Transposon-derived mutants of *Pseudomonas* strains altered in indole acetic acid production: Effect on nodulation and plant growth in green gram (*Vigna radiata* L.)

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ABSTRACT

Pseudomonas strains CPS63 and MPS78 produced 18.1 and 19.2 $\mu\text{g ml}^{-1}$ of indole acetic acid (IAA) at 2 days of growth. Low producers as well as over producer IAA mutants were derived from *Pseudomonas* strains CPS63 and MPS78 by Tn5 mutagenesis. Inoculation of selected IAA mutants on green gram (*Vigna radiata*) seeds showed stunting effect on root and shoot growth of seedlings at 5 and 10 days. Coinoculation studies of *Pseudomonas* strains with *Bradyrhizobium* strain S24 and IAA over producer mutants, i.e. CPS63-20 and MPS78-107 resulted in more nodule formation in green gram as compared to wild type *Bradyrhizobium* strain at 50 days of growth. Significant gains in plant dry weights, i.e. 2.0-3.06 times increase in comparison to uninoculated control plants, were observed on coinoculation of IAA low producer mutants CPS63-27, MPS78-92 and MPS78-166. Better performance of IAA low producer mutants in enhancing plant dry weights of green gram than to IAA over producer mutants indicated that overproduction of IAA by bacteria might inhibit shoot growth in green gram. [Physiol. Mol. Biol. Plants 2008; 14(4) : 315-320] E-mail : sindhuss@hau.ernet.in

Key words : *Pseudomonas*, Indole acetic acid, Green gram, Transposon, Nodulation

INTRODUCTION

Beneficial free-living soil bacteria are generally referred as plant growth-promoting rhizobacteria (PGPR) and often found in association with roots of different plants. These bacteria belonging to genera *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Enterobacter*, *Rhizobium* and *Serratia* have a vast potential for use in agriculture as bioinoculants (Sindhu *et al.*, 2002; Ahmad *et al.*, 2008). PGPR can positively influence seedling emergence and plant growth through direct effects by increasing mineralization of insoluble nutrients and subsequent enhancement of their uptake by plants, by production of plant growth-stimulating hormones (i.e. indole acetic acid, gibberellins, cytokinins and ethylene) and by enhancing nodulation as well as nitrogen fixation in legume plants (Dey *et al.*, 2004; Ahmad *et al.*, 2008). PGPR have also been reported to promote plant growth by reducing population of root

colonizing phytopathogens (Sindhu *et al.*, 1999; Goel *et al.*, 2006; Weller, 2007) and through bioremediation of metal-contaminated soils (Rajkumar and Freitas, 2008).

A large number of PGPR strains have been found to produce indole acetic acid (IAA) (Patten and Glick, 1996). These auxins influence many cellular functions and are involved in initiation of lateral and adventitious roots, stimulation of cell division and in elongation of stems and roots (Yang *et al.*, 1993). Root proliferation by bacteria-secreted IAA causes enhancement of nutrients uptake by the associated plants (Lifshitz *et al.*, 1987). However, the observed responses of IAA on plant growth vary from one species of plant to another depending upon the indigenous hormonal level within the treated plants (Loper and Schroth, 1986; Mordukhova *et al.*, 1991). Arshad and Frankberger (1992) reported concentration dependent effect of IAA on plant growth, i.e. low concentration of exogenous IAA can promote, whereas high IAA concentrations can inhibit root growth. Astrom *et al.* (1993) reported that treatment with a cell-free culture filtrate of *P. fluorescens* caused a strong inhibitory effect on root elongation of wheat seedlings.

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Inoculation of canola (*Brassica campestris*) seeds with *Pseudomonas putida* GR12-2, which produced low level of IAA, was found to increase the length of seedling roots by 2 to 4-fold whereas an IAA over producing mutant inhibited root growth of seedlings by 33% (Xie *et al.*, 1996). Suzuki *et al.* (2003) derived spontaneous IAA low-producing mutant HP72LI from *P. fluorescens* strain that did not cause growth reduction of bentgrass root while parent strain HP72 induced root growth reduction. IAA over producing mutant of *P. putida* has also been reported to cause extensive development of adventitious roots on mung bean cuttings (Mayak *et al.*, 1991). Similar ambiguity about influence of IAA on growth of root, shoot or rate of seedling emergence has been reported in other plants (Sarwar and Kremmer, 1995). In the present study, IAA over producer and low producer mutants were derived by Tn5 mutagenesis of *Pseudomonas* strains CPS63 and MPS78. Inoculation of these *Pseudomonas* mutants showed stunting effect on the development of root and shoot at 5 and 10 days of seedling growth in green gram (*Vigna radiata*). Coinoculation of IAA-producing *Pseudomonas* strains/mutants with *Bradyrhizobium* sp. *Vigna* strain increased nodule number and plant dry weights in comparison to *Bradyrhizobium*-inoculated and uninoculated plants.

