Pedosphere **22**(6): 848–859, 2012
ISSN 1002-0160/CN 32-1315/P

Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Effect of Plant Growth-Promoting Rhizobacteria on Growth, Nodulation and Nutrient Accumulation of Lentil Under Controlled Conditions*[1]

M. ZAFAR[1], M. K. ABBASI[1,*2], M. A. KHAN[1], A. KHALIQ[1], T. SULTAN[2] and M. ASLAM[2]

[1] *Department of Soil and Environmental Sciences, The University of Poonch, Rawalakot Azad Jammu and Kashmir (Pakistan)*
[2] *Soil Biology Laboratory, Land Resources Research Program (LRRP), National Agriculture Research Council (NARC), Islamabad (Pakistan)*

(Received April 16, 2012; revised September 18, 2012)

ABSTRACT

Application of plant growth-promoting rhizobacteria (PGPR) has been shown to increase legume growth and development under field and controlled environmental conditions. The present study was conducted to isolate plant growth-promoting rhizobacteria (PGPR) from the root nodules of lentil (*Lens culinaris* Medik.) grown in arid/semi-arid region of Punjab, Pakistan and examined their plant growth-promoting abilities. Five bacterial isolates were isolated, screened *in vitro* for plant growth-promoting (PGP) characteristics and their effects on the growth of lentil were assessed under *in vitro*, hydroponic and greenhouse (pot experiment) conditions. All the isolates were Gram negative, rod-shaped and circular in form and exhibited the plant growth-promoting attributes of phosphate solubilization and auxin (indole acetic acid, IAA) production. The IAA production capacity ranged in 0.5–11.0 μg mL^{-1} and P solubilization ranged in 3–16 mg L^{-1}. When tested for their effects on plant growth, the isolated strains had a stimulatory effect on growth, nodulation and nitrogen (N) and phosphorus (P) uptake in plants on nutrient-deficient soil. In the greenhouse pot experiment, application of PGPR significantly increased shoot length, fresh weight and dry weight by 65%, 43% and 63% and the increases in root length, fresh weight and dry weight were 74%, 54% and 92%, respectively, as compared with the uninoculated control. The relative increases in growth characteristics under *in vitro* and hydroponic conditions were even higher. PGPR also increased the number of pods per plant, 1 000-grain weight, dry matter yield and grain yield by 50%, 13%, 28% and 29%, respectively, over the control. The number of nodules and nodule dry mass increased by 170% and 136%, respectively. After inoculation with effective bacterial strains, the shoot, root and seed N and P contents increased, thereby increasing both N and P uptake in plants. The root elongation showed a positive correlation ($R^2 = 0.67$) with the IAA production and seed yield exhibited a positive correlation ($R^2 = 0.82$) with root nodulation. These indicated that the isolated PGPR rhizobial strains can be best utilized as potential agents or biofertilizers for stimulating the growth and nutrient accumulation of lentil.

Key Words: indole acetic acid, inoculation, nodules, nutrient uptake, phosphate solubilization

Citation: Zafar, M., Abbasi, M. K., Khan, M. A., Khaliq, A., Sultan, T. and Aslam, M. 2012. Effect of plant growth-promoting rhizobacteria on growth, nodulation and nutrient accumulation of lentil under controlled conditions. *Pedosphere.* **22**(6): 848–859.

The natural agricultural ecosystems depend largely on the beneficial microorganisms that are present in the soil rhizosphere and help crops to maintain growth and sustain productivity (Rosas *et al.*, 2009). These beneficial microorganisms are important components of plant productivity and soil fertility because of their participation in several key processes such as those involved in biological control of plant pathogens, nutrient cycling and seedling establishment (Jeffries *et al.*, 2003; Rosas *et al.*, 2009). Recent studies have confirmed that a number of bacterial species, mostly associated with the plant rhizosphere, are beneficial for plant growth, yield and crop quality. They have been called plant growth-promoting bacteria (PGPB) and include strains in the genera *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Rhizobium* and *Serratia* (Bashan and de-Bashan, 2005; Esitken *et al.*, 2010).

The plant-promoting effect of PGPR has been attributed to the release of metabolites that directly stimulate growth. Several mechanisms have been postulated to explain how PGPR benefit the host plant, including 1) producing plant growth regulators or phytohormones such as indole acetic acid (IAA), cytokinins and gibberellins (Glick, 1995; Marques *et al.*,

*[1]Supported by the University of Azad Jammu and Kashmir, Pakistan and the Pakistan Agriculture Research Council, Pakistan (No. ALP NR-27).
*[2]Corresponding author. E-mail: kaleemabbasi@yahoo.com.

2010); 2) enhancing asymbiotic N_2 fixation (Şhin *et al.*, 2004; Khan, 2005); 3) solubilizing inorganic phosphate and mineralization of organic phosphate and/or other nutrients (Glick, 1995); and 4) antagonistic effects against phytopathogenic microorganisms by the production of siderophores, the synthesis of antibiotics, enzymes and/or fungidical compounds and/or competition with detrimental microorganisms (Dey *et al.*, 2004; Lucy *et al.*, 2004).

Generally, plant growth-promoting bacteria occur in soil, but their population numbers are not sufficient to compete with other bacterial strains that are commonly present and well established in the rhizosphere. Therefore, inoculation of plants with target microorganisms is necessary to take advantage of their beneficial properties for plant yield enhancement (Igual *et al.*, 2001). Consequently, it is important to isolate and characterize bacteria from local conditions that can be used as potential inoculant in the same area where they are obtained. The advantage of using natural soil isolates over the genetically manipulated or those isolated from a different environment is their better adaptation and success rate when inoculated into the plant rhizosphere (Chen *et al.*, 2006).

Lentil (*Lens culinaris* Medik.) is a widespread leguminous plant of great agricultural and economic importance. It is a protein-rich pulse, grown mainly in developing countries as a rain-fed crop in nutrient-poor soils and often subjected to intermittent drought during the growth period and/or terminal drought during the reproductive phase (Gahoonia *et al.*, 2005). Lentil is the second most important grain legume crop in Pakistan and is cultivated on an area of 82 000 hectares (26% of the entire pulse area) with an annual grain production of 30 000 t (Malik *et al.*, 1988; Hafeez *et al.*, 2000). Most of the cultivation of lentil occurs on marginal and unfertile lands with very small indigenous rhizobial populations. Inoculation with active *Rhizobium* strains is required to increase yields through N_2 fixation (Athar, 1998; Hafeez *et al.*, 2000).

The aims of the present study were to isolate *Rhizobium* strains from lentil grown on soils in the arid region of Pakistan, to screen them for their plant growth-promoting properties, and to test selected species on lentil in order to evaluate their effects on growth, root nodulation and N and P assimilation in the roots, shoots and grains of lentil.

