Pak. J. Bot., 47(4): 1553-1558, 2015.

ISOLATION, IDENTIFICATION OF ANTAGONISTIC RHIZOBACTERIAL STRAINS OBTAINED FROM CHICKPEA (*CICER ARIETINUM* L.) FIELD AND THEIR *IN-VITRO* EVALUATION AGAINST FUNGAL ROOT PATHOGENS

SHAZIA SHAHZAMAN[1*], M. INAM-UL-HAQ[1], TARIQ MUKHTAR[1] AND M. NAEEM[2]

[1]*Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan*
[2]*Department of Entomology, PMAS-Arid Agriculture University Rawalpindi, Pakistan*
[*]*Corresponding author e-mail: shahzia_abbasi207@yahoo.com*

Abstract

Plant growth promoting rhizobacteria (PGPR), are associated with roots, found in the rhizosphere and can directly or indirectly enhance the plant growth. In this study soil was collected from rhizosphere of chickpea fields of different areas of Rawalpindi division of Pakistan. PGPR were isolated, screened and characterized. Eight isolates of rhizobacteria (RHA, RPG, RFJ, RC, RTR, RT and RK) were isolated from Rawalpindi division and were characterized. The antagonistic activity of these PGPR isolates against root infecting fungi (*Fusarium oxysporum* and *Verticillium* spp.,) was done and production of indole acetic acid (IAA), siderophore and P-solubilization was evaluated. The isolates RHA, RPG, RFJ, RC, RRD and RT were found to be positive in producing siderophore, IAA and P-solubilization. Furthermore, most of the isolates showed antifungal activity against *Fusarium oxysporum*, and *Verticillium* spp. The rhizobacterial isolates RHA, RPG, RFJ, RC, RRD, RTR, RT and RK were used as bio-inoculants that might be beneficial for chickpea cultivation as the rhizobacterial isolates possessed the plant growth promoting characters i.e. siderophore, IAA production, phosphate solubilization. In i*n vitro* tests, *Pseudomonas* sp. and *Bacillus* spp. inhibited the mycelial growth of the fungal root pathogens. The isolates (RHA and RPG) also significantly increased (60-70%) seed germination, shoot length, root length of the chickpea. The incidence of fungi was reduced by the colonization of RHA and RPG which enhanced the seedling vigor index and seed germination. The observations revealed that isolates RHA and RPG is quite effective to reduce the fungal root infection in greenhouse, and also increases seed yields significantly. These rhizobacterial isolates appear to be efficient yield increasing as well as effective biocontrol agent against fungal root pathogen.

Key words: Rhizobacteria, Biocontrol, Vigor index, Biopesticide.

Introduction

Bacteria, which live attached to plants, have the potential to promote plant growth (Compant *et al*., 2010; George *et al*., 2012; Beneduzi *et al*., 2013). These micro-organisms have attracted attention because of the need to reduce the use of chemicals, especially when considering the context of sustainable agriculture and environmental protection (Vale *et al*., 2010). One of the strategies is to exploit the benefits that several microorganisms may give to plants when added as inoculants (Lucy *et al*., 2004). Bacteria promoting plant growth can act directly, through one or more mechanisms, including biological nitrogen fixation (Ashraf *et al*., 2011; Verma *et al*., 2013), hormones production such as zeatin, gibberellins and auxins (Cassan *et al*., 2009), phosphate solubilization (Rodriguez *et al*., 2004; Krey *et al*., 2013), or act indirectly by means of biological control of pathogens (Wang *et al*., 2009). In addition, some studies point out to the preferred action with antagonistic microorganisms or adapted isolates, due to easier colonization and the lower risk to introduce exogenous organisms (Enebak *et al*., 1998; Khalid *et al*., 2004). Several studies have shown the positive effects of antagonistic bacteria inoculation in plants, e.g. sugarcane (*Saccharum* spp.), leading to increased contribution of biological nitrogen fixation, promote root development, increased biomass and productivity (Oliveira *et al*., 2003); soybean (*Glycine max* (L.) Merr.), with bacteria capable to inhibit growth and sporulation of pathogenic fungi (Assumpcao *et al*., 2009); tomato (*Lycopersicum esculentum* L.), with bacteria increasing plant height, leaf area, leaf number, together with fresh and dry plant weight (Barretti *et al*., 2008). Benefits provided by the inoculation of chickpea (*Cicer arietinum L*.) with selected strains have also been described, including the promotion of growth due to the increased availability of nutrients, provided by inoculation with *B. subtilis* in seeds (Canbolat *et al*., 2006).