MATERIALS AND METHODS

Bacterial strains

Bradyrhizobium sp. *Vigna* strain S24 used in the present study was obtained from the Department of Microbiology and maintained on yeast extract mannitol agar (YEMA) medium. *Pseudomonas* strains CPS63 and MPS78 were maintained on Luria Bertani (LB) agar medium (Sambrook *et al.*, 1989). *E. coli* strain S17-1pSUP2021 (containing transposon Tn5) procured from Dr. A. Puhler, Bielefeld University, Germany was used for transposon mutagenesis and maintained on LB agar medium containing kanamycin (50 µg ml⁻¹).

Determination of indole acetic acid (IAA) production

Indole acetic acid production in different *Pseudomonas* strains and mutants was determined using Salkowski reagent as described by Gordon and Weber (1951). The fresh mass of *Pseudomonas* strains/mutants was transferred into the 5 ml LB broth supplemented with 100 µg ml⁻¹ L-tryptophan. The inoculated broths were incubated at 28° ± 1 °C for 2 and 4 days. Two ml growth suspension was taken from these broths and centrifuged for 5 min at 10,000 rpm. Then equal amount of Salkowski

reagent (0.5 M FeCl₃ in 35 % perchloric acid) was added in the supernatant. The contents were mixed by shaking and allowed to stand at room temperature for 30 min till the development of pink color. For quantitative estimation of IAA, optical density of the culture broth was taken at 500 nm. Uninoculated broth served as control. Auxin concentration values were obtained by preparing standard curve from IAA solution (0 - 100 µg ml⁻¹) and amount of IAA produced is expressed as µg IAA secreted per unit of optical density.

Intrinsic antibiotic resistance pattern of *Pseudomonas* strains

Intrinsic antibiotic resistance pattern of the *Pseudomonas* strains CPS63 and MPS78 was determined on LB plates containing different concentrations of the antibiotics using the procedure as described by Sindhu *et al.* (1999). The antibiotic concentrations used were 50 µg ml⁻¹, except for nalidixic acid and tetracycline (used at 20 µg ml⁻¹). After solidification of the medium, *Pseudomonas* strains were spot inoculated on plates and incubated at 28° ± 1 °C for 3 days. Based on appearance of growth on antibiotic-containing plates, the antibiotic resistance pattern for each strain was determined.

Transposon mutagenesis of IAA-producing *Pseudomonas* strains

Pseudomonas strains CPS63 and MPS78 were mutagenized using *E. coli* strain S17-1 having transposon Tn5 on suicidal plasmid pSUP2021. Conjugation between the *E. coli* (donor strain) and *Pseudomonas* sp. (recipient strain) was carried out as per the method used by Garg and Dadarwal (1991). The conjugation mixture was suspended in 10 ml saline and dilution plated (upto 10⁻⁶) on LB plates containing antibiotics: ampicillin (Ap) and kanamycin (Km) each at 50 µg ml⁻¹ concentration for strain CPS63 whereas tetracycline (Tc) and kanamycin at 20 and 50 µg ml⁻¹ concentration, respectively for strain MPS78. Colonies of Ap^r + Km^r mutants for strain CPS63 and Tc^r + Km^r mutants for strain MPS78 that appeared after 6 days of incubation were purified by streaking on the same medium plates. Isolated colonies obtained after third streaking were transferred to LB slants and maintained for subsequent studies.

Effects of *Pseudomonas* strains and mutants on seedling growth of green gram

Healthy seeds of green gram [*Vigna radiata* (L.) Wilczek] cv. Asha were surface sterilized with acidic alcohol

(H₂SO₄: ethanol, 7:3 v/v) for 3 min followed by washing with repeated changes of sterilized-distilled water (Sindhu *et al.*, 1999). The surface sterilized seeds were inoculated with broth culture of *Pseudomonas* strains and mutants for about 40 min. Inoculated seeds were kept for germination on plain water agar plates (0.8 %) at 28 ± 1 °C under controlled conditions. Uninoculated seeds treated with only LB broth were sown in agar plates as control. Root and shoot length was measured at 5 and 10 days after inoculation.