MATERIALS AND METHODS

Sampling

Root samples with the adjacent soil cores were collected from twenty fields of the Narrowal District, Punjab, Pakistan at the flowering stage during 2008. This area has not been explored before and the isolation was studied first time. The samples were collected approximately 15 cm on either side of a plant to a 30 cm depth. The physical and chemical characteristics of the soil samples are presented in Table I. The rhizosphere soil and roots were slowly lifted before carefully removing the soil from the roots to avoid damage to the roots. Thereafter, the twenty samples were placed in plastic bags with proper tagging and brought to the Soil Biology Laboratory, Land Resources Research Program (LRRP), National Agriculture Research Council (NARC), Islamabad, for further studies.

TABLE I

Physical and chemical properties of the soil before and after cultivation of lentil

Soil parameter	Before sowing	After harvest
Electrical conductivity (dS m^{-1})	0.60	0.64
pH (H_2O, 1:2.5)	7.70	7.16
Organic matter (g kg^{-1})	8.3	8.8
Total N (g kg^{-1})	0.4	0.5
Available P (mg kg^{-1})	10.67	11.77
Available K (mg kg^{-1})	277	285
NO_3^--N (mg kg^{-1})	2.42	2.54

Nodule collection

In the laboratory, plant shoots were removed with a surgical blade and then the roots were washed under running water with a screen underneath to catch the detached nodules. The healthy, pink and undamaged nodules were detached from the roots by cutting the roots 0.5 cm on either side of the nodule. Before isolation, nodules were surface sterilized by immersing them in 95% ethanol for 5–10 s and then were washed for 3–4 times in sterilized distilled water. After washing, the nodules were dipped in 3% hydrogen peroxide (H_2O_2) for 3–4 min and washed for 5–7 times in distilled sterilized water (Somasegaran *et al.*, 1982).

Isolation and purification of Rhizobium strains

The nodules were aseptically crushed in a drop of autoclaved, distilled water on a sterile, glass slide. The nodule extract was streaked onto yeast mannitol (YMA) agar plates supplemented with Congo red as a dye (Nelson and Child, 1981) in such a way that the nodule material was serially diluted. Then, the plates were incubated at 28±2 °C for 2–3 d under fluorescent light. The off-white gummy colonies appeared on the third day and showed sufficient growth after 5–7 d. Single colonies from each isolate was selected

and re-streaked on fresh YMA agar plates (Vincent, 1970). Subculturing was carried out 4–5 times until the culture became pure. The pure culture was considered as presumptive *Rhizobium* ssp. at this stage on the basis of not picking up the red color of Congo red dye.

Phenotypic characterization of bacterial isolates

A total of 23 bacterial isolates were characterized for different morphological and biochemical characteristics. For morphological characterization, purified bacterial isolates were initially characterized on the basis of their colony color, shape, size, elevation, form, and surface texture. Investigations on the morphology and structure of the isolates were conducted under a phase contrast light microscope and the Gram stain reaction was also observed under a light microscope (Vincent, 1970).

For biochemical characterization, five bacterial isolates of the 23 strains characterized, namely *Lens culinaris* LCA-1, LCA-2, LCA-3, LCA-4 and LCA-5, were screened for triple sugar iron and sugar fermentation (Bisen and Verma, 1994). The ability of each isolate to solubilize phosphate was examined based on the appearance of clear zones (halos) surrounding the bacterial colonies, on Pikovskaya's medium containing tricalcium phosphate ($Ca_3(PO_4)_2$) as the inorganic P source (Pikovskaya, 1948). Soluble phosphorus was quantified using the phosphomolybdate blue method (Murphy and Riley, 1962). The available phosphorus was quantified by using a Spectronic-20 spectrophotometer set at a 880 nm wavelength. Extracellular production of IAA was carried out by growing cultures as described by Okon *et al.* (1977). After one week of growth, qualitative estimation of IAA was performed with Fe-$HClO_4$ and Fe-H_2SO_4 reagents. The ethyl acetate oxidation method (Tien *et al.*, 1979) was used for the quantitative estimation of IAA.

In vitro experiment

The effect of the isolated *Rhizobium* spp. strains that were considered potential PGPR on seed germination and plant growth of lentil was tested in a growth chamber (*in vitro*). Six treatments, namely, 1) uninoculated (control), 2) LCA-1, 3) LCA-2, 4) LCA-3, 5) LCA-4 and 6) LCA-5, were organized in a completely randomized design (CRD) with three replication. Five surface sterilized seeds of lentil cultivar Masoor-2002 were grown in sterilized Petri plates with filter paper in the lower lid. The seed in each treatment, except the control, was inoculated with 1 mL of inoculum and wetted with 2–3 mL sterilized water. The Petri plates were incubated at 29 °C. Fifteen days after sowing (DAS), the shoot and root lengths of the plants were recorded.

Hydroponic experiment

The hydroponic experiment was conducted in the Hoagland solution (Winaro and Lie, 1979) to investigate the effect of five newly isolated strains and one previously tested positive NARC strain on the shoot and root lengths of lentil. NARC strain was previously used in many studies and therefore was used here for comparison with the newly isolated strains. The experiment was laid out in a completely randomized design (CRD) with three replications and seven treatments. The treatments were: 1) uninoculated (control), 2) LCA-1, 3) LCA-2, 4) LCA-3, 5) LCA-4, 6) LCA-5 and 7) NARC strain. One liter of nutrient solution in the pots was used for this experiment. Oxygen was provided to the roots through a needle syringe. Seedlings were grown in a sterilized sand tray and were inoculated before being transferred to the nutrient solution by dipping their roots to respective suspension cultures. Then, 1 mL suspension culture of each strain was added to the nutrient solution except the control (inoculum free). Four plants were grown in each pot for three weeks. The pH was checked daily and maintained at 6.8 with HCl or NaOH.

Greenhouse pot experiment

A pot experiment was conducted in 2008 to examine the effect of the isolated *Rhizobium* strains (PGPR) on the growth, nodulation, nutrient concentration and nutrient uptake of lentil. Lentil cultivar Masoor-2002 was used as the host plant to evaluate the effect of selected PGPR. The experiment was carried out using sterilized soil collected from an experimental area of the NARC with the characteristics presented in Table I. The six isolated strains from the hydroponic experiment and one mixture of two most promising strains were tested. Nitrogen was applied in the form of urea at 20 kg N ha^{-1} in two-split doses (initially during sowing and during flowering stage). Phosphorus was applied as 40 kg P ha^{-1} as single superphosphate (SSP). Large sized pots (21 cm diameter) that could hold 8 kg of soil were used in this experiment. The experiment included 8 treatments: 1) uninoculated (control), 2) LCA-1, 3) LCA-2, 4) LCA-3, 5) LCA-4, 6) LCA-5, 7) NARC strain and 8) a mixture of LCA-1 and LCA-5 (50:50, w/w) as these two strains showed maximum IAA production, increased P solu-

bility and increased growth under *in vitro* as well as hydroponic conditions. The treatments were arranged in a completely randomized design (CRD) and were replicated three times. Seedlings were grown in sterilized sand trays. One week-old four seedlings were inoculated with the respective strains by dipping the roots into the suspension cultures (106 cells mL^{-1}) for 30 min and then were transferred to the pots. In addition, one mL of suspension culture of strains was added to the respective pots except the control. The number of nodules per plant was recorded at the flowering stage. Plants were harvested at maturity (108 d) and plant growth characteristics, *i.e.*, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and nutrient uptake of the crop, were recorded.