In addition, there are reports of strains of *Azospirillum brasilens*e increasing chickpea yield by 24% to 30%, compared to the non-inoculated control (Hungria *et al*., 2010). Concomitantly, the plant is supposed to influence the population structure of indigenous rhizobacteria as well as the population dynamics of introduced biocontrol agents (BCAs). Under certain conditions, many compounds present in the root exudates (sugar, amino acids, or organic acids) stimulate a positive chemotactic response in bacteria (Somers *et al*., 2004).

Smalla *et al*. (2001) demonstrated for the first time that roots of each model plant species are colonized by its own bacterial communities using cultivation-independent methods on three phylogenetically different and economically important crops – strawberry (*Fragaria ananassa* Duch.), potato (*Solanum tuberosum* L.), and oilseed rape (*Brassica napus* L.).

Material and Methods

Isolation of biological material: Rhizobacteria and Fungi were isolated from chickpea roots and rhizosphere samples. For the isolation, chickpea roots from field located in the Rawalpindi division were collected, surface-sterilized, aseptically cut, and then transferred to Petri dish plates containing semi-solid Potato Dextrose Agar (PDA) medium. The fungi *Fusarium oxysporum* and *Verticillium* spp. were isolated from infected roots. For rhizobacteria isolation, serial dilution of rhizospheric soil samples was done and 0.1 ml aliquot was placed on

Nutrient Agar medium. Bacterial isolates were identified with reference to Bergy's manual (David *et al*., 2005).

Morphological and biochemical characterization: Morphology of each colony of rhizobacteria from composite cultures was examined on NA plates. The colonies appearing different in morphology were streaked on separate NA plates. Characteristics of colonies such as shape, size, color and odor were recorded after 3 days of incubation. The isolates were purified by re-streaking.

PGPR characters

Phosphate solubilization: Pikovskya's plates were prepared and streaked in triplicates with the culture of these isolates and incubated for 7 days on 28°C. The appearance of halozone around the colony was observed which is characteristic of P-solubilization. Data was recorded after examining the plates (Pikovaskya, 1948).

Production of siderophore: For production of siderophore, rhizobacterial isolates were assayed on the chrome azurole S (CAS) agar as illustrated by Clark & Bavoil (1994). Chrome azurole S agar plates were prepared and inoculated by test organism and incubated for 5 days at 30°C. Yellow–orange halo formation around the colony was considered positive for siderophore production.

Production of indole acetic acid: Indole acetic acid (IAA) was qualitatively analyzed according to Kuss *et al*. (2007). Supplemented with 2 mg/mL of tryptophan, 2 mL of LB medium was inoculated with single bacterial colonies and incubated at 30°C for 72 h at 180 rpm. IAA was determined by mixing 1 mL of cell free culture filtrate (obtained by centrifugation at 10,000 rpm for 5 minutes) with 1 mL of Salkowski's reagent (Loper & Schroth, 1986) and incubated at room temperature for 30 min. and appearance of reddish to pinkish color of the liquid was considered as IAA production. The isolates were categorized as good producer (+++), medium producer (++), weak producer (+) and non-producer (-).

Antagonism assay against phytopathogenic fungi: For antifungal activities against *Fusarium oxysporum* and *Verticillium* spp. all the 8 isolates were assayed using PDA media. In a Petri plate having PDA, rhizobacterial isolates were streaked 3cm opposite to root pathogenic fungi, incubated for 4 to 7 days at 25±2°C. Distance between fungal colony and bacteria was considered as zone of inhibition and was measured in mm.

