academicJournals

Vol. 7(11), pp. 948-959, 12 March, 2013
DOI 10.5897/AJMR12.1055
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Evaluation of plant growth-promoting traits of *Burkholderia* and *Rhizobium* strains isolated from Amazon soils for their co-inoculation in common bean

Silvia Maria de Oliveira Longatti[1,3], Leandro Marciano Marra[2,3] and Fatima Maria de Souza Moreira[1,2,3*]

[1]Agricultural Microbiology Department, Federal University of Lavras, Caixa Postal 3037, Cep: 37.200-000, Lavras MG, Brazil.
[2]Soil Science Department, Federal University of Lavras, Caixa Postal 3037, Cep: 37.200-000, Lavras MG, Brazil.
[3]Universidade Federal de Lavras – UFLA, Departamento de Ciência do Solo. Caixa Postal 3037, Cep: 37.200-000, Lavras, MG, Brazil.

Accepted 8 March, 2013

Several processes that promote plant growth were investigated in diazotrophic bacteria isolated from soils of various land-use systems in the western Amazon region using siratro and bean as the trap species. The processes observed in the 17 studied strains were as follows: Inhibition of phytopathogenic fungal growth; free-living nitrogen fixation in semi-solid nitrogen-free LO medium; solubilisation of insoluble calcium and aluminium inorganic phosphates in solid media and auxin production based on a colorimetric assay with or without the addition of L-tryptophan. The ability to use phenol as the sole carbon source in solid media and antibiotic resistance, based on the disk diffusion method, was also evaluated. Nearly all of the selected strains, including *Rhizobium* strains, were able to fix nitrogen as free-living bacteria. Some *Burkholderia fungorum* strains had a strong ability to solubilise calcium phosphate, whereas strains UFLA 04-229 and UFLA 04-217 displayed high levels of indole-3-acetic acid synthesis in the absence (12.59 µg mL^{-1}) or presence (29.08 µg mL^{-1}) of L-tryptophan, respectively. Four of the studied strains potentially biodegrade the pollutant compound phenol. *Rhizobium* strains were more tolerant to antibiotics than *Burkholderia* strains. The *Rhizobium* strains, which are highly efficient at fixing nitrogen in symbiosis with the common bean plant, and the CIAT 899^T strain demonstrated the ability to perform other processes that promote plant growth. Co-inoculation of CIAT 899^T and UFLA 04-155 (*B. fungorum*) enhanced significantly the dry matter of nodules and shoot and P contents in relation to single inoculation with CIAT 899^T.

Key words: Biological nitrogen fixation, solubilisation of inorganic phosphates, plant growth hormones, phenol biodegradation, antibiotic resistance.

INTRODUCTION

Soil is a complex, heterogeneous and dynamic environment where a number of processes that are important to life occur. These processes are mediated by soil's vast microbial diversity, which may contribute to plant growth. Among these processes, the biological fixation of atmospheric nitrogen (BFN) is mediated by several genera of prokaryotes that can live freely in soil or live symbiotically or in association with plants and other organisms. BFN is important in agricultural systems and in natural ecosystems as it increases nitrogen content

*Corresponding author. E-mail: fmoreira@dcs.ufla.br. Tel: 55 35 3829 12 54. Fax: 55 35 3829 12 51.

in these environments.

Among the nitrogen-fixing bacteria, the 15 genera that symbiotically fix nitrogen in legumes nodules stand out. Among these, only *Azorhizobium* and *Burkholderia* are also able to fix nitrogen as free-living cells (Dreyfus et al., 1988; Moreira et al., 2006; Elliott et al., 2007).

Several studies have been developed to select bacterial strains that efficiently fix nitrogen in symbiosis with the common bean plant (*Phaseolus vulgaris* L.) to increase the productivity of this crop. These studies have revealed a high diversity of strains that could potentially be used as inoculants (Ferreira et al., 2000; Soares et al., 2006; Ferreira et al., 2009).

In addition to their ability to fix atmospheric nitrogen, it is of agricultural interest to identify other important processes in these bacteria, including nodulating bacteria, that may directly or indirectly promote the growth of both legume and non-legume plants. However, in most studies that report plant growth promoters, it is most common to find associative rhizosphere and endophytic bacteria, such as *Pseudomonas*, *Azospirillum*, *Pantoea*, *Paenibacillus* and *Acinetobacter* (Monteiro et al., 2009; Ogut et al., 2010; Collavino et al., 2010) than symbiotic bacteria. Examples of additional processes of interest are the solubilisation of normally insoluble inorganic phosphates, the ability to inhibit the growth of phytopathogens and the production of plant growth hormones.

This potential has been revealed in some studies, showing that, for example, inoculating with *Rhizobium leguminosarum* biovar *phaseoli* promotes soil phosphate solubilisation, increasing the production of maize (*Zea mays* L.) and lettuce (*Lactuca sativa* L.) (Chabot et al., 1996), and that the phosphorus content in chickpea (*Cicer arietinum* L.) and barley (*Hordeum vulgare* L.) increases by 100% and 125%, respectively, when inoculated with *Mesorhizobium mediterraneum*, which solubilises inorganic phosphate (Peix et al., 2001a). The ability to inhibit phytopathogenic fungi is another process that has been reported for these bacteria. Among the 15 *Rhizobium* strains tested, two significantly reduced root rot in bean plants artificially infected with *Fusarium solani* f. sp. *phaseoli* (Buonassisi et al., 1986).

Furthermore, these bacteria are actively involved in the synthesis of plant growth hormones such as auxin, which was confirmed in culture media (Hameed et al., 2004; Boiero et al., 2007) and they promoted growth of radish plants (*Raphanus sativus* L.) in soil (Antoun et al., 1998). A high positive correlation between the production of indole-3-acetic acid (IAA) from L-tryptophan *in vitro* and grain yield in mustard (*Brassica juncea* L.) has also been observed (Asghar et al., 2002).

The synergism between the plant growth promoting bacteria with nitrogen-fixing Leguminosae - nodulating bacteria is another option for the use of these bacteria and it could be an alternative to optimize the BFN (Figueiredo et al., 2008; Camacho et al., 2001) and increase uptake of other nutrients (Karlidag et al., 2007; Mantelin and Touraine, 2004).

In addition to promoting plant growth, some bacteria, including those that can form nodules, have the ability to decompose natural and synthetic toxic substances such as petroleum, pesticides and dyes. *Rhizobium* and *Burkholderia* species reportedly have the potential to degrade phenolic compounds (Wei et al., 2008; Cobos-Vasconcelos et al., 2006). Therefore, some research has focused on selecting bacteria with this ability in order to use them to clean up dangerous spills and toxic deposits.

In order for bacteria to carry out these processes, they have to compete with a vast diversity of soil microbes for resources and overcome antagonistic relationships where, generally, a wide range of antibiotics are involved.

The ability of nitrogen-fixing bacteria to perform other processes, as well as to adapt to environmental conditions, increases the value of strains that potentially can improve plant growth and environmental quality, directly or indirectly. Therefore, the objectives of this study were: to verify the *in vitro* ability of *Burkholderia* and *Rhizobium* strains isolated from Amazonian soils to perform different biotechnological processes; to determine their resistance to several antibiotics and to verify the effects of their co-inoculation with CIAT 899^T in the growth of common beans.

MATERIALS AND METHODS

Strains studied

This study investigated seventeen strains from the Soil Microbiology Laboratory Collection from the Federal University of Lavras, Brazil, and CIAT 899^T, which is approved by MAPA (Ministério da Agricultura, Pecuária e Abastecimento - Ministry of Agriculture, Livestock and Supply, Brazil) as an inoculant for common bean cultures (Table 1). Fourteen of the strains were isolated from siratro nodules (Lima et al., 2009). The *Rhizobium etli* strains UFLA 02-86, UFLA 02-68 and UFLA 02-100, which were isolated from bean nodules, were highly effective in symbiosis with this species (Pereira et al., 1998; Soares et al., 2006). Excepting UFLA 02-86, UFLA 02-68 and UFLA 02-100, all of the strains were studied with respect to their symbiosis with the common bean and to their tolerance do acidity and high Al concentrations by Ferreira et al. (2012).

