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Effect of plant growth promoting Rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum* L.) under in *Vitro* conditions

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ABSTRACT : Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. Five isolates of PGPR designated as Pseudomonas aeruginosa strain BHUPSB02, Pseudomonas putida strain BHUPSB04, Bacillus subtilis strain BHUPSB13, Paenibacillus polymyxa strain BHUPSB17 and Bacillus boronophilus strain BHUPSB19 were successfully isolated and characterized through 16S rDNA gene sequencing. Subsequently, an experiment was conducted under plant growth chamber where chickpea plants are grown in plastic cups containing soil and mixed with isolates of PGPR to investigate the effect of PGPR on the growth of chickpea plant. Isolates of PGPR induced production of plant hormones (indole acetic acid), phosphate solubilization and ammonia production to enhanced plant growth. Most of isolates resulted in a significant increase in shoot length, root length and dry matter production of shoot and root of chickpea seedlings. Therefore, present study suggests that PGPR isolates viz. BHUPSB02, BHUPSB04 and BHUPSB13 may be used as biofertilizers to enhance the growth and productivity of chickpea.

Key words: Chickpea, PGPR, IAA, Phosphate solubilization

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. A large array of bacteria including species of Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus and Serratia have reported as PGPR to enhance plant growth (Kloepper *et al*., 1989; Okon and Labandera-Gonzalez, 1994; Glick, 1995). The direct growth promotion of plants by PGPR entails either providing the plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect plant growth promotion occurs by PGPR due to preventing deleterious effects phytopathogenic microorganisms. The exact mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indoleacetic acid, gibberellic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993; Glick, 1995), (ii) asymbiotic N_2 fixation (Boddey and Dobereiner, 1995), (iii) antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), antibiotics (Shanahan *et al*., 1992) and cyanide (Flaishman *et al*., 1996), (iv) solubilization of mineral phosphates and other nutrients (De Freitas *et al*., 1997; Gaur, 1990). In addition to these traits, plant growth promoting bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizospheric soil (Cattelan *et al*., 1999). The good results obtained in vitro cannot always be dependably reproduced under field conditions (Chanway and Holl, 1993; Zhender *et al*., 1999). The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects on plant. The environmental factors include climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil. To achieve the maximum growth promoting interaction between PGPR and nursery seedlings it is important to discover how the rhizobacteria exerting their effects on plant and whether the effects are altered by various environmental factors, including the presence of other microorganisms (Bent *et al*., 2001). Therefore, it is necessary to develop efficient strains for chickpea production. One possible approach is to explore soil microbial diversity for PGPR having combination of PGP activities and well adapted to particular soil environment. So keeping in view the above constrains, the present study was designed to screen certain rhizospheric bacterial isolates belonging to the genera *Pseudomonas* and *Bacillus*, for their multiple plant growth promoting activities.

Chickpea (*Cicer arietinum*) is the most important staple food in several developing countries and chemical fertilizers are the most important input required for chickpea cultivation. In order to make its cultivation sustainable and less dependent on chemical fertilizers, it is important to know now to use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that could contribute to the improvement of

chickpea growth. Thus the aim of this study was to determine the effect of plant growth promoting rhizobacteria for improvement of germination and plant Growth of chickpea (*Cicer arietinum* L.).

MATERIAL AND METHODS

Bacterial culture and Identification. The rhizospheric soil samples were collected from chickpea growing field of Eastern Uttar Pradesh, India. The bacterial strains were isolated on their respective media; Pseudomonas on King's B medium and Bacillus on nutrient agar. *Bacillus* and *Pseudomonas* were biochemically characterized as per standard methods of Cappuccino and Sherman (1992) and Aneja (2003). Bacterial cultures were also molecularly characterized by 16S rDNA partial gene sequencing. Bacterial cultures were maintained on the respective slants media and store at 4°C further use. All media and chemical ingredient were purchased from Hi-media Pvt. Ltd. Mumbai.