Soil and plant analyses

Total nitrogen in plant shoot, root and seed was determined by Kjeldhal digestion, distillation and titration method (Bremner and Mulvaney, 1982). Phosphorus content was determined with the vanadomolybdate yellow color method using a spectrophotometer set at 440 nm (Jackson, 1967) following wet digestion with a 4:1 mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$). Soil electrical conductivity (EC) was determined from the saturation extract collected from the saturated paste (Rhoades, 1982). Soil pH was measured with a glass electrode at a soil to water ratio of 1:2.5. Soil organic carbon (SOC) was measured using the modified Mebius method (Nelson and Sommers, 1982). Total N was determined by the Kjeldahl digestion, distillation and titration method (Bremmer and Mulvaney, 1982). After extraction of the soil samples with 200 mL of 1 mol L^{-1} KCl, followed by filtration through Whatman No. 40 filter paper, NH_4^+-N of the extract was determined by using the steam distillation and titration method of Keeney and Nelson (1982). NO_3^--N was calculated by subtracting NH_4^+-N from the total mineral N. Available P was determined by the Olsen extraction method (Olsen and Sommers, 1982) and available K was extracted with 1 mol L^{-1} ammonium acetate, adjusted to pH 7, and was determined flamephotometrically (Simard, 1993).

Statistical analysis

An analysis of variance was carried out using the M-STAT C software. If the analysis of variance showed significant strain effects, the least significant difference (LSD) test was applied to compare the means at the 0.05 level of significance (Steel and Torrie, 1980).

RESULTS

Characteristics of isolated plant growth-promoting rhizobacteria

All strains were Gram-negative and fast-growing motile rods, curved rods or straight rods of different sizes. All the colonies had a circular surface except LCA-4, which was irregular, and were either creamy, glossy white, slimy white, white or shiny white in appearance (Table II). The colonies appeared on the YMA agar plates were characteristically indicative of PGPR, which is a desired feature for promotion of plant growth. All isolates were able to produce indole acetic acid (IAA) (Table II). The amount of IAA produced varied with the type of strains and ranged in 0.5–11 μg mL^{-1}. Isolate LCA-1 produced the IAA (11.0 μg mL^{-1}), followed by LCA-5 (8 μg mL^{-1}) and LCA-4 (4 μg mL^{-1}). Similarly, all isolates were able to

TABLE II

Morphological and biochemical characteristics of the strains (plant growth-promoting rhizobacteria) isolated

Characteristic	Strain isolated				
	LCA-1	LCA-2	LCA-3	LCA-4	LCA-5
Color	Creamy	Glossy white	Slimy white	white	Shiny white
Surface form	Circular	Circular	Circular	Irregular	Circular
Elevation	Lowly convex	Raised	Lowly raised	Highly raised	Raised
Surface margin	Entire	Entire	Entire	Entire	Entire
Surface texture	Dry	Dry	Dry	Smooth	Dry
Gram staining	Negative	Negative	Negative	Negative	Negative
Shape	Rod	Curved rod	Straight rod	Rod	Rod
Triple sugar iron	K/A[a)]	K/A	K/A	K/A	K/A
Sugar fermentation	Brown	Brown	Brown	Brown	Brown
P solubilized (mg L^{-1})	16	4	3	9	13
Indole acetic acid production (μg mL^{-1})	11	3	0.5	4	8

[a)] Glucose fermentation only.

form a clear zone on Pikovskaya's agar by mobilizing tricalcium phosphate and were thereby considered as P solubilizers (Table II), but differences in P-solubilizing capacity among the strains were substantial, ranging in 3–16 mg L^{-1}. Isolate LCA-1 had the highest P solubilization capacity (16 mg L^{-1}), followed by LCA-5 (13 mg L^{-1}) and LCA-4 (9 mg L^{-1}). On the basis of these morphological and biochemical characteristics, the isolates under investigation were identified as PGPR as described in many other studies.

Plant growth-promoting properties under in vitro and hydroponic conditions

The *in vitro* tests showed that the isolated PGPR strains stimulated plant growth. Relative increases in shoot and root lengths over the control (due to PGPR application) were 54%–133% and 52%–172%, respectively. However, there were differences among the bacterial strains in their responses to these tests. For example, shoot length ranged in 3.68–5.58 cm and root length in 2.93–5.26 cm. Strain LCA-5 produced the highest shoot length (5.58 cm) while the highest root length (5.26 cm) was recorded for LCA-1 (Table III). Similarly, the PGPR strains enhanced plant growth when tested under hydroponic conditions. Shoot and root lengths in the PGPR treatments ranged in 13.6–16.4 and 3.67–5.96 cm, compared with 11.76 and 2.61 cm, respectively, in the control (Table III). The relative increases in shoot and root lengths were 15%–39% and 41%–128%, respectively, over the uninoculated control. The highest value of shoot length was recorded for LCA-5 and that of root length for LCA-1.

TABLE III

Effect of isolated plant growth-promoting rhizobacteria (LCA-1–LCA-5) on shoot and root lengths of lentil under *in vitro* and hydroponic conditions

Treatment	*In vitro* conditions		Hydroponic conditions	
	Shoot length	Root length	Shoot length	Root length
	cm			
Control	2.4 c[a)]	1.9 c	11.8 c	2.6 d
LCA-1	5.6 a	5.3 a	15.2 ab	6.0 a
LCA-2	4.3 ab	3.3 b	14.8 ab	3.7 cd
LCA-3	4.9 ab	3.1 b	14.5 b	4.9 ab
LCA-4	3.7 bc	2.9 b	15.2 ab	4.0 bc
LCA-5	5.6 a	3.1 b	16.4 a	5.6 a
NARC strain			13.6 b	4.2 bc
LSD[b)] ($P \leq 0.05$)	1.602	0.394	1.678	1.112

[a)]Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[b)]Least significant difference.