In addition to these strains, others strains were used as positive controls for some of the tests: BR5401^T and ORS571^T served as positive controls for free-living biological nitrogen fixation, and BR11001^T and BR11080 ^{T} served as positive controls for auxin production.

Antifungal activity

The antagonistic activity of the strains against *Fusarium oxysporum f.* sp. *phaseoli* was studied following the methods of Peix et al. (2001b) with some modifications. Fungal mycelia grown on 5 mm discs in pH 6.8 PDA (Potato Dextrose Agar, Difco) were placed in the centre of a Petri dish containing the same culture medium. Isolated colonies from each bacterium were streaked onto three areas of the plate around the disc. Each strain was tested in triplicate (3 plates/strain). Mycelial growth in the absence of bacteria

Table 1. Origin, characteristics and identification of bacterial strains isolated from western Amazonian soils using siratro and bean as the trap plants and CIAT 899^T as the inoculant for the common bean (*Phaseolus vulgaris*).

Strains	Location/ LUS[(1)]	Symbiotic efficiency in the fixation of N_2 in the common bean [(2)]	Growth characteristics in 79 medium					Identification	Access No. in GenBank (NCBI)
			[(3)]G.R.	[(4)]pH	Colony Colour	[(5)]Abs. ind.	[(6)]Ø (mm)		
CIAT 899^T	Colombia	High	F	acidic	yellow	no	> 2	*Rhizobium tropici*	---
UFLA 02-68	RO/C	High	F	neutral	white	yes	> 2	*R. etli* bv. *mimosa*	EF158572
UFLA 02-86	RO/C	High	F	neutral	white	yes	> 2	*R. etli* bv. *phaseoli*	---
UFLA 02-100	RO/C	High	F	neutral	white	yes	>2	*Rhizobium etli*	AY465886
UFLA 04-173	AM/AG	High	I	neutral	white	no	2	*Rhizobium* sp.	JF412047
UFLA 04-195	AM/FA	High	F	acidic	yellow	yes	5	*Rhizobium* sp.	JF412048
UFLA 04-202	AM/P	High	F	neutral	cream	yes	4	*Rhizobium* sp.	JF412049
UFLA 04-122	AM/PF	Medium	F	acidic	cream	yes	5	*Burkholderia fungorum*	JF412046
UFLA 04-226	AM/AF	No nodules	F	acidic	yellow	no	3	*Burkholderia fungorum*	JF412050
UFLA 04-217	AM/FA	No nodules	F	neutral	cream	no	5	*Burkholderia fungorum*	---
UFLA 04-155	AM/FI	No nodules	F	acidic	yellow	no	3	*Burkholderia fungorum*	GU144382
UFLA 04-228	AM/FI	No nodules	F	acidic	yellow	yes	5	*Burkholderia fungorum*	JF412052
UFLA 04-229	AM/P	Medium	F	neutral	cream	no	3	*Burkholderia fungorum*	JF412053
UFLA 04-231	AM/P	No nodules	F	acidic	yellow	no	4	*Burkholderia fungorum*	JF412054
UFLA 04-232	AM/P	No nodules	F	neutral	yellow	yes	2	*Burkholderia fungorum*	JF412055
UFLA 04-233	AM/AG	No nodules	F	acidic	yellow	no	3	*Burkholderia fungorum*	JF412056
UFLA 04-234	AM/AG	No nodules	F	neutral	cream	no	3	*Burkholderia* sp.	JF412057
UFLA 04-21	AM/AG	Medium	F	acidic	yellow	yes	4	*Burkholderia* sp.	FJ534643

AM: Amazônia state, RO: Rondônia state, [(1)]LUS: Land-use systems - FA: Secondary forest in an advanced stage of regeneration, AG: Agriculture, PF: Primary forest, P: Pasture, AF: Agroforest, FI: Secondary forest in an initial stage of regeneration, C: Crop/beans. [(2)](Pereira et al., 1998; Soares et al., 2006; Ferreira et al., 2009, 2012). Growth characteristics in 79 medium: [(3)]G.R. Growth rate – F: fast (2 to 3 days) – I: intermediate (4 to 5 days); [(4)]pH of the culture medium after growth; [(5)]Absorption indicator; [(6)]Colony diameter.

served as the control. The plates were incubated for 3 to 7 days at 28°C. The results were evaluated by looking for a zone of inhibition in fungal growth in the presence of bacteria.

Free-living nitrogen fixation

To evaluate free-living nitrogen fixation, the strains and positive controls BR 5401^T and ORS 571^T, which are *Azorhizobium doebereinerae* and *Azorhizobium caulinodans* strains (Moreira et al., 2006; Dreyfus et al., 1988), respectively, were inoculated in the centre of vials (total volume 10 mL) containing 5 mL of semi-solid, nitrogen-free LO culture medium (Dreyfus et al., 1983). LO medium is composed of 10 g L^{-1} sodium lactate, 1.67 g L^{-1} K_2HPO_4, 0.87 g L^{-1} KH_2PO_4, 0.05 g L^{-1} NaCl, 0.1 g L^{-1} $MgSO_4.7H_2O$, 40 mg L^{-1} $CaCl_2$, 4 mg L^{-1} $FeCl_3$, 5 mg L^{-1} $MoO_4Na.2H_2O$, 10 mg L^{-1} biotin, 20 mg L^{-1} nicotinic acid, 10 mg L^{-1} pantothenic acid, 2 ml L^{-1} micronutrient solution (0.2 g $Na_2MoO_4.2H_2O$, 0.235 g $MnSO_4.H_2O$, 0.28 g H_3BO_3, 0.008 g $CuSO_4.5H_2O$ and 0.024 g $ZnSO_4.7H_2O$ dissolved in 200 ml of distilled water), 5 ml L^{-1} bromothymol blue (0.5% in 0.2 N KOH), pH 7.0. Mannitol was also tested as a carbon source by substituting sodium lactate in the LO medium by it. Each strain was tested in triplicate (3 vials/strain).

The flasks were incubated for 3 to 7 days in the dark at 28°C until a typical pellicle formed near the surface of the media. The inoculated samples were compared to the positive controls; inoculating strains that lead to pellicle formation during this period were considered free-living nitrogen-fixing bacteria, whereas those that did not form a pellicle were not considered free-living nitrogen-fixing bacteria.

Solubilisation of insoluble calcium and aluminium inorganic phosphates

Two experiments were carried out to establish whether the strains could solubilise calcium phosphate (P-Ca) or

aluminium phosphate (P-Al). Solubilising activity (solubilisation ability and potential) was evaluated in GES medium, which was composed of 10 g L^{-1} glucose, 0.1g L^{-1} KNO_3, 100 mL L^{-1} soil extract, 2 mL L^{-1} $MgSO_4$ (10%), 2 mL L^{-1} $CaCl_2$ (1%), 1 mL L^{-1} NaCl (10%), 2 mL L^{-1} micronutrient solution (the same used in LO medium), 4 mL L^{-1} Fe-EDTA (1.64%) and 15 g L^{-1} agar (Sylvester-Bradley et al., 1982). In the first experiment, P-Ca was obtained by adding 50 mL of a 10% K_2HPO_4 solution and 100 mL of a 10% $CaCl_2$ solution in 850 mL of culture medium (all autoclaved separately) to produce an insoluble phosphate precipitate. In the second experiment, 3.04 g L^{-1} of $AlH_6O_{12}P_3$ was added. In the treatment containing P-Ca, the pH was adjusted to 6.8, whereas the pH was adjusted to 4.5 in the treatment with P-Al. To obtain the inocula, the strains were grown in liquid culture medium 79 (Fred and Waksman, 1928), which was composed of 0.1 g L^{-1} K_2HPO_4, 0.4 g L^{-1} KH_2PO_4 , 0.2 g L^{-1} $MgSO_4.7H_2O$, 0.1 g L^{-1} NaCl, 10.0 g L^{-1} mannitol and 0.4 g L^{-1} yeast extract, at a pH of 6.8. Saline solution (0.85%) was added to the cultures to adjust the concentration of cells to an optical density at 600 nm (OD_{600}) of 0.5. Twenty microlitres of cell suspension was spotted at three equidistant points on a plate containing media and the phosphate precipitate, thus resulting in three colonies per plate, with the experiment being repeated in triplicate for each strain (three plates). The diameter of the solubilisation halo (a translucent area around the colony) was measured using a digital calliper daily during 18 days. These measurements were used to obtain the solubilisation index (SI), which was determined by the following equation: S.I = Halo diameter (mm) / Colony diameter (mm) (Berraquero et al., 1976).