Germination of inoculated chickpea seeds: Rhizobacterial cell suspension was prepared at 30°C for 24 hours at 150 rpm in Nutrient Both (NB) medium. Then the suspension was centrifuged at 5000 xg and re-suspended in saline solution (0.85% NaCl). The concentration was adjusted to 2×10^8 CFU/mL, with spectrophotometer, to OD600. Prior to treatment with rhizobacteria, seeds of chickpea were surface disinfested with 1% NaOCl for 2 min, rinsed in distilled water, and dried on absorbent paper towels. The dried seeds were treated with rhizobacterial suspension and dried again before being plated on the media (Shiomi *et al*., 2008). To confirm the disinfection, samples of seeds were germinated in Petri dishes with PDA medium. The amount of rhizobacterial cells in the seed was measured by doing plate counts (CFU). Bacteria were extracted by using 0.85% saline solution agitated by vortexing (Silva & Reis, 2004). Seeds were germinated in an incubator at 28°C, on filter paper moistened with distilled water. Evaluation of length (cm) and volume (cm^3) of the root and of the hypocotyls were made after 4 and 7 days (Cassan *et al*., 2009).

Preparation of rhizobacterial inoculum: Rhizobacterial isolates developed in nutrient broth on rotary shaker at 180 rpm, 28°C for 24 h. Centrifuge the suspension at 5,000 rpm in 50ml sterile plastic tubes for 10 min. To make final concentration of 10^8 cfu/ml, a fresh pellets-suspension was prepared in distilled water (Idris *et al.,* 2007).

Pot experiment: A pot experiment was designed in university green house to estimate the efficacy of rhizobacteria as a bio-control agent against phytopathogenic fungi. Eight treatments with four replications were prepared. Five susceptible chickpea cultivars; Wanhaar, Bital-98, Desi, Channa-2008 and Balkasar were sown in a plastic pot of 7 cm diameter sterilized with (3% Sodium hypochlorite), filled 2/3 with sterile soil mixture and with peat (V/V). Soil mixture was sterilized three times at 120°C for 1h. Seeds were surface sterilized with 2% sodium hypochlorite for 3 min and rinsed three times with sterile distilled water and then dried.

To ensure uniform surface coating with test isolates, thorough soaking of the seeds was done in a bacterial suspension having 10^8 bacteria/ml. For wilt incidence, pots with natural soil were kept under observation for 12 weeks followed by post sowing comparison with the control pots. After 12 weeks of germination, parameters under observation were; seed germination (SG), Disease Incidence (DI), Shoot Length (SL), Root Length (RL), Fresh Weight of Shoot (FWS), Dry Weight of Shoot (DWS) Fresh Weight of Root (FWR) Dry Weight of Root (DWR).

Results

PGPR isolation: From the rhizosphere soils of chickpea fields, eight bacterial isolates were successfully isolated from different areas of Rawalpindi division (Table 1) nominated as RHA, RPG, RFJ, RC, RRD, RTR, RT and RK.

Table 1. Description of the PGPR isolates.

S. No.	Isolates	Rhizosphere soil location	Chickpea variety in the field
1.	RHA	Hassan abdal	Channa 2008
2.	RPG	Pindigheb	Balkasar, Bital 98
3.	RFJ	Fatehjang	Bital 98, Desi
4.	RC	Chakwal	Desi
5.	RRD	Doltala	Wanhaar, Channa 2008
6.	RTR	Tarnol	Channa 2008, Balkasar
7.	RT	Taxila	Desi, Bital 98
8.	RK	Kahuta	Wanhaar, Desi

Morphological characteristics of PGPR isolates: As revealed (Table 2), the morphological characteristics of PGPR isolates varied widely. Raised, round shaped colonies with smooth shiny surface and smooth margin were produced by all the isolates. Similar in being odorless, all isolates differed in color. Diameter of the colonies of isolates varied from 0.2 to 2 mm.

Microscopic observation of Rhizobacterial isolates: Microscopic observations were made to examine the characteristics of rhizobacterial isolates such as form, Gram reaction and motility (Table 3). Rod shapes were observed in four isolates while the rest of four isolates appeared spherical. All the isolates were found motile and Gram negative in reaction.

IAA production and phosphorus solubilization: PGPR isolates were investigated for the production of IAA and solubilization of phosphorus. As shown in Table 4, isolates RPG, RHA, RC, RTR, RT and RK has shown the production of IAA. Isolate RPG was found to be fine producer of IAA. On the converse, in comparison to the weak producer isolates RTR, RT and RK, RHA and RC were found to be a medium producer of IAA. On the other hand, all isolate had ability to solubilize the phosphorus (Table 4).

Production of siderophore: Siderophores were produced by 6 isolates (RHA, RPG, RFJ, RC, RRD, and RT) which is definite by the expansion of orange halos surrounding the colonies (Table 4).