Characterization of rhizobacteria for plant growth promoting activities

Production of Indole acetic acid. Indole acetic acid (IAA) production was detected as described by Brick *et al*., (1991). *Pseudomonas* and *Bacillus* cultures were grown separately on their respective media with 100 and 200 µg/ml of L-tryptophan at 30°C for 48 h. Fully grown cultures were centrifuged at 8000 rpm for 10 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink colour indicates IAA production.

Phosphate solubilization by bacteria. All isolates were first screened on Pikovskaya's agar plates for phosphate solubilization as described by Gaur (1990). Bacterial cultures were inoculated on centre of agar plate through inoculation loop under aseptic condition. Inoculated plates were incubated for 3 days at 30°C. Halo zone was obtained on Pikovskaya's agar plates. This halo zone showed positive phosphate solubilization ability.

Production of ammonia. Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48–72 h at 36 ± 2°C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

Chickpea seed germination and plant growth. Seeds of chickpea (*Cicer arietinum* L.) cultivar C-235 were obtained from Indian Institute of Pulse Research (IIPR), Kalyanpur, Kanpur, Uttar Pradesh, India. Healthy seeds were surface sterilized with 0.1% $HgCl_2$ for 2 min and rinsed six times with sterile distilled water. Bacillus and *Pseudomonas* were grown in respective broth on shaking incubator (180 rpm) at 28 ± 2°C for 24 h. The surface sterilized seeds of chickpea were inoculated in broth culture of *B. subtilis, P. polymyxa, B. boronophillus, P. aeruginosa* and *P. putida* for 30 min. Five Seeds were sown at 5 cm depth in 250 g sterilized soil containing plastic pot. A control treatment was also maintained without inoculated seed. Pots were kept in to plant growth chamber for 21 days. The experiment was setup in 3 replication with 6 treatments. All seeds were germinated. After 21days, chickpea plants were harvested. Shoot and root length were recorded in centimeter of each plant. Then plants were dried in an oven at 65°C for 3 days. After this shoot and root dry weight were recorded in gram.

Statistical analysis. The data were analyzed statistically by using SPSS software version 12.0. Comparisons of means were performed by the Fisher's Protected LSD test at $P \leq 0.05$.

RESULT

Isolation and Characterization of PGPR. Five bacterial isolates were successfully isolated from the rhizosphere of chickpea. They were molecularly characterized by 16S rDNA partial gene sequencing and their accession number were allotted as GU124826 (*Pseudomonas aeruginosa* strain BHUPSB02), GU124834 (*Pseudomonas putida* BHUPSB04), GU124818 (*Bacillus subtilis* strain BHUPSB13), GU124838 (*Paenibacillus polymyxa* strain BHUPSB17) and GU124815 (*Bacillus boronophilus* strain BHUPSB19) by National Centre for Biotechnology Information (NCBI) GenBank.

Plant growth promoting activities. Screening results of PGPR traits are depicted in Table 1. Bacterial isolates of PGPR were tested for the quantitative estimation of IAA in presence of different concentrations of tryptophan. Production of IAA as tryptophan is a key precursor in IAA biosynthesis. IAA production was increased on increasing the concentration of tryptophan from 100 to 200 µg ml^{-1}. Bacterial strain P. putida BHUPSB04 showed maximum significant concentration of IAA 25.65 µg ml^{-1} followed by *P. aeruginosa* BHUPSB02 (21.35 µg ml^{-1}), B. subtilis BHUPSB13 (16.23 µg ml^{-1}), *P. polymyxa* BHUPSB17 (15.79 µg ml^{-1}) and *B. boronophillus* BHUPSB19 (11. µg ml^{-1}) at 100 µg ml^{-1} trptophan in broth after 48 hours incubation. Similar trend of IAA production was also recorded in bacterial strain at 200 µg ml^{-1} trptophan in broth after 48 hours incubation.

Table 1 : Production of IAA, phosphate solubilization and production ammonia by PGPR.

PGPR strain	IAA (µg ml^{-1}) at tryptophan		Phosphate solubilization	Ammonia
	100 µg ml^{-1}	200 µg ml^{-1}		
P. aeruginosa	21.35^{c}	35.36^{c}	+++	+
P. putida	25.65^{d}	48.46^{d}	++	+
B. subtillis	16.23^{b}	36.38^{c}	++	+
P. polymyxa	15.79^{b}	29.30^{b}	++	+
B. boronophillus	11.71^{a}	25.63^{a}	+	+

(+) positive, (–) negative test, IAA-Indole acetic acid; Mean values in each column with the same superscript (s) do not differ significantly by LSD (P=0.05).