Based on the SIs, the strains were classified as having a low (SI < 2.00), medium (2.00 ≤ SI < 4.00) or high (SI ≥ 4.00) solubilisation ability. Based to the onset of solubilisation, the strains were also classified as early (when the onset of solubilisation occurred until the third day), late (when the onset of solubilisation occurred after the third day) or non-solubilising (when solubilisation was not visible within 18 days).

Production of the growth hormone auxin

To determine whether the bacteria could produce indole-3-acetic acid (IAA), the experimental strains and the positive controls *Azospirillum brasilense* (BR 11001^T) and *A. lipoferum* (BR 11080^T) (Tarrand et al., 1978; Radwan et al., 2002) were grown in Dygs medium, which contains 2.0 g L^{-1} glucose, 2.0 g L^{-1} malic acid, 1.5 g L^{-1} bacteriological peptone, 2.0 g L^{-1} yeast extract, 0.5 g L^{-1} K_2HPO_4, 0.5 g L^{-1} $MgSO_4.7H_2O$ and 1.5 g L^{-1} glutamic acid. After growth, the cultures were centrifuged, resuspended and adjusted to an OD_{600} of 0.5 using saline solution (0.85%), as described before. Aliquots of the bacterial solutions (500 µL) were inoculated in Erlenmeyers with 20 mL of Dygs medium (without L-tryptophan or supplemented with 100 mg L^{-1} of L-tryptophan) and incubated for 72 h at 30°C with constant stirring. Each strain was tested in triplicate (3 Erlenmeyers/strain). To quantify the indolic compounds produced after this period, the cultures were centrifuged at 17,792 g for 10 min and 3 mL of supernatant was removed and mixed with 2 mL of Salkowski reagent (Sarwar and Kremer, 1995). This mixture was placed in the dark for 30 min to develop a pink colour, which is indicative of IAA production. The colour intensity was determined in a spectrophotometer at 535 nm, following the methods described by Asghar et al. (2002). The concentration of IAA was estimated using a standard curve previously prepared with 0, 25, 50, 100, 150, 200 and 300 µg mL^{-1} IAA (Sigma-Aldrich) in sterilised, uninoculated culture media (Radwan et al., 2002).

Growth in media containing different concentrations of phenol as the sole carbon source

The ability to use phenol as the sole carbon source was verified in Petri dishes containing an inorganic salt medium, according to the methods of Cobos-Vasconcelos et al. (2006), modified by supplementing the medium with 1, 2, 5, 6, 8 or 10 mM phenol filtered through a membrane with 0.2 µm pores. The inorganic salt medium contained 0.57 g L^{-1} $(NH_4)_2SO_4$, 0.13 g L^{-1} KH_2PO_4, 0.065 g L^{-1} $MgSO_4.7H_2O$ and 2 mL L^{-1} of micronutrient solution (0.04 g L^{-1} $CuSO_4.5H_2O$, 1.20 g L^{-1} $ZnSO_4.7H_2O$, 1.40 g L^{-1} H_3BO_3, 1.00 g L^{-1} $Na_2MoO_4.2H_2O$, 1.175 g L^{-1} $MnSO_4.H_2O$) and 15.00 g L^{-1} agar at pH 6.8. After subculturing the strains onto plates (each strain subcultures in three plates), the cultures were incubated for 4 days at 28°C. After the incubation, the plates were evaluated for growth.

Bacterial antibiotic resistance

Bacterial resistance to different antibiotics was evaluated by the saturated disc diffusion technique in Petri dishes containing solid medium 79 (15.0 g/L agar, pH 6.8). The antibiotics studied were azithromycin (15 µg), streptomycin (10 µg), erythromycin (15 µg), ampicillin (10 µg), chloramphenicol (30 µg), rifamycin (30 µg), kanamycin (30 µg), nalidixic acid (30 µg), clarithromycin (15 µg), amoxicillin (10 µg), gentamicin (10 µg) and vancomycin (30 µg) (Cecon ^{TM}, Brazil).

Bacteria were grown in liquid medium 79 for three days with constant stirring. After the incubation, 100 µL of each bacterial culture was spread onto Petri dishes containing the solid medium 79. Subsequently, using sterile forceps, three discs saturated with different antibiotics were added to each plate. Each strain was tested in triplicate (3 plates/strain).The discs were lightly pressed and kept away from one another to prevent the inhibition zones from overlapping. The plates were inverted and incubated for three days at 28°C. After this period, the diameter of the growth inhibition halo (a translucent area around the disc) was measured using a digital calliper.

Effects on *Phaseolus vulgaris* nodulation, growth and nutrient accumulation

The experiment was carried out in a greenhouse during June and July of 2010 using Leonard's jars. The top of the jar contained a 1:2 mixture of sand (150 mL) and vermiculite (300 mL), the bottom contained a mineral solution previously described in Hoagland and Arnon (1950). This solution had a low concentration of mineral nitrogen (21 mg L^{-1}) obtained from $NH_4H_2PO_4$ and KNO_3.

The statistical design was completely randomised, with factorial 2 X 7 and three replications. The first factor was the inoculation or not with *Rhizobium tropici* CIAT 899^T, the strain approved by the Ministry of Agriculture, Livestock and Supply as inoculant for beans. Second factor was inoculation with strains selected as plant growth promoters (PGP): UFLA 04-226, UFLA 04-217, UFLA 04-155, UFLA 04-232, UFLA 04-234 e BR11001^T and no inoculation. All the strains, in the second factor, are not able to establish symbiosis with beans, however they are able to promote plant growth by other processes. Strains UFLA 04-217 and BR11001^T have high IAA production. Strain UFLA 04-217 has a medium solubilisation index (I.S.) for P-Ca. Strains UFLA 04-155 and UFLA 04-232 have high I.S. for P-Ca and both have a low IAA production. UFLA 04-226 has low IAA production and medium I.S. for P-Ca. UFLA 04-234 has intermediate IAA production and low I.S for P-Ca. All these strains are able to fix N_2 in the free-living state.

The bean cultivar used was the BRS-MG Talismã, carioca grain type, launched in 2002; it is resistant to anthracnose and common mosaic virus, and is also moderately resistant to angular leaf spot (Ramalho et al., 2002). Before planting, seeds were superficially disinfested with 70% ethanol for 5 min and 1% sodium hypochlorite for 3 min and then they were washed six times in sterile distilled water. Once germinated, four seeds were transferred to each

Table 2. Onset and solubilisation index (S.I.) of calcium phosphate by *Rhizobium* and *Burkholderia* strains grown in GES medium.

Strains (*Rhizobium* sp.)	On. Sol.* (days)	S.I. (mm)		Strains (*Burkholderia* sp.)	On. Sol. (days)	S.I. (mm)	
		Initial	End			Initial	End
CIAT 899^T	3	1.93**	1.92	UFLA 04-122	3	1.74	2.80
UFLA 02-68	18	1.15	-	UFLA 04-226	3	2.20	2.09
UFLA 02-86	9	1.18	1.36	UFLA 04-217	3	2.27	3.12
UFLA 02-100	9	1.22	1.24	UFLA 04-155	3	2.67	4.55
UFLA 04-173	GNFH	-	-	UFLA 04-228	3	2.18	2.20
UFLA 04-195	NG	-	-	UFLA 04-229	3	1.00	1.43
UFLA 04-202	GNFH	-	-	UFLA 04-231	3	2.45	3.19
				UFLA 04-232	3	2.09	4.30
				UFLA 04-233	3	2.09	4.61
				UFLA 04-234	3	1.73	1.89
				UFLA 04-21	3	2.00	3.40

*Onset of Solubilisation. **S.I. = halo diameter (mm) / colony diameter (mm). NG: No growth. GNFH: Grew but did not form a halo by the 18th day.

Leonard's jar and each seed was inoculated with $1x10^8$ rhizobia cells obtained from exponential growth cultures ($1x10^8$ cells mL^{-1}). After six days of germination, thinning was performed leaving only two plants per jar.