Effect of coinoculation of *Pseudomonas* strains/mutants with *Bradyrhizobium* strain S24 on plant growth

Healthy seeds of green gram (*Vigna radiata*) were surface sterilized with acidic alcohol as described earlier. For preparing chillum jar assemblies, thoroughly washed coarse river sand was used to fill the upper assembly of the jars. The lower jar assemblies were half-filled with quarter-strength of Sloger's nitrogen-free mineral salt solution (Sloger, 1969). The whole assembly was sterilized in the autoclave at 15 lbs psi for 3 hr. Surface-sterilized seeds were inoculated with broth culture of *Bradyrhizobium* sp. *Vigna* strain S24 alone or as coinoculant by mixing culture broth of the *Pseudomonas* strains/mutants with *Bradyrhizobium* strains in a ratio of 1:1 (v/v). Two ml of the mixed inoculum was used for inoculation of 15 seeds and culture was allowed to adsorb on the seeds for half an hour. Inoculated seeds were sown in sterilized chillum jar assemblies in triplicate. The jars were kept in a net house under day light conditions. Quarter strength Sloger's nitrogen-free mineral salt solution was used for watering as and when required. The plants were uprooted at 40 and 50 days after sowing (DAS). The plant samples were used for determining nodule number, nodule fresh weight and plant dry weight.

RESULTS AND DISCUSSION

Pseudomonads are predominantly found in rhizosphere of cereal and legume crops. These rhizobacteria have immense potential in agriculture for use as biofertilizer, biocontrol agent and in bioremediation due to their plant growth-promoting ability, antagonistic activity and degradation of pollutants (Ahmad *et al.*, 2008). Production of plant growth hormones particularly IAA has long been considered as an important attribute of PGPR strains and different bacterial strains were found to produce IAA (Patten and Glick, 1996).

Indole acetic acid production and antibiotic resistance pattern of *Pseudomonas* strains

Pseudomonas strains CPS63 and MPS78 produced indole acetic acid in LB broth and the intensity of color in centrifuged growth supernatants changed depending upon the amount of IAA produced by a particular strain. The amount of IAA produced by *Pseudomonas* strain CPS63 was found 18.1 µg ml⁻¹ of supernatant at 2 days and its production increased to 22.2 µg ml⁻¹ of supernatant at 4 days of growth (Table 1). In strain MPS78, IAA produced was 19.2 µg ml⁻¹ of supernatant at 2 days and its amount increased to 24.0 µg ml⁻¹ at 4 days of growth.

The spot inoculation of *Pseudomonas* strains onto LB medium plates supplemented with different antibiotics showed that *Pseudomonas* strain CPS63 was resistant to ampicillin and spectinomycin whereas strain MPS78 showed resistance to ampicillin, spectinomycin and tetracycline. Both the strains were found sensitive to the antibiotics chloramphenicol, streptomycin, kanamycin, neomycin, trimethoprim, nalidixic acid and rifampicin.

Derivation of mutants and screening for variation in IAA production

Tn5 mutagenesis of *Pseudomonas* strains CPS63 and MPS78 yielded kanamycin resistant mutants at a frequency of approximately 10⁻⁵ for each donor. The failure to obtain any spontaneous kanamycin resistant colonies after plating of parent culture on antibiotic incorporated plates suggested that all the mutants have

Table 1. Production of indole acetic acid by Tn5 induced mutants of *Pseudomonas* strains CPS63 and MPS78.

Culture/mutant	IAA production (µg ml ⁻¹)	
	2 d	4 d
Control	0.0	0.0
<i>Pseudomonas</i> strain CPS63	18.1	22.2
CPS63-20	25.8	29.4
CPS63-27	17.3	21.4
<i>Pseudomonas</i> strain MPS78	19.2	24.0
MPS78-107	23.4	27.8
MPS78-92	10.4	10.6
MPS78-166	14.5	14.8

arisen from Tn5 insertion. A total of 200 ($Ap^r + Km^r$) mutants from *Pseudomonas* strain CPS63 were screened for variation in level of IAA production. Most of the mutants i.e., 61.4 % showed little variation in amount of IAA produced under cultural conditions and behaved as parent-type. Only 8.4 % mutants showed increased level of IAA and 30.2 % had decreased capacity of IAA production (data not shown). Over producer mutant CPS63-20 and low producer mutant CPS63-27 were selected for further studies (Table 1). Similarly, another 200 ($Tc^r + Km^r$) mutants were selected from the Tn5 mutagenesis of *Pseudomonas* strain MPS78. Only 10.2 % mutants showed increased IAA production and 33.4 % produced less amount of IAA. One IAA over producer *Pseudomonas* mutant MPS78-107 and two low producer mutants MPS78-92 and MPS78-166 were selected.