Antagonism assay against phytopathogenic fungi: To determine the isolates with capability of inhibiting the growth of *Verticillium spp.* and *F. oxysporum*, antagonism assay was conducted. Several isolates were found to inhibit *F. oxysporum* and *Verticillium spp.* with varying level of inhibition. Among 8 PGPR isolates that significantly promoted plant growth of chickpea seedlings, there were only 3 isolates (RHA, RPG and RRD) that were strongly capable of inhibiting *F. oxysporum* and only 1 isolate (RTR) strongly inhibited *Verticillium* spp. (Table 5).

Table 2. Morphological characteristics of 2-day-old colony of Rhizobacterial isolates.

Isolates	Form	Size (mm)	Plane(surface)	Edges	Color
RHA	Round	0.9-1.1	Smoot Shiny	Smooth	Off white
RPG	Round	1-1.5	Smoot Shiny	Smooth	Cream
RFJ	Round	1-1.5	Smoot Shiny	Smooth	Yellowish
RC	Round	0.9-1.1	Smoot Shiny	Smooth	Yellowish
RRD	Round	1.8-2.0	Smoot Shiny	Smooth	Cream
RTR	Round	0.5-1.0	Smoot Shiny	Smooth	Off white
RT	Round	0.2-0.5	Smoot Shiny	Smooth	Yellowish
RK	Round	0.9-1.1	Smoot Shiny	Smooth	Cream

Table 3. Microscopic observation of rhizobacterial isolates collected from chickpea rhizosphere.

Isolates	Form	Gram reaction	Motility
RHA	Rod	-	+
RPG	Spherical	-	+
RFJ	Rod	-	+
RC	Rod	-	+
RRD	Spherical	-	+
RTR	Spherical	-	+
RT	Spherical	-	+
RK	Rod	-	+

Table 4. Production of IAA, siderophore and phosphorus solubilization by PGPR isolates.

Isolates	IAA Production	Phosphorus solubilization	Siderophore production
RHA	++	Positive	Positive
RPG	+++	Positive	Positive
RFJ	-	Positive	Positive
RC	++	Positive	Positive
RRD	-	Positive	Positive
RTR	+	Positive	Negative
RT	+	Positive	Positive
RK	+	Positive	Negative

(-) = No production; (+) = Weak producer; (++) = Medium producer; (+++) = Good producer

Table 5. Antagonism assay of PGPR isolates against phytopathogenic fungi.

Isolates	Inhibition (%) of phytopathogenic fungi by rhizobacterial isolates	
	F. oxysporum	*Verticillium* spp.
RHA	67	25
RPG	65	32
RFJ	38	18
RC	35	23
RRD	69	34
RTR	39	62
RT	37	20
RK	22	21

Small inhibition percentage (<30%); modest inhibition percentage (30% to 40%); Strong inhibition percentage (>40%)

Pots assay: These tests prove that susceptible cultivar reacts to fungal root pathogen with a high occurrence of *Fusarium* and *Verticillium* spp. There was 100% disease on wilted plants after 12 weeks of sowing. Seeds treated with RHA and RPG isolates showed significant reduction in percentage of wilted plants, from 60–70 % (Tables 6-10) evaluate to plants from seeds treated with the Rhizobacterial isolates towards fungal root pathogen, low disease severity with less yellowish foliage was noted. It is demonstrated here that the growth of chickpea was enhanced in pots under controlled conditions on treatment with rhizobacterial isolate.

Table 6. *In vitro* effect of rhizobacterial inoculation on Desi variety of chickpea.

Isolates	Growth parameters							
	SG (%)	DI (%)	SL (cm)	RL (cm)	FWS (gm)	DWS (gm)	FWR (gm)	DWR (gm)
C	38	2.5	2.57	2.57	0.22	0.12	0.06	0.03
RHA	67.3	12.5	3	5.4	0.37	0.26	0.1	0.05
RPG	70.7	11	5.27	2.37	0.36	0.21	0.07	0.07
RFJ	50	10.5	2.67	2.6	0.36	0.23	0.08	0.05
RC	49.7	11	3.97	3.37	0.32	0.22	0.08	0.06
RRD	34.3	13	5.97	4.3	0.28	0.19	0.09	0.04
RTR	48.3	12.5	4.6	3.67	0.34	0.25	0.09	0.05
RT	51.7	10.5	9.13	6.4	0.4	0.3	0.08	0.06
RK	33	11	7.17	4.87	0.37	0.28	0.1	0.08

Table. 7. *In vitro* effect of rhizobacterial inoculation on Balkasar variety of chickpea.