Plants were harvested during the flowering period at 42 days to assess the following variables: number of nodules (NN), nodule dry matter (NDM), shoot dry matter (SDM) and nitrogen accumulation in shoots (NAS). The nitrogen and phosphorus accumulated in shoots was calculated by multiplying the weight of dry shoots by the nitrogen and phosphorus content, which was measured by the semi micro-kjedahl method as described by Sarruge and Haag (1979). P was analyzed according to the method of Malavolta et al. (1997).

Statistical analysis

The statistical analyses were performed using the Sisvar program, version 5.3 (Ferreira, 2008) and the Scott-Knott test (5% probability) to compare mean values. All tests were completely randomised and performed in triplicate. The values of variables, number of nodules (NN) and nodules dry matter (NDM) were previously transformed by the formula $(X + 0.5)^{0.5}$.

RESULTS AND DISCUSSION

Antifungal activity

None of the strains were able to inhibit the growth of *Fusarium oxysporum* f. sp. phaseoli, although some studies have shown that *Rhizobium* strains can inhibit the growth of phytopathogenic fungi, including *Fusarium oxysporum* (Buonassisi et al., 1986; Chao, 1990; Arfaoui et al., 2006).

Free-living nitrogen fixation

Almost all of the *Rhizobium* species, including three of the six strains that are highly efficient at fixing nitrogen when grown symbiotically with the common bean (Ferreira et al., 2012), and UFLA 04-21 (*Burkholderia* sp.), which is an efficient nitrogen fixer when in symbiosis with siratro (*Macroptilium atropurpureum*) (Lima et al., 2009), fixed nitrogen while free-living. Among the 18 strains studied, only UFLA 04-229 (*Burkholderia fungorum*), UFLA 02-68 (*R. etli* bv. *mimosae*), UFLA02-86 (*R. etli* bv. *phaseoli*) and UFLA 02-100 (*Rhizobium etli*) did not fix nitrogen while free-living.

The nitrogen-fixing strains formed a pellicle near the surface of the LO medium containing the two tested carbon sources (lactate and mannitol), with the exception of the positive controls BR 5401^T and ORS 571^T, which did not form a pellicle when mannitol was used in place of sodium lactate, because the *Azorhizobium* strains do not use mannitol as a carbon source (Moreira et al., 2006; Dreyfus et al., 1988). As of yet, free-living nitrogen fixation has not been reported for the *Rhizobium* genus. This characteristic has only been reported for the nodulating genera *Azorhizobium* and *Burkholderia* (Dreyfus et al., 1983; Elliott et al., 2007). This study reports nitrogen fixation by free-living bacteria of four *Rhizobium* strains, UFLA 04-195, UFLA04-202, UFLA04-173 and the *Rhizobium tropici* strain CIAT 899^T, which is highly efficient at BNF when grown symbiotically with the bean plant (Graham et al., 1994).

Solubilisation of insoluble calcium and aluminium inorganic phosphates

Of the 18 *Burkholderia* and *Rhizobium* strains, 15 (83%) solubilised P-Ca in solid media, with the *Burkholderia fungorum* strains UFLA 04-155, UFLA 04-232 and UFLA 04-233 showing a high rate of solubilisation (SI > 4) (Table 2). Other studies have also reported that *Burkholderia* strains have a high solubilising potential both *in vitro* and when tested to promote the growth of the common bean (Peix et al., 2001b, Collavino et al.,

2010). All P-Ca-solubilising strains were early solubilisers, with the exception of the *Rhizobium etli* strains UFLA 02-68, UFLA 02-86, UFLA 02-100, which were late solubilisers. Other researchers have found that *Bradyrhizobium*, *Rhizobium* and *Sinorhizobium* strains did not solubilise P-Ca, whereas *Mesorhizobium mediterraneum* strains effectively solubilise P-Ca in solid medium with varying efficiency (Peix et al., 2001a).

In this study, nine (50%) of the 18 evaluated strains had the ability to solubilise P-Al. All of these strains had low solubilisation ability, with SIs ranging from 1.00 to 1.52, and were late solubilisers (Table 3). These results differ from previously published results, showing that a greater number of unidentified nodulating bacteria, from Amazonian soils, were able to solubilise P-Al than P-Ca (Hara and Oliveira, 2004). However, they found that bacteria with a strong solubilisation ability were infrequent, among 88 bacteria studied, 39% solubilised P-Ca, but only one did so with a high solubilisation rate (SI > 4), and 67% had a weak ability to solubilise P-Al. In another study, also using isolates from Amazonian soils, only strains with a weak ability to solubilise P-Ca and P-Al were found (Hara and Oliveira, 2005).

The *Burkholderia fungorum* strains UFLA 04-122, UFLA 04-217, UFLA 04-155, UFLA 04-228, UFLA 04-226 and UFLA 04-229 and the *Burkholderia* sp. strain UFLA 04-21 solubilised both P-Ca and P-Al (Tables 2 and 3).

The ability to promote plant growth by phosphate solubilisation (Chabot et al., 1996; Peix et al., 2001a, b) shows how much is promising the use of bacteria as inoculants in agricultural crops for both legumes and non-legumes. There is a need to further study the use of symbiotic and free-living diazotrophic bacteria as phosphate solubilizers, focusing on methods to select isolates that are not only capable of fixating atmospheric nitrogen but also solubilising insoluble inorganic phosphates, among other processes.

Production of the growth hormones auxin

All of the strains were able to synthesise IAA when L-tryptophan was added to the culture media (Figure 1). Twelve of the experimental strains and the controls synthesised IAA in the absence of L-tryptophan. The strains UFLA 04-217, UFLA 04-226, UFLA 04-234 (*Burkholderia fungorum*), UFLA 04-173 (*Rhizobium* sp.), UFLA 02-68 (*R. etli* bv. *mimosae*), and UFLA 02-100 (*R. etli*) did not produce IAA in the absence of L-tryptophan. Nodulating bacteria synthesise IAA through three pathways, indole-3-acetamide (IAM), indole-3-pyruvate (IpyA) and tryptamine (TAM) (Patten and Glick, 1996; Theunis et al., 2004), of which the IpyA pathway is independent of L-tryptophan, whereas the remaining two pathways use the amino acid as a precursor. The *Rhizobium* strains that did not produce IAA in the absence of L-tryptophan probably do not possess an active indole-3-pyruvate (IpyA) pathway in these growth conditions.

In this study, IAA production varied from 0.00 to 12.59 µg mL^{-1} in media that was not supplemented with L-tryptophan, and the maximum production was reached by the strain UFLA 04-229 (*Burkholderia fungorum*), which did not significantly differ from the positive control BR 11001^{T} (*Azospirillum brasilense*). Striking differences were observed when the media was supplemented with L-tryptophan. Under these conditions, the *Burkholderia fungorum* strains UFLA 04-217, UFLA 04-122, UFLA 04-234 and UFLA 04-229 showed increased IAA production.

In soil, the exposure of roots to exogenous bacterial IAA can affect plant growth in several ways, from pathogenesis and growth inhibition to phytostimulation (Spaepen et al., 2007). IAA, as well as other hormones, stimulate plant growth within a narrow concentration range; outside this beneficial range, lower concentrations are inefficient and higher concentrations become toxic (Biswas et al., 2000). Studies have found that *Rhizobium leguminosarum* producing 171.17 µg mL^{-1} of IAA impaired the development of lettuce seedlings. On the other hand, isolates of *Bradyrhizobium* sp. producing 1.2 to 3.3 µg mL^{-1} of IAA increased seedling vigour in comparison to an uninoculated treatment (Schlindwein et al., 2008). In our study, the values observed for nodulating and endophytic bacteria were not as high as those cited by other authors (Schlindwein et al., 2008; Kumar and Ram, 2012) indicating were that these strains may act as phytostimulators. However, these authors utilized culture media with different composition.

The use of phenol as the sole carbon source

The strains UFLA 02-86 (*R. etli* bv. *phaseoli*), UFLA 02-68 (*R. etli* bv. *mimosae*) and UFLA 02-100 (*Rhizobium etli*) grew in all of the concentrations analysed, and UFLA 04-234 (*Burkholderia fugorum*) grew in concentrations lower than 5 mM (Table 4). Another *Rhizobium* strain and some nitrogen-fixing *Burkholderia tropicalis* strains have been also reported to degrade phenolic compounds (Wei et al., 2008; Cobos-Vasconcelos et al., 2006).

