalig A (2002) Plant growth regulating substances in the rhizozphere: microbial production and functions. Adv Agron 62:146–151

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770

Bin L, Smith DL, Ping-Qui F (2000) Application and mechanism of silicate bacteria in agriculture and industry. Guizhou Sci 18:43–53

Biswas JC, Ladha JK, Dazzo FB (2000) Rhizobial inoculation influences seedling vigor and yield of rice. Agron J 92:880–886

Boddey LH, Hungria M (1994) Classificação de estirpes de *Bradyrhizobium japonicum* em genótipo I e II baseada nas características fenotípicas e genotípicas. In: Simpósio Brasileiro Sobre Microbiologia de Solo, Londrina. Resumos. Londrina: LAPAR, p 66

Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–252

Bremner JM (1965) Total nitrogen. In: Black CA (ed) Methods of soil analysis chemical and microbiological properties. Part-2. Agronomy 9. American Society of Agronomy, Madison, pp 1149–1178, ASIN: B000H94EHO

Camacho M et al (2001) Co-inoculation with *Bacillus* sp CECT 450 improves nodulation in *Phaseolus vulgaris*. Can J Microbiol 47:1058–1062

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680

Chanway CP (1998) Bacterial endophytes: ecological and practical implications. Sydowia 50:149–170

Chen C, Bauske EM, Musson G, Rodriguez-Kabaña R, Kloepper JW (1994). Biological control of *Fusarium* on cotton by use of endophytic bacteria. Biol Control 5:83–91

Cooper JB, Long SR (1994) Morphogenetic rescue of *Rhizobium meliloti* nodulation mutants by trans-zeatin secretion. Plant Cell 6:215–225

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária. Centro Nacional de Pesquisa em Solos) (1997) Manual de Métodos de Análise de Solos. Rio de Janeiro, 1997, v.2

Evans ML (1984) Functions of hormones at the cellular level of organization. In: Scott TK (ed) Hormonal regulation of development, II. Springer-Verlag, Berlin, pp 23–62, ISBN-10:0387101969

Ferguson BJ, Mathesius U (2003) Signaling interactions during nodule development. J Plant Growth Regul 22:47–72

Ghosh S et al (2003) Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, *Brassica campestris*. Plant Physiol Biochem 41:277–281

Glick BR (1995) The enhancement of plant-growth by free-living bacteria. Can J Microbiol 41:109–117

Hoagland DR, Arnon DI (1950) The water culture method of growing plants without soil. University of California, Berkeley, 39 pp, ASIN B00088T A3W

Izumi K et al (1988) Levels of IAA, cytokinin, ABA and ethylene in rice plants as affected by giberellin biosynthesis inhibitor Unincazole-P. Plant Cell Physiol 29:97–104

Kloepper JW, Schroth MN, Miller TD (1980) Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078–1082

Lambrecht M, Okon Y, Vande Broek A, Vanderleyden J (2000) Indole-3-acetic acid: a reciprocal signaling molecule in bacteria-plant interactions. Trends Microbiol 8:298–300

Lorteau MA, Ferguson BJ, Guinel FC (2001) Effects of cytokinin on ethylene production and nodulation in pea (*Pisum sativum*) cv.Sparkle. Physiol Plant 112:421–428

Nefedieva EE (2003) The influence of impulse pressure on the phytohormone content, growth and crop productivity of buckwheat plants (*Fagopyrum esculentum* Moench. cv. *Aromat*). Pac J Sci Technol 3:123–135

Pan B, Bai YM, Leibovitch S, Smith DL (1999) Plant growth-promoting rhizobacteria and kinetin as ways to promote corn growth and yield in short season areas. Eur J Agron v 11:179–186

Rosendahl L, Jochimsen BV (1995) Uptake of indolacetic acid in symbiosomes from soybean (*Glycine max* L.) root nodules. In: Nitrogen fixation: fundamentals and applications. Proceedings. 10. International Congress of Nitrogen Fixation, 11, St.Petersburg. Resumos. St. Petersburg, Russian Academiy of Sciences, Russia, p. 336

Silva VN, Silva LESF, Figueiredo MVB (2006) Atuação de rizóbios com rizobactérias promotora de crescimento em plantas na cultura do caupi (*Vigna unguiculata* L. Walp). Acta Sci Agron 28:407–412

Srinivasan M, Petersen DJ, Holl FB (1996) Influence of indoleacetic-acid-producing *Bacillus* isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under gnotobiotic conditions. Can J Microbiol 42:1006–1014

Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by *Paenibacillus polymyxa*. Soil Biol Biochem 31:1847–1852

Vessey JK (2003) Plant growth-promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Wilson DO, Reisenauer HM (1963) Determination of leghemoglobin in legumes nodules. Anal Biochem 6:27–30

Yurekli F, Turkan I, Banu Porgali Z, Topcuoglu SF (2001) Indoleacetic acid, gibberellic acid, zeatin and abscisic acid levels in NaCl-treatment tomato species differing in salt tolerance. Isr J Plant Sci 49:269–277

Zhang F et al (1996) Plant growth-promoting rhizobacteria and soybean [*Glycine max* (L.) Merr.]. Nodulation and fixation at suboptimal root zone temperatures. Ann Bot 7:453–459