Maximum significant IAA production was recorded in *P. putida* strain BHUPSB04 as compared others bacterial strains at both concentration of tryptophan. Studies on agar plates revealed that phosphate solubilizing microorganisms formed halo zones by solubilizing suspended tricalcium phosphate on Pikovskaya's agar plates. Generally, halo zone was formed around the bacterial colony. The results showed that the bacterial *P. aeruginosa* strain BHUPSB02 was formed maximum halo zone around the colony followed by BHUPSB13 and BHUPSB17 as compare to BHUPSB19. All strains of bacteria were showed positive for ammonia production

Length and Dry Weight of Shoot and Root. The PGPR isolates significantly affected the length of chickpea seedlings. Results reveal that the shoot length increased in PGPR treated plants over uninoculated control. The highest shoot length 15.6 cm plant^{-1} was recorded in treatment of *P. putida* BHUPSB04 isolate followed by statistically at par values due to isolates *P. aeruginosa* BHUPSB02 (14.5 cm plant^{-1}). *B. subtilis* BHUPSB13, *P. polymyxa* BHUPSB17 and *B. boronophillus* BHUPSB19 showed significantly higher shoot length over control. A significant increase in shoot dry matter of chickpea seedling was observed in response to PGPR isolates. The highest shoot dry matter was recorded in isolate BHUPSB02 (9.3 mg plant^{-1}) followed by BHUPSB04 (9.1 mg plant^{-1}) over control. Also PGPR strains BHUPSB13, BHUPSB17 and BHUPSB19 were showed statistically significant increase of shoot dry weight over control. Root length ranged from 3.87 to 9.2 cm plant^{-1}. The isolate BHUPSB02 produced the highest root length (9.2 cm plant^{1}) followed by BHUPSB04 and BHUPSB17 in comparison to control and other isolates. BHUPSB13 and BHUPSB19 also showed significant increase in root length, respectively over control (Table 2).

Table 2 : Effect of plant growth promoting rhizobacteria on the growth of chickpea plants.

Treatment	Shoot length plant^{-1} (cm)	Root length plant^{-1} (cm)	Shoot dry weight plant^{-1} (mg)	Root dry weight plant^{-1} (mg)
Control	9.5 ± 1.2^{a*}	3.87 ± 1.1^{a}	6.5 ± 0.6^{a}	4.6 ± 0.3^{a}
P. aeruginosa	14.5 ± 1.2^{c}	9.2 ± 1.4^{d}	9.3 ± 0.8^{d}	7.5 ± 0.8^{cd}
P. putida	15.6 ± 1.1^{cd}	8.4 ± 1.1^{cd}	9.1 ± 1.1^{d}	7.3 ± 1.0^{c}
B. subtillis	12.2 ± 1.6^{b}	6.7 ± 1.2^{b}	8.5 ± 0.7^{c}	6.5 ± 0.8^{c}
P.polymyxa	12.6 ± 2.1^{b}	7.5 ± 1.4^{c}	7.8 ± 1.0^{bc}	6.3 ± 1.2^{c}
B.boronophillus	11.8 ± 1.8^{b}	5.8 ± 0.8^{b}	7.1 ± 1.1^{b}	5.6 ± 0.8^{b}

*Values are the mean ± SD, Mean values in each column with the same superscript (s) do not differ significantly by LSD ($P \leq 0.05$).

A significant variation in root dry weight was observed in response to different PGPR isolates. The isolate BHUPSB02 produced the highest root dry weight (7.5 mg plant^{-1}) followed by BHUPSB04 (7.3 mg plant^{-1}), BHUPSB13 (6.5mg plant^{-1}) and BHUPSB17 (6.3 mg plant^{-1}) in comparison to control and other isolates. In this study, all isolates significantly increased shoot length, root length and dry matter production of shoot and root of seedlings.