Bacterial resistance to antibiotics

All of the strains were resistant to at least three of the antibiotics tested (Table 5). Four strains, including the *Rhizobium* sp. UFLA 04-195, which was highly efficient in the BNF in symbiosis with the bean plant (Ferreira et al., 2012), were resistant to ten of the 12 antibiotics tested. This gives them a selective advantage over other microorganisms and may make these strains more competitive in soil, which is an indispensable characteristic for the establishment of symbiosis and may contribute, at least in part, to its success as an inoculant.

Rhizobium strains were resistant to 75% of the antibiotics tested, meanwhile *Burkholderia* strains were

Table 3. Onset and solubilisation index (S.I) of aluminium phosphate by *Rhizobium* and *Burkholderia* strains grown in GES medium.

Strains (*Rhizobium* spp.)	On. Sol.* (days)	S.I. (mm)		Strains (*Burkholderia* spp.)	On. Sol. (days)	S.I (mm)	
		Initial	End			Initial	End
CIAT 899^T	GNFH	-	-	UFLA 04-122	6	1.00	1.15
UFLA 02-68	GNFH	-	-	UFLA 04-226	6	1.00	1.39
UFLA 02-86	GNFH	-	-	UFLA 04-217	6	1.40	1.52
UFLA 02-100	GNFH	-	-	UFLA 04-155	6	1.03	1.15
UFLA 04-173	GNFH	-	-	UFLA 04-228	6	1.27	1.29
UFLA 04-195	6	1.21**	1.29	UFLA 04-229	6	1.36	1.24
UFLA 04-202	6	1.32	1.41	UFLA 04-231	NG	-	-
				UFLA 04-232	GNFH	-	-
				UFLA 04-233	NG	-	-
				UFLA 04-234	NG	-	-
				UFLA 04-21	9	1.30	1.32

*Onset of solubilisation. **S.I. = Halo diameter (mm) / colony diameter (mm). NG: No growth. GNFH: Grew but did not a form halo by the 18th day.

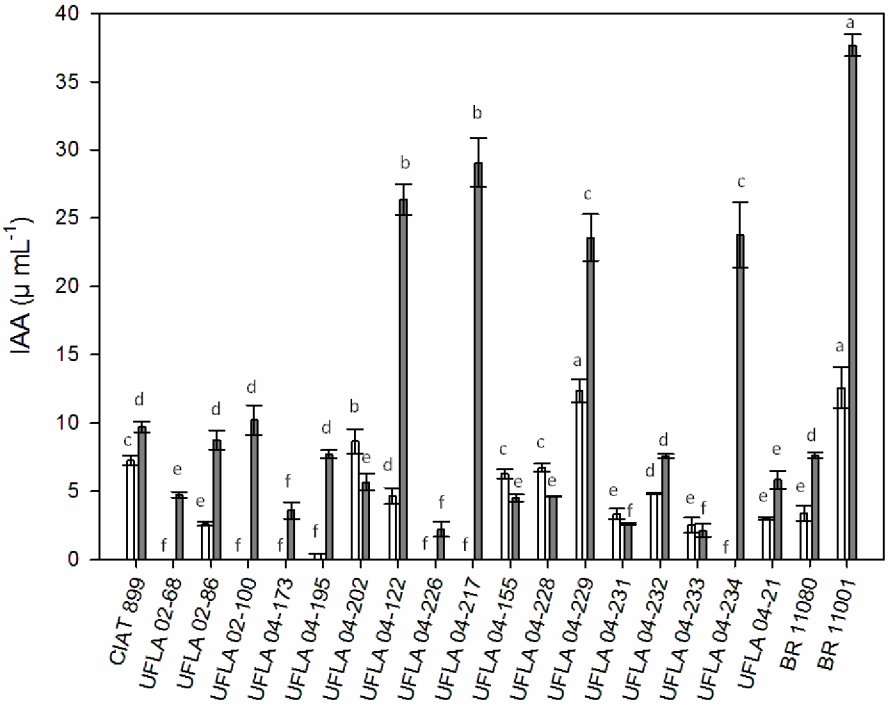

Figure 1. Production of indole-3-acetic acid (IAA) by *Rhizobium* and *Burkholderia* strains grown in Dygs medium in either the presence of 100 mg L^{-1} of L-tryptophan (grey bars) or in the absence of the amino acid (white bars). Values followed by the same letter under the same treatment were not significantly different according to the Scott-Knott test at 5% probability.

resistant to only 36%. *Rhizobium* and *Burkholderia* are both resistant to Chloramphenicol, Amoxicillin, and largely resistant to Ampicillin Vancomycin. Ahmad et al. (2001) also found a greater number of isolates resistant for chloramphenicol followed by amoxicillin and ampicillin. However, these isolates belonged to *Bradyrhizobium* genus.

All of the strains were sensitive to kanamycin and gentamicin. The *R. etli* bv. *phaseoli*, *R. etli* bv. *mimosae* and *Rhizobium etli* strains were resistant to rifamycin. Rhizobia isolated from leguminous tree species from Uruguay showed a high sensitivity to rifamycin, among other antibiotics (Frioni et al., 2001).

These results indicate that, in general, nitrogen-fixing

Table 4. Ability of *Rhizobium* and *Burkholderia* strains to grow in media containing different phenol concentrations as the sole carbon source.

Strains	Phenol (mM)						Strains	Phenol (mM)					
(*Rhizobium* spp.)	1	2	5	6	8	10	(*Burkholderia* spp.)	1	2	5	6	8	10
CIAT 899^T	-	-	-	-	-	-	UFLA 04-122	-	-	-	-	-	-
UFLA 02-68	+	+	+	+	+	+	UFLA 04-226	-	-	-	-	-	-
UFLA 02-86	+	+	+	+	+	+	UFLA 04-217	-	-	-	-	-	-
UFLA 02-100	+	+	+	+	+	+	UFLA 04-155	-	-	-	-	-	-
UFLA 04-173	-	-	-	-	-	-	UFLA 04-228	-	-	-	-	-	-
UFLA 04-195	-	-	-	-	-	-	UFLA 04-229	-	-	-	-	-	-
UFLA 04-202	-	-	-	-	-	-	UFLA 04-231	-	-	-	-	-	-
							UFLA 04-232	-	-	-	-	-	-
							UFLA 04-233	-	-	-	-	-	-
							UFLA 04-234	+	+	+	-	-	-
							UFLA 04-21	-	-	-	-	-	-

strains that are symbiotic with the common bean are well adapted to overcome the amensalistic relationships found among the antibiotic-producing soil populations (Tables 1 and 5) , as 8 out of the 12 antibiotics tested are produced by microorganisms.

Relationships between the different plant growth-promoting processes

The *Rhizobium* spp. strains UFLA 04-195, UFLA 04-202 and UFLA 04-173, the *R. etli* bv. *mimosae* strain UFLA 02-68, the *R. etli* bv. *phaseoli* strain UFLA 02-86 and the *Rhizobium etli*_strain UFLA 02-100, which are highly efficient at fixing nitrogen in the common bean (Ferreira et al., 2009, 2012; Soares et al., 2006), as well as the CIAT 899^T strain, were able to perform other processes that promote plant growth.

The strains UFLA 04-195 and UFLA 04-202 solubilised aluminium phosphate and synthesised IAA independently of the addition of the precursor amino acid (L-tryptophan), UFLA 04-173 synthesises IAA in the presence of L-tryptophan and CIAT 899^T solubilises calcium phosphate and produces IAA regardless of the addition of L-tryptophan. UFLA 04-195, UFLA 04-202, UFLA 04-173 and CIAT 899^T also fix nitrogen when free-living.

The strains UFLA 02-86, UFLA 02-68 and UFLA 02-100 (*Rhizobium etli*) solubilise calcium phosphate; the first strain also synthesises IAA regardless of the presence of L-tryptophan and the last two synthesise IAA in the presence of the amino acid. These three strains also potentially degrade phenol.