Isolates	Growth parameters							
	SG (%)	DI (%)	SL (cm)	RL (cm)	FWS (gm)	DWS (gm)	FWR (gm)	DWR (gm)
C	47.7	15.5	1.87	2.03	0.15	0.05	0.05	0.03
RHA	62.7	14.3	4	2.87	0.21	0.1	0.07	0.03
RPG	70	13	3.3	2.8	0.26	0.12	0.09	0.05
RFJ	55.7	14	3.7	3.07	0.29	0.14	0.06	0.03
RC	45.3	13.4	4.5	3.27	0.26	0.13	0.08	0.04
RRD	38	12.4	5.2	3.77	0.31	0.15	0.06	0.06
RTR	48	14	3.9	3.4	0.22	0.1	0.1	0.05
RT	45	10.4	10.8	6.13	0.23	0.11	0.09	0.07
RK	35.3	11.4	12.2	8.73	0.33	0.16	0.07	0.04

Table 8. *In vitro* effect of rhizobacterial inoculation on Wanhar variety of chickpea.

Isolates	Growth parameters							
	SG (%)	DI (%)	SL (cm)	RL (cm)	FWS (gm)	DWS (gm)	FWR (gm)	DWR (gm)
C	48.6667	16.67	2.07	1	0.103	0.06	0.05	0.0367
RHA	67.6667	15.3	3.1	1.267	0.17	0.09	0.06	0.0633
RPG	71	14.5	3.5	1.2	0.14	0.08	0.09	0.05
RFJ	52	14.83	2.5	1.267	0.137	0.05	0.07	0.06
RC	51	13.83	4.1	1.93	0.163	0.08	0.29	0.08
RRD	38	13.33	3.2	1.2	0.13	0.1	0.1	0.63
RTR	48.667	15	3.1	1.43	0.113	0.11	0.07	0.05
RT	48.333	15	3.1	1.43	0.113	0.11	0.07	0.05
RK	38.333	13.33	3.7	2.567	0.267	0.11	0.07	0.06

Table 9. *In vitro* effect of rhizobacterial inoculation on Bital-98 variety of chickpea.

Isolates	Growth parameters							
	SG (%)	DI (%)	SL (cm)	RL (cm)	FWS (gm)	DWS (gm)	FWR (gm)	DWR (gm)
C	50.7	13.7	2.1	1.43	0.23	0.08	0.08	0.027
RHA	70	12.4	3.43	1.93	0.33	0.11	0.1	0.037
RPG	70.7	12.9	4.2	2.43	0.35	0.14	0.11	0.057
RFJ	54	11.8	3.9	2.07	0.37	0.12	0.1	0.05
RC	53	12.5	3.7	1.7	0.26	0.18	0.12	0.057
RRD	34	11.4	5.1	2.67	0.37	0.16	0.11	0.03
RTR	51	11	3.5	2.1	0.34	0.14	0.11	0.037
RT	50.7	11.4	5.1	2.67	0.37	0.16	0.11	0.03
RK	37.3	10.9	4.2	2	0.41	0.15	0.1	0.027

Table 10. *In vitro* effect of rhizobacterial inoculation on Channa-2008 variety of chickpea.

Isolates	Growth parameters							
	SG (%)	DI (%)	SL (cm)	RL (cm)	FWS (gm)	DWS (gm)	FWR (gm)	DWR (gm)
C	54	13.67	2.1	1.43	0.23	0.08	0.08	0.027
RHA	69	12.43	3.5	2	0.333	0.11	0.13	0.04
RPG	68.67	12.93	3.5	2.4	0.34	0.14	0.11	0.06
RFJ	47.67	11.33	3.77	2.1	0.36	0.12	0.1	0.05
RC	50.1	10.5	4.0	1.89	0.23	0.15	0.12	0.02
RRD	33.4	11.2	3.4	1.76	0.33	0.17	0.16	0.04
RTR	44	9.8	3.2	2.0	0.42	0.13	0.10	0.03
RT	49.2	10.3	3.8	1.7	0.28	0.10	0.12	0.05
RK	36	11.8	4.1	2.5	0.46	0.14	0.1	0.04