Plant growth-promoting properties under greenhouse conditions

Plant morphological characteristics. Based on the results of the *in vitro* and hydroponic tests, in addition to 5 isolated strains including an uninoculated control, a mixture of two promising strains, LCA-1 and LCA-5, and a positive tested NARC strain were included in the pot experiment to evaluate their plant growth-promoting abilities. Plant morphological characteristics (*i.e.*, shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight and root dry weight) were tested in this experiment (Table IV). Application of PGPR significantly ($P \leq 0.05$) increased lentil growth compared with the control. The relative increase ranged between 38% and 65% in shoot length, 3% and 43% in shoot fresh weight and 11% and 63% in shoot dry weight over the uninoculated control. Shoot length and weight increments differed considerably among the PGPR strains and were extremely high either with LCA-5 or LCA-1 or with the mixture of both. The tallest shoot was recorded for LCA-5, followed by the mixture LCA-1+LCA-5, greatest shoot fresh weight for the mixture LCA-1+LCA-5 and greatest shoot dry weight for LCA-1 and LCA-1+LCA-5. The mixture did not enhance the growth over the best strain. The inoculated plants developed significantly ($P \leq 0.05$) greater root length and root mass than the uninoculated plants (Table IV). All the seven tested bacterial strains induced greater root length than the uninoculated control. Increases ranged from 26% to 74% for root length, from 22% to 54% for root fresh weight and from 43% to 92% for root dry weight. Similar to shoots, length and weight increases in root growth differed considerably among the PGPR strains and were extremely high for either LCA-1 or the mixture LCA-1+LCA-5. The highest root length was recorded for LCA-1 and the highest root fresh and dry weights for the mixture of strains LCA-1 and LCA-5. The previously tested NARC strain did not produced an increase in any of the morphological characteristics when compared to the newly isolated strains and had either equal or lower values than the uninoculated control for most of the parameters studied. These results indicated that inoculation with each of the bacterial strains responded positively to all the tests, indicating a high potential of growth-promoting capacities.

Yield and yield attributes. Yield and yield components of lentil showed a similar trend to that recorded for the growth parameters (Table V). Application of the PGPR strains significantly ($P \leq 0.05$) increased the number of pods per plant, 1 000-grain

TABLE IV

Effect of isolated plant growth-promoting rhizobacteria (LCA-1–LCA-5) on growth characteristics of lentil grown in pots under greenhouse conditions

Treatment	Shoot			Root		
	Length	Fresh weight	Dry weight	Length	Fresh weight	Dry weight
	cm	g		cm	g	
Control	25.0 d[a)]	2.6 d	1.1 d	11.7 f	1.9 d	0.8 e
LCA-1	38.2 b	3.3 b	1.7 a	20.3 a	2.7 b	1.3 b
LCA-2	34.6 c	2.7 c	1.4 b	15.9 cd	2.3 c	1.1 d
LCA-3	35.2 c	2.7 cd	1.3 c	15.1 de	2.3 c	1.1 d
LCA-4	35.7 c	2.6 cd	1.2 cd	16.4 c	2.3 c	1.2 c
LCA-5	41.3 a	3.3 b	1.5 b	19.1 b	2.6 b	1.3 b
NARC strain	35.8 c	2.6 cd	1.3 c	14.7 e	2.3 c	1.1 d
LCA-1+LCA-5	39.4 ab	3.6 a	1.7 a	18.8 b	2.8 a	1.4 a
LSD[b)] ($P \leq 0.05$)	1.980	0.182	0.122	1.097	0.077	0.055

[a)] Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[b)] Least significant difference.

weight, total biomass yield, dry matter yield and grain yield as compared with the uninoculated control. The increases in yield characteristics relative to uninoculated control ranged from 31% to 87% for number of pods, from 4% to 22% for 1 000-grain weight, 14% to 50% for total biomass yield, 12% to 51% for dry matter yield and 21% to 39% for grain yield. Increases in yield components and biomass varied considerably among the isolated strains and were extremely high for LCA-1 or the mixture LCA-1+LCA-5. The highest number of pods and 1 000-grain weight were recorded for the mixture of strains LCA-1 and LCA-5 while biomass, dry matter and grain yield were highest for LCA-1. The variation among different treatments with regard to 1 000-grain weight and grain yield was attributed to selection of grains on the basis of their size.

Nodulation. The lentil plants developed significantly ($P \leq 0.05$) more root nodules when inoculated with PGPR (Table VI). In the uninoculated control, only an average of 12 nodules per plant were recorded, but increased significantly to 28–32 nodules per plant following the application of PGPR. The relative increase in nodule number ranged from 137% to 170%. LCA-1 induced the highest nodule number per plant (32), followed by LCA-5 (31), and the difference between the two strains was non-significant. Nodule fresh and dry weights showed a similar increasing trend. Increases relative to the uninoculated control ranged from 193% to 254% for nodule fresh weight and 82% to 136% for nodule dry weight. Similar to nodule numbers, fresh and dry weights of nodules differed considerably among PGPR strains and were extremely high either with LCA-1 or with the mixture LCA-1+LCA-5. A significant correlation ($r = 0.54$) was found between IAA production and nodule number.

Nutrient accumulation. Plants from all treatments were chosen for analysis of nutrients (N and P) in shoots, roots and grains. Lentil grains had the hig-

TABLE V

Effect of isolated plant growth-promoting rhizobacteria (LCA-1–LCA-5) on yield and yield attributes of lentil grown in pots under greenhouse conditions

Treatment	Number of pods per plant	1000-grain weight	Total biomass yield	Dry matter yield	Grain yield
		g		g plant^{-1}	
Control	10.80 d[a)]	34.0 f	3.19 e	2.14 f	1.05 e
LCA-1	18.00 b	39.5 b	4.79 a	3.24 a	1.46 a
LCA-2	15.00 c	37.8 d	3.95 c	2.64 d	1.32 cd
LCA-3	14.33 c	38.1 cd	3.77 d	2.50 e	1.27 d
LCA-4	14.50 c	37.7 d	3.79 d	2.49 e	1.30 cd
LCA-5	17.17 b	39.2 c	4.75 a	3.00 b	1.46 a
NARC strain	14.17 c	35.4 e	3.65 d	2.40 e	1.26 d
LCA-1+LCA-5	20.33 a	41.4 a	4.24 b	2.85 c	1.38 bc
LSD[b)] ($P \leq 0.05$)	1.111	0.743	0.464	0.109	0.109

[a)] Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[b)] Least significant difference.

TABLE VI

Effect of isolated plant growth-promoting rhizobacteria (LCA-1–LCA-5) on number and mass of root nodules of lentil grown in pots under greenhouse conditions

Treatment	Number of nodules per plant	Fresh weight of nodules	Dry weight of nodules
		mg plant^{-1}	
Control	11.8 d[a)]	13.1 e	11.2 f
LCA-1	32.0 a	45.5 b	25.4 ab
LCA-2	29.0 bc	38.8 d	21.8 d
LCA-3	28.0 c	38.3 d	21.8 c
LCA-4	28.8 c	38.7 d	22.4 cd
LCA-5	31.2 a	44.5 c	25.3 b
NARC strain	28.8 c	38.5 d	20.3 e
LCA-1+LCA-5	30.0 b	46.3 a	26.4 a
LSD[b)] ($P \leq 0.05$)	1.111	0.795	1.000

[a)] Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[b)] Least significant difference.

hest nutrient contents among all plant organs, with N content of 2.26%–2.95% and P content ranging between 0.52% and 0.83% (Table VII). In the shoots, the N content was 1.30%–1.92% and P content 0.42%–0.71% while the roots exhibited lower N and P contents that ranged in 1.19%–1.46% and 0.32%–0.44%, respectively. Compared to the uninoculated control, application of PGPR strain significantly ($P \leq 0.05$) increased plant N content and the relative increase ranged in 12%–66% for shoot N, 13%–44% for root N and 6%–38% for grain N. Similarly, P content increased by 11%–81% for the shoots, 14%–57% for the roots and 18%–89% for the grains. Plant N and P increases differed considerably among the PGPR strains and were extremely high either for LCA-1 or LCA-5.