Four of the strains that are highly efficient at BNF when in symbiosis with the common bean (UFLA 04-195, UFLA 02-86, UFLA 02-68 and UFLA 02-100) were resistant to 83% of the antibiotics studied.

It was also found that the *Rhizobium* spp. UFLA 04-195, UFLA 04-202 and UFLA 04-173 and the *Rhizobium tropici* strain CIAT 899^T had a faster growth rate in pH 5.0 than in pH 6.0 or 6.9, when cultivated in media with a pH of 5.0, thus demonstrating acid tolerance. These strains were also tolerant to aluminium levels up to 0.5 mmol L^{-1}, and the strains UFLA 04-202 and CIAT 899^T were tolerant up to 1 mmol L^{-1} (Ferreira et al., 2012). The strain UFLA 04-202, which is highly efficient at BNF during symbiosis with the common bean, performed the most plant growth-promoting processes.

The three strains that are highly efficient in the solubilisation of calcium phosphate (*Burkholderia fungorum* strains UFLA 04-155, UFLA 04-233 and UFLA 04-232) were also able to synthesise IAA, both in the presence and absence of L-tryptophan; UFLA 04-155 also solubilises aluminium phosphate. However, these three strains were only resistant to 33% of the antibiotics studied, thus indicating a low resistance to various antibiotics.

Free-living diazotrophic strains had a stronger ability to solubilise calcium phosphate. The highest level of IAA synthesis in the absence of L-tryptophan was detected in UFLA 04-229 (*Burkholderia fungorum*), but this strain has medium efficiency at symbiotic nitrogen fixation with the common bean. The free-living diazotrophic strain UFLA 04-217 (*Burkholderia fungorum*) showed the highest level of IAA synthesis in the presence of L-tryptophan. Bacteria that have symbiotic efficiency with the common bean plants (UFLA 02-68, UFLA02-86 and UFLA 02-100) are also able to degrade pollutants such as phenol.

Effects on *Phaseolus vulgaris* nodulation, growth and nutrient accumulation

Additionally to the diverse strategies to increase N_2-fixation by CIAT 899^T (*Rhizobium tropici*) in common beans (Fernández-Luqueño et al., 2012), the co-inoculation of this strain with plant growth promoting

Table 5. Resistance of *Rhizobium* and *Burkholderia* strains to different antibiotics.

	Strains	AZI	STR	ERY	AMP	CHL	RFM	KAN	NAL	CLA	AMO	GEN	VAN	Σ(R)
		---------- Inhibition Halo (mm)----------												
(*Rhizobium* spp.)	CIAT 899^T	R(a)	R(a)	R(a)	R(a)	R(a)	16.63(c)	16.90(c)	R(a)	R(a)	R(a)	10.40(b)	8.91(b)	8
	UFLA 02-68	R(a)	R(a)	R(a)	R(a)	R(a)	R(a)	11.66(c)	R(a)	R(a)	R(a)	8.43(b)	R(a)	10
	UFLA 02-86	R(a)	R(a)	R(a)	R(a)	R(a)	R(a)	13.32(c)	R(a)	R(a)	R(a)	8.26(b)	R(a)	10
	UFLA 02-100	R(a)	R(a)	R(a)	R(a)	R(a)	R(a)	13.92(c)	R(a)	R(a)	R(a)	8.48(b)	R(a)	10
	UFLA 04-173	7.07(b)	R(a)	R(a)	R(a)	R(a)	14.47(d)	22.53(e)	R(a)	R(a)	R(a)	10.92(a)	9.53(a)	7
	UFLA 04-195	R(a)	R(a)	R(a)	R(a)	R(a)	15.00(c)	23.56(d)	R(a)	R(a)	R(a)	11.75(b)	10.86(b)	10
	UFLA 04-202	R(a)	R(a)	R(a)	R(a)	R(a)	16.43(c)	21.48(d)	R(a)	R(a)	R(a)	11.17(b)	11.91(b)	8
(*Burkholderia* spp.)	UFLA 04-122	27.26(g)	18.26(d)	12.48(c)	R(a)	R(a)	20.74(e)	23.04(f)	31.73(i)	15.10(d)	R(a)	9,11(b)	R(a)	4
	UFLA 04-226	26.84(e)	17.79(c)	12.46(b)	R(a)	R(a)	21.15(d)	21.86(d)	33.41(f)	16.65(c)	R(a)	12.08(b)	R(a)	4
	UFLA 04-217	25.79(e)	R(a)	R(a)	R(a)	R(a)	13.85(c)	21.17(d)	30.72(f)	14.36(c)	R(a)	11.41(b)	R(a)	6
	UFLA 04-155	28.63(f)	21.10(d)	13.67(c)	R(a)	R(a)	21.47(d)	26.78(e)	31.30(g)	22.67(d)	R(a)	11.90(b)	R(a)	4
	UFLA 04-228	25.85(f)	18.79(e)	14.75(d)	7.71(b)	R(a)	18.82(e)	24.33(f)	30.47(g)	15.66(d)	R(a)	10.94(c)	R(a)	3
	UFLA 04-229	R(a)	9.00(b)	R(a)	R(a)	R(a)	17.57(c)	20.82(d)	9.19(b)	R(a)	R(a)	8.56(b)	R(a)	7
	UFLA 04-231	24.26(f)	13.79(c)	13.93(c)	R(a)	R(a)	18.58(d)	22.27(e)	33.02(g)	15.30(c)	R(a)	10.75(b)	R(a)	4
	UFLA 04-232	25.43(h)	17.75(e)	14.79(c)	R(a)	R(a)	19.54(f)	23.77(g)	34.01(i)	16.37(d)	R(a)	9.95(b)	R(a)	4
	UFLA 04-233	24.85(f)	19.15(d)	15.20(c)	R(a)	R(a)	22.67(e)	25.25(f)	30.90(g)	15.20(c)	R(a)	9.81(b)	R(a)	4
	UFLA 04-234	28.30(g)	18.82(d)	14.50(c)	R(a)	R(a)	22.26(e)	24.62(f)	32.72(h)	15.85(c)	R(a)	11.32(b)	R(a)	4
	UFLA 04-21	24.82(e)	20.68(d)	14.50(c)	R(a)	R(a)	20.57(d)	27.62(f)	31.84(g)	15.95(c)	R(a)	10.04(b)	R(a)	4
	Σ(R)	7	8	9	17	18	3	0	7	8	18	0	14	

Antibiotic resistant bacteria (absence of halo = 0.00 =R), azithromycin (AZI), streptomycin (STR), erythromycin (ERY), ampicillin (AMP), chloramphenicol (CHL), rifamycin (RFM), kanamycin (KAN), nalidixic acid (NAL), clarithromycin (CLA), amoxicillin (AMO), gentamicin (GEN) and vancomycin (VAN). In each line, values followed by the same letter are not significantly different according to the Scott-Knott test at 5% probability.

rhizobacteria arises as a promising biotechnology (Camacho et al., 2001; Figueiredo et al., 2008). Our results shows that co-inoculation of CIAT 899^T with UFLA 04-155 (*Burkholderia fungorum*) enhanced significantly nodule number, shoot dry matter and P accumulation of beans in relation to inoculation with CIAT 899^T alone (Figure 2). Co-inoculation of CIAT 899^T with BR11001 also increased dry matter of nodules however it had no effect on shoot dry matter but enhanced P accumulation. The other strains had detrimental effects on nodule and shoot dry matter and nutrient contents when co-inoculated with CIAT 899^T. The single inoculation of all strains (except CIAT 899^T) have no effect on shoot dry matter and N and P accumulation, probably because the experimental conditions (N-limitation) were suitable for the expression of nitrogen fixation and they were not able to supply N for plants. Few reports were found about the co-inoculation of CIAT 899^T with other PGPB species, in common beans and, they reported positive effects with *Paenibacillus polymyxa* strain Loutit on nodulation and nitrogen fixation in pots with soil (Figueiredo et al., 2008) and, with *Bacillus* sp. strain CECT 450 on nodule number and dry matter in both axenic and field conditions (Camacho et al., 2001).