DISCUSSION

The ability of bacteria to produce IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant. Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities (Arshad and Frankenberger, 1993; Glick, 1995). Production of IAA by *Bacillus* and *Pseudomonas* is a general characteristic of our test isolates. Bacterial strain *Pseudomonas putida* BHUPSB04 showed maximum significant concentration of IAA followed by *P. aeruginosa* BHUPSB02, *B. subtilis* BHUPSB13, *P. polymyxa* BHUPSB17 and *B. boronophillus*. Similarly higher level of IAA production by Pseudomonas was recorded by other workers (Xie *et al*., 1996). Another important trait of PGPR, that may indirectly influence the plant growth, is the production of ammonia. All the isolates were able to produce ammonia. Phosphate solubilization was most frequently encountered by *P. aeruginosa* strain BHUPSB02 followed by *P. putida* strain BHUPSB04, *B. subtilis* BHUPSB13, *P. polymyxa* BHUPSB17 and least by *B. boronophillus* BHUPSB19. *P. aeruginosa* produces largest halos around their colonies within 2 days of incubation than other isolates. Several species of fluorescent pseudomonas such as *P. fluorescens* NJ101 (Bano and Musarrat, 2004), *P. aeruginosa* (Jha *et al*., 2009) and *Bacillus* sp. (Ahmad *et al*., 2008) were reported as good phosphate solubilizers. Our results suggested that PGPR are able to enhance the production of IAA, solubilization of phosphorus and ammonia production, thereby improving growth of chickpea plant. *Pseudomonas putida* and *Pseudomonas aeruginosa* were observed most efficient inoculations for enhancement of shoot and root length and dry matter followed by *Bacillus subtilis*, *Paenibacillus polymyxa* and *Bacillus boronophillus* over control. Other workers have been reported the enhancement of shoot and root length and dry matter by inoculation of plant growth promoting rhizobacteria for chickpea (Mishra *et al*., 2010). Plant growth of chickpea was enhanced due proper root colonization of plant growth promoting rhizoabcteria which provide plant hormones (IAA), phosphorus and ammonia to plant. Similarly PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to chickpea that represent a possible mechanism of plant growth promotion under field condition (Verma *et al*., 2001; Verma *et al*., 2010).The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable chickpea cultivation in India and other developing countries. Further investigations, including efficiency test under green house and field conditions needed to clarify the role of PGPR as biofertilizers that exert beneficial effects on plant growth and development.

CONCLUSION

In the present study we have found the five bacterial strains was significantly enhanced biomass production of chickpea. Therefore we suggest that the use of PGPR isolates BHUPSB02, BHUPSB04 and BHUPSB13 as effective biofertilizers might be beneficial for chickpea cultivation.

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REFERENCES

Ahmad, F., Ahmad, I., and Khan M.S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiology Research*, **163:** 173–181.

Aneja, K.R. (2003). Experiments in Microbiology, Plant pathology and Biotechnology, fourth ed. New Age International Publishers, Daryaganj, New Delhi.

Arshad, M. and Frankenberger Jr., W.T. (1993). Microbial production of plant growthregulators. In: Blaine, F., Metting, Jr. (Eds.), Soil Microbial Ecology. Marcel and Dekker, Inc., New York, pp. 307–347.

Bano, N. and Musarrat, J. (2004). Characterization of a novel carbofuran degrading Pseudomonas sp. with collateral biocontrol and plant growth promoting potential. FEMS *Microbiology Letter*, **23:** 13-17.

Bent, E., Tuzun, S., Chanway, C.P. and Enebak, S. (2001). Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. *Candian Journal of Microbiology*, **47:** 793–800.

Boddey, R.M. and Dobereiner, J. (1995). Nitrogen fixation associated with grasses and cereals: recent progress and perspectives for the future. *Fertilizer Research*, **42:** 241–250

Brick, J.M., Bostock, R.M. and Silverstone, S.E. (1991). Rapid in situ assay for indoleacetic acid production by bacteria immobilized on nitrocellulose membrane. *Applied and Environmental Microbiology*, **57:** 535–538.