Discussion

By colonizing plant roots, PGPR proved to be beneficial for growth and development of plant in a variety of ways. For an effective PGPR, colonization of roots by bacteria is must for its establishment in the rhizosphere at specific densities of its population is required for beneficial effects. The exact mechanism of stimulating plant growth by PGPR is not yet established clearly, but promotion of uptake of mineral nutrient, phytohormones production, phosphate solubilization activation and deleterious organism's suppression are believed to be the hypothesis to be involved (Kloepper *et al.*, 2004). Literature has described many auxins that occur naturally, till now, the most common auxin that has been studied the most is indole-3- acetic acid (IAA) it is considered interchangeable with the auxins in many scientific literatures (Liu *et al.*, 1992, Neeru *et al*., 2000). For the regulation of the plant development, IAA may play the role of important signaling molecule. Six out of eight isolates produced IAA (Table 4). Only RPG isolate came out as good producer of IAA (Table 4). Different species and strains of PGPR, depending upon condition of the culture, stage of its growth and availability of substrate can vary in producing IAA (Spaepen *et al.*, 2007). Moreover, rhizospheric isolates in comparison with isolates from bulk soil can produce auxins much more efficiently (Patten & Glick., 1996).

Phosphorus is one of the major nutrients required by plants, phosphorous stands second to nitrogen. At first instance, insoluble phosphates are available to plants as most phosphorous in soil that plants are unable to utilize (Sarwar & Frankenberger, 1994). The bacterial aid in availability of iron and phosphorous as soluble minerals to plants for growth has been an area of interest to agricultural microbiologists. Plant growth promoting mechanism of PGPR has been reported under field conditions in providing consumable phosphate to rice by solubilizing the precipitated phosphates (Jetiyanon & Kloepper, 2002). Phosphate solubilizing bacteria are found in abundance in the rhizosphere than in non-rhizospheric soil (Whipps, 2001). All isolates we used in our experiments solubilized phosphate in rhizosphere soil (Table 4). The important thing to be noted here is that the bacteria that solubilize phosphate in soil are bacilli but their numbers are much less than other bacteria that colonize rhizospheric soil commonly (Goldstein *et al.*, 1994).

It is demonstrated here that the 6 PGPR isolates, that already are IAA producers, produced Siderophore such as RHA, RPG, RFJ, RC, RRD, and RT. Based on those isolates that produced Siderophore , it has been determined that, phytopathogenic fungal growth was inhibited by producers of Siderophore as bioactive compound (Table 5). These results suggest that, Siderophore produced by the isolates had a suppressing effect on the growth of phytopathogenic fungi such as *F. oxysporum*, *Verticillium* spp. The inhibition of fungal pathogens can also be related to the ammonia released by bacteria, as reported by Fravel (1988) with Enterobacter cloacae on *Verticillium dahliae* and *Pythium ultimum*. In Brazil, among the field phytopathogens that affect chickpea, *F. verticilliodes* is the most common (Peixoto *et al*., 1998). The following pathogens are also common: *Glomerella. graminicola*, *Bipolaris maydis* (Pinto, 1998) and *Cercospora zeae-maydis*, and have become more important in recent decades in Brazil (Pereira *et al*., 2005).

Among the strains tested in our study, RHA and RPG were the most efficient in the inhibition against all fungi tested. They are known to have an antagonist effect against various fungi, for example, *F. moniliforme*, *F. graminearum*, *M. phaseolina* (Pal *et al*., 2001), *F. oxysporum* (Araujo & Guerreiro, 2010). One of the advantages of the biological control strategy using rhizobacteria is that they can act in the same niche, in direct competition with the pathogens (Bacon & Hinton, 2002).

It can be concluded from the above discussion that PGPR enhance the plant growth. Collectively, our results indicated that as chickpea growth was enhanced by the use of PGPR isolates RHA and RPG as inoculants/ biopesticide, by producing IAA, phosphate solubilization, Siderophore production and have antifungal activity against phytopathogenic fungi, they might prove beneficial for the cultivation of chickpea.

References

Araujo, F.F. and R.T. Guerreiro. 2010. Bioprospecção de isolados de Bacillus promotores de crescimento de milho cultivado em solo autoclavado e natural. *Ciênc Agrotec*., 34(4): 837-844.