Plant nutrient uptake. Total N and P uptake by lentil in response to different PGPR isolates is presented in Fig. 1. N uptake in plants without inoculation was 47 mg plant^{-1}; it significantly ($P \leq 0.05$) increased to the range between 60 and 105 mg plant^{-1} with PGPR application, demonstrating a 28%–123% increase over the control. Plant N increases varied significantly among the PGPR strains and were extremely high for either LCA-1 or LCA-5 or the mixture of both strains. P uptake by plants in the control was 13 mg plant^{-1}, which significantly ($P \leq 0.05$) increased to 17–35 mg plant^{-1} with PGPR application. Plant P increments varied significantly among the PGPR strains and were extremely high for either strain LCA-1 or LCA-5.

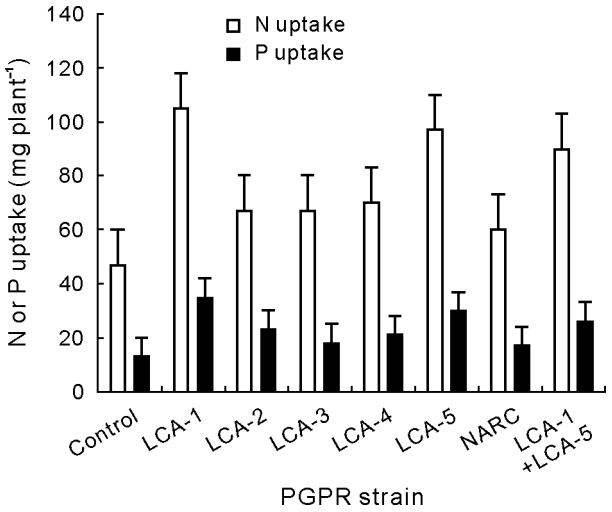

Fig. 1 Effect of plant growth-promoting rhizobacteria (PGPR) on total N and P uptake (shoot + root + grain) of lentil grown in pots under greenhouse conditions.

Changes in soil properties

After crop harvest, the soil was analyzed to exa-

TABLE VII

Effect of isolated plant growth-promoting rhizobacteria (LCA-1–LCA-5) on shoot, root and grain N and P contents of lentil grown in pots under greenhouse conditions

Treatment	N content			P content		
	Shoot	Root	Grain	Shoot	Root	Grain
	g kg^{-1}					
Control	1.16 e[a)]	10.5 d	21.3 f	3.8 f	2.8 e	4.4 f
LCA-1	1.92 a	15.1 a	29.5 a	7.1 a	4.4 a	8.3 a
LCA-2	1.30 d	12.2 c	25.1 d	5.5 cd	3.6 bcd	6.1 cde
LCA-3	1.33 d	13.1 b	26.2 c	4.3 e	3.4 cd	5.5 de
LCA-4	1.45 c	13.3 b	25.9 cd	5.0 d	3.4 cd	6.2 cd
LCA-5	1.85 ab	14.6 a	28.7 ab	6.4 b	4.0 ab	7.6 ab
NARC strain	1.32 d	11.9 c	22.6 e	4.2 ef	3.2 de	5.2 ef
LCA-1+LCA-5	1.80 b	14.6 a	28.3 b	5.8 c	3.8 bc	6.9 bc
LSD[b)] ($P \leq 0.05$)	1.22	0.77	0.95	0.55	0.55	0.93

[a)] Values followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[b)] Least significant difference.

mine any changes induced by the PGPR application during the course of experiment. The post harvest soil samples showed relative increases in electrical conductivity, organic matter content, total N, available P and available K, whereas pH decreased to some extent compared to the pre-sowing soil (Table I). The extent and rate of changes in soil properties were small.

Correlations among PGPR strains and plant characteristics

The root growth (length) of the lentil plants had a significant and positive correlation with the IAA production ($R^2 = 0.67$, $P \leq 0.05$) (Fig. 2a). A significant correlation was also detected between root nodules produced with different PGPR strains and grain yield of lentil ($R^2 = 0.82$, $P \leq 0.05$) (Fig. 2b). Similarly, N and P uptake also showed a significant correlation with grain yield ($R^2 = 0.67$, $P \leq 0.05$; $R^2 = 0.82$, $P \leq 0.05$) (Fig. 3).

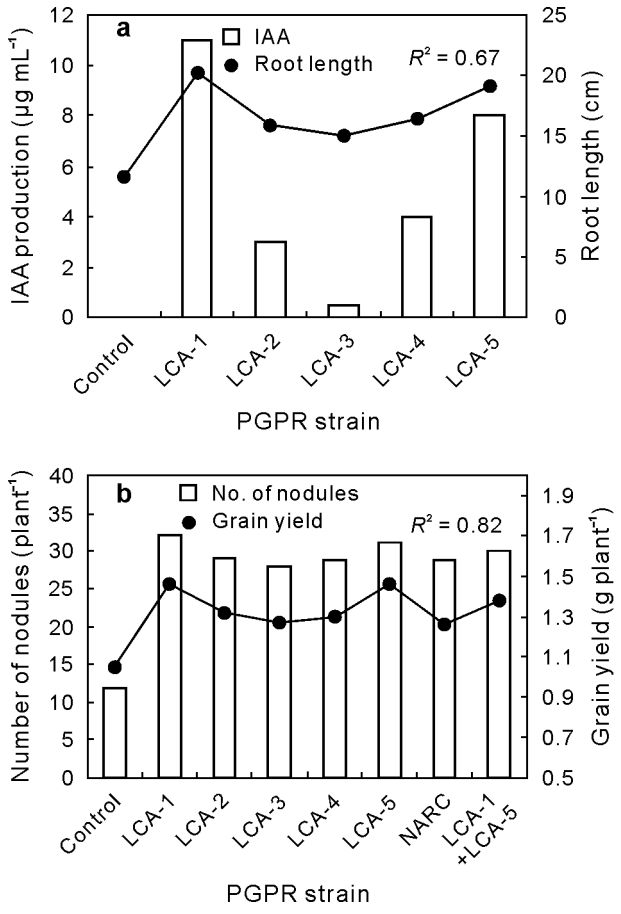

Fig. 2 Correlations of indole acidic acid (IAA) production (a), root length and number of nodules per plant (b) with grain yield of lentil grown in pots under greenhouse conditions with application of different plant growth-promoting rhizobacteria (PGPR) strains.