Cappuccino, J.C. and Sherman, N. (1992). In: Microbiology: A Laboratory Manual, third ed. Benjamin/cummings Pub. Co. New York, pp. 125–179.

Cattelan, A.J., Hartel, P.G. and Fuhrmann, J.J. (1999). Screening of plant growth promoting rhizobacteria to promote early soybean growth. *Soil Science Society of American Journal*, **63:** 1670–1680.

Chanway, C.P. and Holl, F.B. (1993). First year yield performance of spruce seedlings inoculated with plant growth promoting rhizobacteria. *Canadian Journal of Microbiology*, **39:** 1084–1088.

De Freitas J.R., Banerjee, M.R. and Germida, J.J. (1997). Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). *Biology and Fertility of Soils*, **24:** 358–364.

Flaishman, M.A., Eyal, Z.A., Zilberstein, A., Voisard, C. and Hass, D. (1996). Suppression of Septoria tritci blotch and leaf rust of wheat by recombinant cyanide producing strains of Pseudomonas putida. *Molecular and Plant Microbe Interaction*, **9:** 642–645.

Gaur, A.C. (1990). Physiological functions of phosphate solubilizing micro-organisms. In: Gaur, A.C. (Ed.), Phosphate Solubilizing Micro-organisms as Biofertilizers. Omega Scientific Publishers, New Delhi. pp. 16–72.

Glick B. R. (1995). The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology*, **41:** 109–117.

Jha, B.K., Pragash, M.G., Cletus, J., Raman, G. and Sakthivel, N. (2009). Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent pseudomonad strains, Pseudomonas aeruginosa, P. plecoglossicida and P. mosselii. *World Journal of Microbiology and Biotechnology*, **25:** 573-581.

Kloepper, J.W., Lifshitz, R. and Zablotowicz, R.M. (1989). Free-living bacteria inocula for enhancing crop productivity. *Trends Biotechnolology*, **7:** 39-44.

Mirza, M.S., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P. and Malik, K.A. (2001). Isolation, partial characterization and effect of plant growth promoting bacteria on micropropagated sugarcane in vitro. *Plant and Soil*, **237:** 47-54.

Okon, Y. and Labandera-Gonzalez, C.A. (1994). Agronomic applications of Azospirillum. In: Ryder, M.H., Stephens, P.M., BOWen, G.D. (Eds.), Improving Plant Productivity with Rhizosphere Bacteria. *Commonwealth Scientific and Industrial Research Organization*, Adelaide, Australia, pp. 274–278.

Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiology*, **17:** 362–70.

Scher, F.M. and Baker, R. (1982). Effect of Pseudomonas putida and a synthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. *Phytopathology*, **72:** 1567–1573.

Shanahan, P., O'Sullivan, D.J., Simpson, P., Glennon, J.D. and O'Gara, F. (1992). Isolation of 2,4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Applied Environmental Microbiology*, **58:** 353–358.

Verma, S.C., Ladha J.K. and Tripathi, A.K. (2001). Evaluation of plant growth promoting and coloization, ability of endophytic diazotrophs from deep water rice. *Journal of Biotechnology*, **91:** 127-141.

Verma, J.P., Yadav, J. and Tiwari, K.N. (2010). Application of Rhizobium sp. BHURC01 and plant growth promoting rhizobactria on nodulation, plant biomass and yields of chickpea (Cicer arietinum L.). *International Journal of Agriculture Research*, **5:** 148-156.

Xie, H., Pasternak, J.J. and Glick, B.R. (1996). Isolation and characterization of mutants of plant growth promoting rhizobacterium Pseudomonas putida GR 12-2 that over produce indoleacetic acid. *Current Microbiology*, **32:** 67–71.

Zhender, G.W., Yao, C., Murphy, J.F., Sikora, E.R., Kloepper, J.W., Schuster, D.J. and Polston, J.E. (1999). Microbeinduced resistance against pathogens and herbivores: evidence of effectiveness in agriculture. In: Agarwal, A.A., Tuzun, S., Bent., E. (eds.), Induced Plant Defenses Against Pathogens and Herbivores: Biochemistry, Ecology and Agriculture. APS Press, St Paul, MN, p. 33.