Ashraf, M.A., M. Rasool and M.S. Mirza. 2011. Nitrogen fixation and indole acetic acid production potential of bacteria isolated from rhizosphere of sugarcane (*Saccharum officinarum* L.). *Adv. Biol. Res*., 5(6): 348-355.

Assumpcao, L.C., P.T. Lacava, A.C.F. Dias, J.L. Azevedo and J.O.M. Menten. 2009. Diversidade e potencial biotecnológico da comunidade bacteriana endofítica de semente de soja. Pesq Agropec. *Bras*., 44: 503-510.

Bacon, C.W. and D.M. Hinton. 2002. Endophytic and biological control potential of Bacillus mojavensis and related species. *Biological Control*, 23(3): 274-284.

Beneduzi, A., F. Moreira, P.B. Costa, L.K. Vargas, B.B. Lisboa, R. Favreto, J.I. Baldani and L.M.P. Passaglia. 2013. Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the South of Brazil. *Appl. Soil Ecol*., 63: 94-104.

Barretti, P.B., R. M. Souza and E. A. Pozza. 2008. Bactérias endofíticas como agentes promotores do crescimento de plantas de tomateiro e de inibição in vitro de Ralstonia solanacearum. Ciênc Agrotec., 4:731–739.

Canbolat, M., S. Bilen, R. Çakmakçi, F. Sahin and A. Aydi. 2006. Effect of plant growthpromoting bacteria and soil compaction on barley seeding growth, nutrient uptake, soil properties and rhizosphere microflora. *Biol. Fertil. Soils*, 42(3): 350-357.

Cassan, F., S. Maiale, O. Masciarelli, A. Vidal, V. Luna and O. Ruiz. 2009. Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. *Eur. J. Soil Biol*., 45: 12-19.

Clark, V.L. and P.M. Bavoil. 1994. *Methods in Enzymology* 235(A), Academic Press, London, pp. 315-372.

Compant, S., C. Clément and A. Sessitsch. 2010. Plant growth-promoting bacteria in the rhizo and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.*, 42: 669-678.

David R. Boone, Richard W. Castenholz, George M. Garrity, Don J. Brenner, Noel R. Krieg, James T. Staley. 2005. Springer Science & Business Media.

Enebak, S.A., G. Wei and J.W. Kloepper. 1998. Effects of plant growth promoting rhizobacteria on loblolly and slash pine seedlings. *Forest Sci*., 44: 139144.

Fravel, D.R. 1988. Role of antibiosis in the biocontrol of plant diseases. *Ann. Rev. Phytopathol*., 26: 75-91.

George, P., A. Gupta, M. Gopal, L. Thomas and G.V. Thomas. 2012. Multifarious beneficial traits and plant growth promoting potential of *Serratia marcescens* KiSII and *Enterobacter* sp. RNF 267 isolated from the rhizosphere of coconut palms (*Cocos nucifera* L.). *World J. Microb. Biot*., 29(1): 109-117.

Goldstein, A.H. 1994. Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. In: *Phosphate in Microorganisms: Cellular and Molecular Biology*. (Eds.): Torriani-Gorini A., E. Yagil and S. Silver. Washington, DC: ASM Press, pp. 197-203.

Hungria, M., R.J. Campo, E.M. Souza and F.O. Pedrosa. 2010. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant Soil*, 331: 413-425.

Idris, E.E.S., D.J. Iglesias, M. Talon and R. Borriss. 2007. Tryptophan-dependent production of Indole-3- Acetic Acid (IAA) affects level of plant growth promotion by Bacillus amyloliquefaciens FZB42. *Mol. Plant Microbe. Interact*, 20: 619-626.

Jetiyanon, K. and J.W. Kloepper. 2002. Mixtures of plant growth promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biol. Contr*., 24: 285-291.

Khalid, A., M. Arshad and Z.A. Zahir. 2004. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol*., 96: 473-480.

Kloepper, J.W., C.M. Ryu and S. Zhang. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*, 94: 1259-1266.

Krey, T., N. Vassilev, C. Baum and B. Eichler-Löbermann. 2013. Effects of long-term phosphorus application and plant-growth promoting rhizobacteria on maize phosphorus nutrition under field conditions. *Eur. J. Soil Biol*., 55: 124-130.