The ability to perform other biotechnological processes that contribute to plant growth, in addition to adapting to various types of stress, adds considerable value to free-living and/or symbiotic diazotrophic bacteria. Our results showed

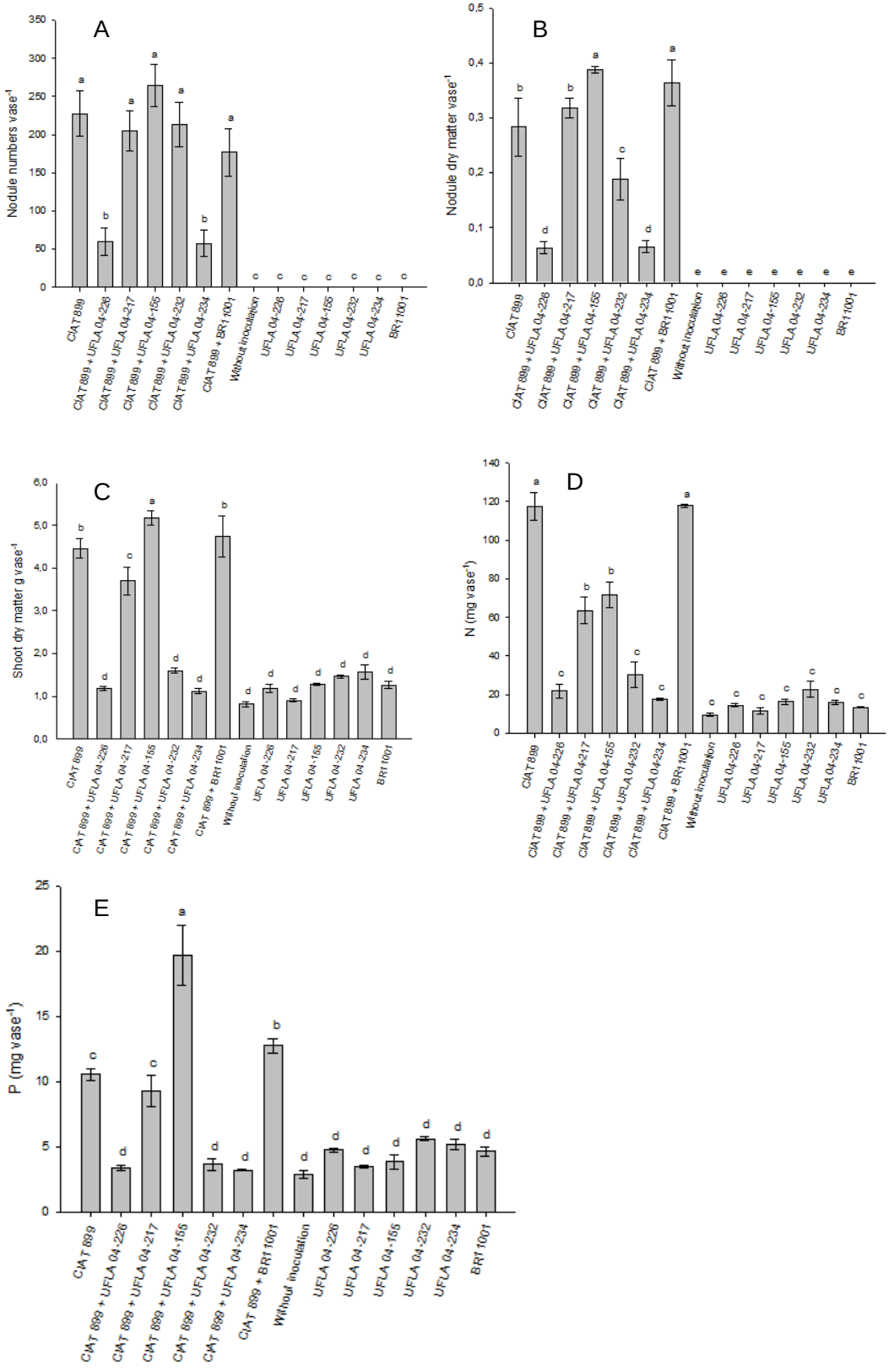

Figure 2. Effects of co-inoculation with *Rhizobium tropici* CIAT 899[T] and different plant growth promoting Bacteria (PGPB) as well as inoculation with CIAT 899[T] and PGPB alone on nodule numbers (A), nodule dry matter (B), shoot dry matter (C), nitrogen (N) and phosphorus (P) accumulation in the shoot of common bean (*Phaseolus vulgaris* L.) (D and E, respectively).

how versatile diazotrophic bacteria are, what must play an important role for environmental sustainability. However, the management of the combined actions of these processes on plant growth in the complex, heterogeneous and dynamic conditions of an edaphic system in the field, should be further evaluated.

ACKNOWLEDGEMENTS

We thank Capes and CNPq for student fellowships, CNPq for a research fellowship and grant, and project GEF/UNEP-GF2715-02 (CSM-BGBD) for financial support. This publication presents part of the findings of the international project “Conservation and Management of Below-Ground Biodiversity” implemented in seven tropical countries—Brazil, Cote d’Ivoire, India, Indonesia, Kenya, Mexico, and Uganda. This project is coordinated by the Tropical Soil Biology and Fertility Institute of CIAT (TSBF-CIAT with co-financing from the Global Environmental Facility (GEF), and implementation support from the United Nations Environment Program (UNEP).

REFERENCES

Ahmad I, Hayat S, Ahmad A, Inam A, Samiullah (2001). Metal and antibiotic resistance traits in *Bradyrhizobium* sp. (cajanus) isolated from soil receiving oil refinery wastewater. World J. Microbiol. Biotechnol. 17:379-384.

Arfaoui A, Sifi B, Boudabous A, El Hadrami I, Chérif M (2006). Identification of *Rhizobium* isolates possessing antagonistic activity against *fusarium oxysporum* f.sp. ciceris, the causal agent of *fusarium* wilt of chickpea. J. Plant Pathol. 88:67-75.

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57-67.

Asghar HN, Zahir ZA, Arshad M, Khaliq A (2002). Relationship between in vitro production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. Biol. Fertil. Soils 35:231-237.

Berraquero FR, Baya AM, Cormenzana AR (1976). Estabelacimiento de indices para el estudio de la solubilizacion de fosfatos por bacterias del suelo. Ars Pharm. 17:399-406.

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

Boiero L, Perrig D, Masciarelli O, Penna C, Cassán F, Luna V (2007). Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Appl. Microbiol. Biotechnol. 74:874-880.

Buonassisi AJ, Copeman RJ, Pepin HS, Eaton GW (1986). Effect of *Rhizobium* spp. on *Fusarium* f.sp*. phaseoli.* Can. J. Plant Pathol. 8(2):140-146.

Camacho M, Santamaria C, Temprano F, Rodriguez-Navarro DN, Daza A (2001). Co-inoculation with *Bacillus* sp. CECT 450 improves nodulation in *Phaseolus vulgaris* L. Can. J. Microbiol. 47:1058-1062.

Chabot R, Beauchamp CJ, Kloepper JW, Antoun H (1996). Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. Plant Soil 184:311-321.

Chao LW (1990). Antagonistic activity of *Rhizobium* spp. against beneficial and plant pathogenic fungi. Lett. Appl. Microbiol. 10:213-215.

Cobos-Vasconcelos D De Los, Santoyo-Tepole F, Juárez-Ramírez C, Ruiz-Ordaz N, Galíndez-Mayer CJJ (2006). Cometabolic degradation of chlorophenols by a strain of *Burkholderia* in fed-batch culture. Enzyme Microb. Technol. 40:57-60.

Collavino MM, Sansberro PA, Mroginski LA, Aguilar OM (2010). Comparison of *in vitro* solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biol. Fertil. Soils 46:727-738.

Dreyfus B, Garcia JL, Gillis M (1988). Characterization of *Azorhizobium caulinodans* gen. nov, sp. nov, a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*. Int. J. Syst. Bacterial. 38:89-98.

Dreyfus BL, Elmerich C, Dommergues, YR (1983). Free-living *Rhizobium* strain able to grow on N_2 as the sole nitrogen source. Appl. Environ. Microbiol. 45:711-713.