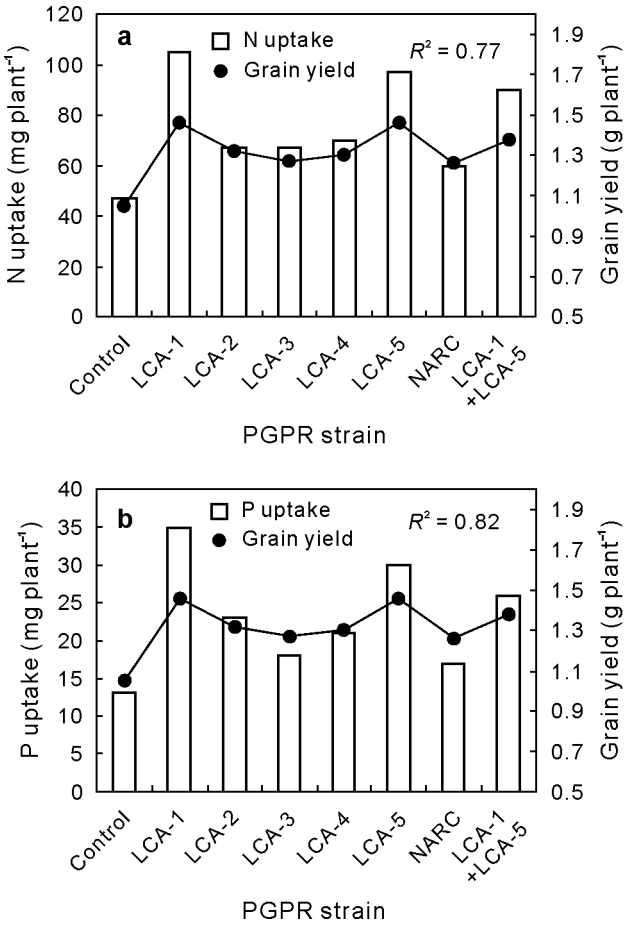

Fig. 3 Correlations of plant N uptake (a) and plant P uptake (b) with grain yield of lentil grown in pots under greenhouse conditions with application of different plant growth-promoting rhizobacteria (PGPR) strains.

DISCUSSION

Plant growth-promoting bacteria isolated from root nodules of lentil often show different physiological characteristics. There are reports that these bacteria are capable of producing biologically active compounds including different enzymes, phytohormones such as auxin and also sidespores (Egamberdiyeva, 2005; Cong *et al.*, 2009; Oswald *et al.*, 2010).

In our study, the bacteria isolated produced various hydrolytic compounds (*i.e.*, IAA production) and were also capable of solubilize P. The isolated strains showed variation in these characteristics and two isolated strains LCA-1 and LCA-5 showed higher concentrations of the growth-promoting substances. The amount of IAA produced varied with the strains and ranged between 0.5–11 µg mL^{-1}. P solubilization differed significantly among strains in the range between 3 and 16 mg L^{-1}. Similar to our findings, variation in IAA production within species and/or strains of the

same species was previously reported (Zahir *et al.*, 2000; Egamberdiyeva, 2008; Abbasi *et al.*, 2011). In a study conducted by Poonguzhali *et al.* (2008), PGPR from the rhizosphere of *Brassica campestris* L. were shown to produce 6.02–29.75 μg mL^{-1} of IAA while Mehnaz *et al.* (2010) reported IAA production of 0.28–5.49 μg mL^{-1} by PGPR from the maize (*Zea mays* L.) rhizosphere. Differences in the production of IAA among bacterial strains can be attributed to various biosynthetic pathways, location of the genes involved, regulatory sequences, and the presence of enzymes to convert active free IAA into conjugated forms (Patten and Glick, 1996).

Five out of twenty-three isolates tested exhibited the potential for P solubilization, an important characteristic for inoculant development. Generally, it has been observed that the organisms isolated from the rhizosphere of legumes are more efficient in solubilizing phosphates than those from the non-rhizosphere soil or from the root zones of non-legumes (Hameed *et al.*, 2004). The ability of PGPR strains to solubilize insoluble P and convert it to an available form is an important characteristic under conditions where P is a limiting factor for crop production.

Application of PGPR under *in vitro* and hydroponic conditions showed that almost all isolated strains possessed properties that would be beneficial in stimulating lentil growth. Yet, there were differences among the bacterial strains in their ability to sustain plant growth. For example, strains LCA-1 and LCA-5 were the most effective in increasing shoot and root growth. The superiority of these two strains over the remaining isolates was due to their higher capacity for IAA production and P solubilization. The growth-promoting potential and variability among strains in this study is almost similar to that reported earlier (Oswald *et al.*, 2010).

The response in lentil growth to the different PGPR strains under greenhouse conditions (pot experiment) was similar to that found under *in vitro* and hydroponic conditions. However, the rate and extent of the growth under *in vitro* and hydroponic conditions were markedly higher. The growth characteristics, *i.e.*, shoot and root lengths, shoot and root fresh weights, and shoot and root dry weights, were measured. Averaged over different strains, relative increase in these growth characteristics due to PGPR were 49%, 17%, 33%, 22%, 24% and 40%. This increased growth of the inoculated plants might be due to the higher N accumulation by bacterial N, N_2 fixation and better root growth. The shoot and root growth, nodule numbers, and nutrient uptake of peas (*Pisum sativium*) and soybean were increased when seeds were inoculated by rhizobacteria (Egamberdiyeva and Höflich, 2004; Figueiredo *et al.*, 2008). Egamberdiyeva (2008) reported that the bacterial strains increased the root, shoot, and dry weights of peas by more than 26% compared to the control. Zahir *et al.* (2003) conducted a series of laboratory experiments on the rhizobial inoculation of wheat and reported increases in root elongation up to 20%, root dry weight up to 13%, shoot elongation up to 38%, and shoot dry weight up to 36%. Similar rates of increase in wheat due to the application of PGPR have also been reported by Çakmakçi *et al.* (2007). Similarly, the increases in dry matter and grain yield due to PGPR was 28%–29% over the control.

The mechanisms of plant growth stimulation by associative bacteria are most probably related to greater mobilization of nutrients, through the production of growth-promoting substances such as IAA and cytokinins (Vikram *et al.*, 2007). The role of auxins and cytokinins in enhancing plant cell division and root development is well known (Arshad and Frankenberger, 1993). Isolated bacterial strains produced auxin (IAA) and stimulated root growth in our study. The increase in root elongation due to IAA production was confirmed in this study by the significant positive correlation between IAA and root elongation ($R^2 = 0.67$) (Fig. 2a).