Kuss, A.V., V.V. Kuss, T. Lovato and F. Ml. 2007. Fixação de nitrogênio e produção de ácido indol acético In vitro por bactérias diazotróficas endofíticas. Pesquisa Agropecuária Brasileira. 42(10): 1459-1465.

Liu, S.T., L.Y. Lee, C.Y. Tai, C.H. Hung, Y.S. Chang, J.H. Wolfram, R. Rogers and A.H. Goldstein. 1992. Cloning of an *Erwinia herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli* HB101. *J. Bacteriol*., 174: 5814-5819.

Loper, J.E. and M.N. Schroth. 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. *Phytopathology*, 76: 386-389.

Lucy, M., E. Reed and B.R. Glick. 2004. Application of free living plant growth promoting rhizobacteria. Antonie van Leewen hoek, 86: 1-25.

Neeru, N., K. Vivek, K. Rishi and M. Wolfgancy. 2000. Effect of P-solubilizing *Azotobacter chroococcum* on N, P, K uptake in p-responsive genotypes grown under greenhouse condition. *J. Plant Nutr. Soil Sci*., 163: 393-398.

Oliveira, A.L.M., E.L. Canuto, V.M. Reis and J.I. Baldani. 2003. Response of micropropagated sugarcane varieties to inoculation with endophytic diazotrophic bacteria. *Braz. J. Microbiol*., 34: 59-61.

Pal, K.K., K.V.B.R. Tilak, A.K. Saxena, R. Dey and C.S. Singh. 2001. Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiol. Res*., 156: 209-223.

Patten, C.L. and B.R. Glick. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol*., 42: 207-220.

Peixoto, A.R., S.B. Torres and N. Karasawa. 1998. Qualidade sanitária de sementes de milho produzidas no submédio São Francisco. *Rev. Bras. Sementes*, 20(1): 12-15.

Pikovaskya, R.I. 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiologiya*, 17: 362-370.

Rodriguez, H., T. Gonzalez, I. Goire and Y. Bashan. 2004. Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. *Naturwissenschaften*, 91: 552-555.

Sarwar, M. and W.T. Frankenberger. 1994. Influence of L-Tryptophan and auxins applied to the rhizosphere on the vegetative growth of *Zea mays* L. *Plant and Soil*, 160: 97-104.

Shiomi, H.F., I.S. Melo and M.T.A. Minhoni. 2008. Seleção de bactérias endofíticas com ação antagônica a fitopatógenos. *Scientia Agraria*., 9: 535-538.

Silva, M.F. and V.M. Reis. 2004. Técnicas usadas para quantificar a população de bactérias diazotróficas inoculadas em sementes de milho (*Zea mays*). Embrapa, Seropédica Sukumaran J, Holder MT (2010) DendroPy: a Python library for phylogenetic computing. *Bioinformatics*, 26: 1569-1571.

Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer and G. Berg. 2001. Bulk and rhizospheric soil bacterial communities studied by denaturing gradient gel electrophoresis: plant dependent enrichment and seasonal shift revealed. *Appl. Environ. Microbiol*., 67: 4742-4751.

Somers, E., J. Vanderleyden and M. Srinivasan. 2004. Rhizosphere bacterial signalling: a love parade beneath our feet. *Crit. Rev. Microbiol*., 304: 205-240.

Spaepen, S., J. Vanderleyden and R. Remans. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS (Fed. Eur. Microbiol. Soc.) *Microbiol. Rev*., 31: 425-448.

Vale, M., L. Seldin, F.F. Araújo and R. Lima. 2010. Plant growth promoting rhizobacteria: fundamentals and applications. In: (Ed.): Maheshwari, D.K. *Plant growth and health promoting bacteria Springer*, Berlin, pp. 21-43.

Verma, J.P., J. Yadav, K.N. Tiwari and A. Kumar. 2013. Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer aritenium* L.) under sustainable agriculture. *Ecol. Eng*., 51: 282-286.

Wang, S., W. Huijun, Q. Junqing, M. Lingli, L. Jun, X. Yanfei and G. Xuewen. 2009. Molecular mechanism of plant growth promotion and induced systemic resistance to tobacco mosaic virus by *Bacillus* spp. *J. Microbiol. Biotechnol*., 19(10): 1250-1258.

Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Expt. Bot*., 52: 487-511.

(Received for publication 15 May, 2014)