Elliott GN, Chen WM, Chou JH, Wang HC, Sheu SY, Perin L, Reis VM, Moulin L, Simon MF, Sutherland JM, Bessi R, de Faria SM, Trinick MJ, Prescott AR, Sprent JI, James EK (2007). *Burkholderia phymatum* is a highly effective nitrogen-fixing symbiont of *Mimosa* spp. and fixes nitrogen ex planta. New Phytol. 173:168–180.

Fernández-Luqueño F, Cabrera-Lazaro G, Méndez-Bautista J, López-Valdez F, Dendooven L (2012). Symbiotic nitrogen fixation in nodules from ten common bean cultivars as a reliable estimator of yield during the early stages. Afr. J. Agric. Res. 7(9):1400-1409.

Ferreira DF (2008). SISVAR: a program for statistical analysis and teaching. Rev. Sympos. 6:36-41.

Ferreira AN, Arf O, Carvalho MAC, Araújo RS, Sá ME, Buzetti S (2000). *Rhizobium Tropici* strains for inoculation of the common bean. Sci. Agric. 57(3):507-512.

Ferreira PAA, Silva MAP, Cassetari A, Ruffini M, Moreira FMS, Andrade MJB (2009). Inoculation with rhizobium strains in beans crop. Ciênc. Rural. 39(7):2210-2212.

Ferreira PAA, Bomfeti CA, Soares BL, Moreira FMS (2012). Efficient nitrogen-fixing *Rhizobium* strains isolated from Amazonian soils are highly tolerant to acidity and aluminium. World J. Microbiol. Biotechnol. 28(5):1947-1959.

Figueiredo MVB, Martinez CR, Burity HA, Chanway CP (2008). Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). World J. Microbiol. Biotechnol. 24:1187-1193.

Fred EB, Waksman SA (1928). Laboratory Manual of General Microbiology. McGraw-Hill Book, New York.

Frioni L, Rodriguez A, Meerhoff M (2001). Differentiation of rhizobia isolated from native legume trees in Uruguay. Appl. Soil Ecol. 16:275-282.

Graham PH, Draeger K, Ferrey ML, Conroy MJ, Hammer BE, Martinez E, Aarons SR, Quinto C (1994). Acid pH tolerance in strains of *Rhizobium* and *Bradyrhizobium*, and initial studies on the basis for acid tolerance of *Rhizobium tropici* UMR1899. Can. J. Microbiol. 40:198-207.

Hameed S, Yasmin S, Malik KA, Zafar Y Hafeez FY (2004). *Rhizobium*, *Bradyrhizobium* and *Agrobacterium* strain isolated from cultivated legumes. Biol. Fertil. Soils 39:179-185.

Hara FAS, Oliveira LA (2004). Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos e álicos de Presidente Figueiredo, Amazonas. Acta Amazon. 34:343-357.

Hara FAS, Oliveira LA (2005). Características fisiológicas e ecológicas de isolados de rizóbios oriundos de solos ácidos de Iranduba, Amazonas. Pesq. Agropec. Bras. 40:667-672.

Hoagland DR, Arnon DI (1950). The water culture method for growing plants without soil. Berkeley: California Agricultural Experiment Station. p. 32.

Karlidag H, Esitken A, Turam M, Sahin F (2007). Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of Apple. Sci. Hortic. 114(1):16-20.

Kumar R, Ram MR (2012). Production of indole acetic acid by *Rhizobium* isolates from *Vigna trilobata* (L) Verdc. P. Afr. J. Microbiol. Res. 6(27):5536-5541.

Lima AS, Nóbrega RSA, Barberi A, Silva K, Ferreira DF, Moreira FMS (2009). Nitrogen-fixing bacteria communities occurring in soils under different uses in the Western Amazon Regionas indicated by nodulation of siratro (*Macroptilium atropurpureum*). Plant Soil 319:

127-145.
Malavolta E, Vitti GC, Oliveira AS (1997). Avaliação do estado nutricional de plantas: princípios e aplicações. 2.edn. Potafos.
Mantelin S, Touraine B (2004). Plant growth-promoting bactéria and nitrate availability: impacts on root development and nitrate uptake. J. Exp. Bot. 55:27-34.
Monteiro JM, Vollú RE, Coelho MRR, Alvino CS, Blank AF, Seldin L (2009). Comparison of the Bacterial Community and Characterization of Plant Growth Promoting Rhizobacteria from different genotypes of *Chrysopogon zizanioides* (L.) Roberty (Vetiver) Rhizospheres. J. Microbiol. 47:363-370.
Moreira FMS, Cruz L, Faria SM, Marsh T, Martinez-Romero E, Pedrosa FO, Pitard RM, Young JPW (2006). *Azorhizobium doebereiner* sp. Nov. Microsymbiont of *Sesbania virgata* (Caz.) Pers. Syst. Appl. Microbiol. 29:197-206.
Ogut M, Er F, Kandemir N (2010). Phosphate solubilization potentials of soil *Acinetobacter* strains. Biol. Fertil. Soils 46:707-715.
Patten CL, Glick BR (1996). Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol. 42:207-20.
Peix A, Rivas-Boyero AA, Mateos PF, Rodriguez-Barrueco C, Martínez-Molina E, Velazquez E (2001a). Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. Soil Biol. Biochem. 33(1):103-110.
Peix A, Mateos PF, Rodriguez-Barrueco C, Martinez-Molina E, Velazquez E (2001b). Growth promotion of common bean (*Pasheolus vulgaris* L.) by a strain of *Burkholderia cepacia* under growth chamber conditions. Soil Biol. Biochem. 33(14):1927-1935.
Pereira EG, Lacerda AM, Lima AS, Moreira FMS, Carvalho D, Siqueira JO (1998). Genotypic, Phenotypic and Symbiotic Diversity Amongst Rhizobia Isolates from *Phaseolus vulgaris* L. Growing in the Amazon Region. Biol. Fertil. Trop. Soils 38:86-87.
Radwan T, Mohamed ZK, Reis VM (2002). Production of indole-3-acetic acid by different strains of *Azospirillum* and *Herbaspirillum* spp. Symbiosis 32(1):39-54.
Ramalho MAP, Abreu AFB, Carneiro JES, Gonçalves FMA, Santos JB, Peloso MJ (2002). O 'Talismã' de sua lavoura de feijoeiro. Santo Antônio de Goiás: Embrapa Arroz e Feijão. 4. (Embrapa Arroz e Feijão. Comunicado Técnico, 36).
Sarwar M, Kremer RJ (1995). Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. Plant Soil 172:261-269.
Sarruge JR, Haag HP (1979). Análises químicas em plantas. Piracicaba: ESALQ/USP.
Schlindwein G, Vargas LK, Lisboa BB, Azambuja AC, Granada CE, Gabiatti NC (2008). Influence of rhizobial inoculation on seedling vigor and germination of lettuce. Ciênc. Rural 38:658-664.
Soares ALL, Ferreira PAA, Pereira JPAR, Vale HMM, Lima AS, Andrade MJB, Moreira FMS (2006). Agronomic efficiency of selected rhizobia strains and diversity of native nodulating populations in Perdões (MG - BRAZIL). II – BEANS. Rev. Bras. Cienc. Solo 30:803-811.
Spaepen S, Vanderleyden J, Remans R (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol. Rev. 31:425-448.
Sylvester-Bradley R, Asakawa N, La Torraca S, Magalhaes FMM, Oliveira LA, Pereira RM (1982). Levantamento quantitativo de microrganismos solubilizadores de fosfatos na rizosfera de gramíneas e leguminosas forrageiras na Amazônia. Acta Amazon. 12:15-22.
Tarrand JJ, Krieg NR, Döbereiner J (1978). A taxonomic study of the *Spirillum lipoferum* group with description of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. Can. J. Microbiol. 24:967-980.
Theunis M, Kobayashi H, Broughton WJ, Prinsen E (2004). Flavonoids, NodD1, NodD2, and nod-box NB15 modulate expression of the y4wEFG locus that is required for indole-3- acetic acid synthesis in *Rhizobium* sp. strain NGR234. Mol. Plant. Microbe 17:1153-1161.
Wei G, Yu J, Zhu Y, Chen W, Wang L (2008). Characterization of phenol degradation by *Rhizobium* sp. CCNWTB 701 isolated from *Astragalus chrysopterus* in mining tailing region. J. Hazard. Mater. 151: 111-117.