The increased nodulation number and mass by PGPR confirmed the selection of compatible strains based on growth performance. Averaged over different strains, the relative increase in nodule numbers by PGPR strains was about 150% over the control. Our results were in agreement with the findings of Egamberdiyeva (2008), who reported that isolated bacterial strains increased the main root nodulation of peas by 35%–75% and the nodulation of lateral roots significantly increased by 200%. We found that the tested isolates were IAA producers as well as P solubilizer. Availability of P in soils is known to initiate nodule formation, increases the number of nodules and is essential for the development and functioning of nodules (Waluyo *et al.*, 2004; Tagoe *et al.*, 2008). A significant and positive relationship existed between root nodulation and seed yield ($R^2 = 0.82$), demonstrating the importance of nodulation in legumes. Figueiredo *et al.* (2008) reported that in legume root nodules, IAA activates the enzyme H^+-ATPase, which is fundamental for energy production in the nodules, and the correlation between the two (IAA and nodule number) in the present study was significant ($R^2 = 0.54$) (data not shown).

The effects of PGPR on N and P contents in lentil when compared with uninoculated control are conclusive and strongly indicate a possible role of PGPR in providing N and P. In our study, PGPR inoculation increased the N content in the roots, shoots and seeds of lentil by an average of 35%, 29% and 25%, respectively. This increase in N content consequently increased N uptake, which certainly was due to the N_2 fixation by the PGPR, since no fertilizer N was applied in the treatments. The N fixed in plant nodules is partly used by the plant as indicated by the rise in N content in the roots and shoots. The increases in N content and N uptake by plants due to inoculation are in agreement with some previous studies (Hafeez *et al.*, 2000; Cong *et al.*, 2010; Osman *et al.*, 2010; Abbasi *et al.*, 2011). Increases in total N content and plant uptake could be due to nitrogen fixation and nitrate reductase activities of PGPR, or to the uptake of NH_4^+ and amino acids produced by PGPR (Osman *et al.*, 2010).

The increases in P content and P uptake in lentil may partially be attributed to the production of a variety of organic acids by the tested PGPR, which decreased the soil pH, leading to the conversion of the non-available P into the available P. Soil microorganisms can also make P available to the plant by producing chelating substances, which leads to solubilization of phosphates (Osman *et al.*, 2010). Phosphate-solubilizing *Pseudomonas fluorescens* was reported to enhance growth, yield, and N and P contents in the shoots and seeds of peanut (Dey *et al.*, 2004). Cong *et al.* (2010) explained that the higher nutrient uptake was due to inoculation with *Rhizobium and Azospirillum* that might attribute to morphological changes in the plant roots, especially increases in the root number, length and thickness.

The uptake of both N and P in lentil played a significant role in lentil seed yield as a significant, positive correlation existed between N uptake and seed yield ($R^2 = 0.77$) and between P uptake and seed yield ($R^2 = 0.82$).

CONCLUSIONS

Beneficial bacterial isolates from the root nodules of lentil grown on the nutrient-deficient soils of the arid/semi-arid region of Punjab, Pakistan produced different biologically active compounds. These microbial strains exhibited substantial plant growth-promoting abilities in addition to nutrient accumulation in lentil. The results of our study indicated that PGPR inoculation significantly increased the root properties (length and mass), shoot characteristics (length and weight), nodulation (number and mass), yield attributes and N and P uptake by the plant. This study also indicated that most of the tested PGPR strains, especially strains LCA-1 and LCA-5, may be used as crop growth regulators or biofertilizer to improve the growth and nutrient accumulation of lentil. The tested isolates were shown to colonize the rhizosphere, produce IAA and solubilize P in the laboratory and greenhouse. Tests under the full range conditions in the field are needed, the research must be proactive, and field trials must be established across a broad range of soil and environment. Also, further studies are needed to test the effects of PGPR inoculation on soil quality over the long term to determine economic as well as biological benefits.

REFERENCES

Abbasi, M. K., Sharif, S., Kazmi, M., Sultan, T. and Aslam, M. 2011. Isolation of plant growth promoting rhizobacteria from wheat rhizosphere and their effect on improving growth, yield and nutrient uptake of plants. *Plant Biosyst.* **145**: 159–168.

Arshad, M. and Frankenberger, J. R. W. P. 1993. Microbial production of plant growth regulators. *In* Metting, F. B. (ed.) Soil Microbial Ecology—Application in Agricultural and Environmental Management. Marcel Dekker, New York. pp. 307–347.

Athar, M. 1998. Drought tolerance by lentil rhizobia (*Rhizobium leguminosarum*) from arid and semiarid areas of Pakistan. *Lett. Appl. Microbiol.* **26**: 38–42.

Bashan, Y. and de-Bashan, L. E. 2005. Bacteria/plant growth-promotion. *In* Hillel, D. (ed.) Encyclopedia of Soils in the Environment. Elsevier, Oxford. pp. 103–115.

Bisen, P. S. and Verma, K. 1994. Handbook of Microbiology. 1st edition. CBS Publishers and Distributors, Dedhli.

Bremner, J. M. and Mulvaney, C. S. 1982. Nitrogen—total. *In* Page, A. L. *et al.* (eds.) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd edition. ASA and SSSA, Madison. pp. 595–624.

Çakmakçi, R., Erat, M., Erdoğan, Ü. and Dönmez, M. F. 2007. The influence of plant growth-promoting rhizobacteria on growth and enzyme activities in wheat and spinach plants. *J. Plant Nutr. Soil Sci.* **170**: 288–295.

Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A. and Young, C. C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* **34**: 33–41.

Cong, P. T., Dung, T. D., Hien, T. M., Hien, N. T., Choudhury, A. T. M. A., Kecskés, M. L. and Kennedy, I. R. 2009. Inoculant plant growth-promoting microorganisms enhance utilisation of urea-N and grain yield of paddy rice in southern Vietnam. *Eur. J. Soil Biol.* **45**: 52–61.

Dey, R., Pal, K. K., Bhatt, D. M. and Chauhan, S. M. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiol. Res.* **159**: 371–394.

Egamberdiyeva, D. 2005. Plant-growth-promoting rhizobacteria isolated from a Calcisol in a semi-arid region of Uzbekistan: biochemical characterization and effectiveness. *J. Plant Nutr. Soil Sci.* **168**: 94–99.

Egamberdiyeva, D. 2008. Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loamy sand soil. *Turk. J. Biol.* **32**: 9–15.

Egamberdiyeva, D. and Höflich, G. 2004. Effect of plant growth-promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region of Uzbekistan. *J. Arid Environ.* **56**: 293–301.

Esitken, A., Yildiz, H. E., Ercisli, S., Donmez, M. F., Turan, M. and Gunes, A. 2010. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Sci. Hort.* **124**: 62–66.

Figueiredo, M. V. B., Martinez, C. R., Burity, H. A. and Chanway, C. P. 2008. Plant growth-promoting rhizobacteria for improving nodulationand nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). *World J. Microbiol. Biotechnol.* **24**: 1187–1193.

Gahoonia, T. S., Omar, A., Sarker, A., Rahman, M. M. and Erskine, W. 2005. Root traits, nutrient uptake, multi-location grain yield and benefit-cost ratio of two lentil (*Lens culinaris*, Medikus.) varieties. *Plant Soil.* **272**: 153–161.

Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* **41**: 109–117.

Hafeez, F. Y., Shah, N. H. and Malik, K. A. 2000. Field evaluation of lentil cultivars inoculated with *Rhizobium leguminosarum* bv. *viciae* strains for nitrogen fixation using nitrogen-15 isotope dilution. *Biol. Fert. Soils.* **31**: 65–69.

Hameed, S., Yasmin, S., Malik, K. A., Zafar, Y. and Hafeez, F. Y. 2004. *Rhizobium, Bradyrhizobium* and *Agrobacterium* strains isolated from cultivated legumes. *Biol. Fert. Soils.* **39**: 179–185.

Igual, J. M., Valverde, A., Cervantes, E. and Velázquez, E. 2001. Phosphate-solubilizing bacteria as inoculants for agriculture: Use of updated molecular techniques in their study. *Agronomie.* **21**: 561–568.

Jackson, M. L. 1967. Soil Chemical Analysis. Prentice-Hall of India Pvt. Ltd, Delhi.

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K. and Barea, J. M. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol. Fert. Soils.* **37**: 1–16.

Keeney, D. R. and Nelson, D. W. 1982. Nitrogen—inorganic forms. *In* Page, A. L. *et al.* (eds.) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd edition. ASA and SSSA, Madison. pp. 643–693.

Khan, A. G. 2005. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *J. Trace Elem. Med. Biol.* **18**: 355–364.

Lucy, M., Reed, E. and Glick, B. R. 2004. Application of free living plant growth-promoting rhizobacteria. *Anton. Leeuw.* **86**: 1–25.

Malik, B. A., Verma, M. M., Rahman, M. M. and Bhattarai, A. N. 1988. Production of chickpeas, lentil, pea and faba bean in South-East Asia. *In* Summerfield, R. J. (ed.) World crops: Cool Season Legumes. Kluwer, Boston. pp. 1095–1110.

Marques, A. P. G. C., Pires, C., Moreira, H., Rangel, A. O. S. S. and Castro, P. M. L. 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biol. Biochem.* **42**: 1229–1235.

Mehnaz, S., Kowalik, T., Reynolds, B. and Lazarovits, G. 2010. Growth promoting effects of corn (*Zea mays*) bacterial isolates under greenhouse and field conditions. *Soil Biol. Biochem.* **42**: 1848–1856.

Murphy, J. and Riley, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* **27**: 31–36.

Nelson, D. W. and Sommers, L. E. 1982. Total carbon. Organic carbon and organic matter. *In* Page, A. L. *et al.* (eds.) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd Edition. ASA and SSSA, Madison. pp. 539–579.

Nelson, L. M. and Child, J. J. 1981. Nitrogen fixation and hydrogen metabolism by *Rhizobium leguminosaru*m isolates in pea root nodules. *Can. J. Microbiol.* **27**: 1028–1034.

Okon, Y., Albercht, S. L. and Burris, R. H. 1977. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Appl. Environ. Microbiol.* **33**: 85–88.

Olsen, S. R. and Sommers, L. E. 1982. Phosphorus. *In* Page, A. L. *et al.* (eds.) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd edition. ASA and SSSA, Madison. pp. 403–430.

Osman, M. E. H., El-Sheekh, M. M., El-Naggar, A. H., Saly, F. and Gheda, S. F. 2010. Effect of two species of cyanobacteria as biofertilizers on some metabolic activities, growth, and yield of pea plant. *Biol. Fert. Soils.* **46**: 861–875.

Oswald, A., Velez, P. C., Dávila, D. Z. and Pineda, J. A. 2010. Evaluating soil rhizobacteria for their ability to enhance plant growth and tuber yield in potato. *Ann. Appl. Biol.* **157**: 259–271.

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

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

Poonguzhali, S., Madhaiyan, M. and Sa, T. M. 2008. Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and phosphorus utilization of plants. *J. Microbiol. Biotechnol.* **18**: 773–777.

Rhoades, J. D. 1982. Soluble salts. *In* Page, A. L. *et al.* (eds.) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd edition. ASA and SSSA, Madison. pp. 167–179.

Rosas, S. B., Avanzini, G., Carlier, E., Pasluosta, C., Pastor, N. and Rovera, M. 2009. Root colonization and growth promotion of wheat and maize by *Pseudomonas aurantiaca* SR1. *Soil Biol. Biochem.* **41**: 1802–1806.

Şhin, F., Çakmakçi, R. and Kantar, F. 2004. Sugar beet and barley yields in relation to inoculation with N_2-fixing and phosphate solubilizing bacteria. *Plant Soil.* **265**: 123–129.

Simard, R. R. 1993. Ammonium acetate-extractable elements. *In* Carter, M. R. (ed.) Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton. pp. 39–42.

Somasegaran, P., Hoben, H. and Halliday, J. 1982. The NIFTAL

(Nitrogen Fixation in Tropical Agricultural Legumes). University of Hawaii, Hawaii.

Steel, R. G. D. and Torrie, J. H. 1980. Principles and Procedure of Statistics. McGrawHill Book Co., New York.

Tagoe, S. O., Horiuchi, T. and Matsui, T. 2008. Effects of carbonized and dried chicken manures on the growth, yield, and N content of soybean. *Plant Soil.* **306**: 211–220.

Tien, T. M., Gaskins, M. H. and Hubbell, D. H. 1979. Plant growth substances produced by *Azosprillum brazilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Appl. Environ. Microbiol.* **37**: 1016–1024.

Vikram, A., Hamzehzarghani, H., Al-Mughrabi, K. I., Krishnaraj, P. U. and Jagadeesh, K. S. 2007. Interaction between *Pseudomonas fluorescens* FPD-15 and *Bradyrhizobium* spp. in peanut. *Biotechnology.* **6**: 292–298.

Vincent, J. M. 1970. The cultivation, isolation and maintenance of rhizobia. *In* Vincent, J. M. (ed.) A Manual for the Practical Study of the Root-Nodule Bacteria. Blackwell Scientific Publications, Oxford. pp. 1–13.

Waluyo, S. H., Lie, T. A. and Mannetje, L. 2004. Effect of phosphate on nodule primordia of soybean (*Glycine max* Merrill) in acid soils in rhizotron experiments. *Indones. J. Agr. Sci.* **5**: 27–44.

Winaro, R. and Lie, T. A. 1979. Competition between Rhizobium strains in nodule formation: interaction between nodulating and non-nodulating strains. *Plant Soil.* **51**: 135–142.

Zahir, Z. A., Abbas, S. A., Khalid, M. and Arshad, M. 2000. Substrate dependent microbially derived plant hormones for improving growth of maize seedlings. *Pakistan. J. Biol. Sci.* **3**: 289–291.

Zahir, Z. A., Arshad, M. and Frankenberger, W. T. 2003. Plant growth promoting rhizobacteria: Applications and perspectives in agriculture. *Adv. Agron.* **81**: 97–168.