Effect of seed inoculation with *Pseudomonas* strains/mutants on root and shoot elongation in green gram

Tn5 derived mutants of *Pseudomonas* strains CPS63 and MPS78 having altered level of IAA production were used for seed inoculation of green gram cv. Asha on plain water agar plates. Both the wild-type *Pseudomonas* strains as well as their mutants showed stunting effect on root growth of seedlings at both 5 and 10 days. *Pseudomonas* mutant CPS63-27 stimulated the shoot growth of green gram seedlings at both 5 and 10 days. All other mutants showed stunting effect on shoot growth of seedlings at both the stages of observation (Table 2).

The range of effective IAA concentrations on growth of seedlings or plant has been reported to vary depending upon the plant species used or to the sensitivity of the plant organ to auxin (Loper and Schroth, 1986). It is possible that the initial stunting effect on seedlings could be due to the contact of bacterial cell with legume seeds or due to synthesis or secretion of excessive amount of IAA (Sarwar and Kremmer, 1995) or some inhibitory agent (cyanide or toxin) produced by the bacterium when grown in synthetic medium (Astrom and Burns, 1989; Bolton and Elliott, 1989). In contrast, Astrom *et al.* (1993) reported that treatment of wheat seedlings with a cell-free culture filtrate of *P. fluorescens* caused a strong inhibitory effect on root elongation but the formation of IAA by the bacterium was too low to contribute to the inhibitory effect induced in plants. It is, therefore, apparent from these results that IAA production by the rhizobacteria beyond a critical limit may be inhibitory for plant growth.

Effect of coinoculation of *Bradyrhizobium* strain S24 with *Pseudomonas* strains/mutants on nodulation and plant growth of green gram

Inoculation of legumes and cereal plants with PGPR strains has been found to show a wide range of effects on plant growth that varied among strains of PGPR. Coinoculation of wild-type *Pseudomonas* strains and mutants enhanced nodule number, nodule fresh weight and plant dry weight in comparison to inoculation with *Bradyrhizobium* alone at 40 days (Table 3). IAA over

Table 2. Effect of seed bacterization with *Pseudomonas* strains/mutants on root and shoot growth of green gram on plain water agar plates.

Treatment	Root length (cm)		Shoot length (cm)	
	5 d	10 d	5 d	10 d
Control	10.4±0.43	15.14±1.00	7.84±0.57	9.58±0.38
<i>Pseudomonas</i> strain CPS63	9.72±0.31	11.75±0.85	8.12±0.42	11.52±2.10
CPS63-20	10.43±0.24	11.50±0.47	5.04±0.52	7.82±1.90
CPS63-27	10.15±0.90	16.10±0.94	8.03±0.88	12.67±2.05
<i>Pseudomonas</i> strain MPS78	10.24±1.00	15.26±0.20	7.14±0.12	9.76±1.30
MPS78-107	10.04±1.23	15.90±0.20	5.73±1.20	6.24±0.43
MPS78-92	9.81±0.99	19.23±1.02	5.32±1.10	7.42±0.60
MPS78-166	9.51±0.89	15.53±0.92	5.44±0.99	7.94±1.20

Values are average of three plants (Mean ± S.E.)

Table 3. Effect of coinoculation of green gram with *Pseudomonas* strains/mutants and *Bradyrhizobium* strain S24 on symbiotic parameter at 40 and 50 days of plant growth.

Treatment	Nodule number (per plant)		Nodule fresh weight (mg plant ⁻¹)		Plant dry weight (mg plant ⁻¹)	
	40 DAS	50 DAS	40 DAS	50 DAS	40 DAS	50 DAS
Control	-	2	-	14	360	518
<i>Bradyrhizobium</i> sp. S24	35	42	202	238	830	1100
<i>Pseudomonas</i> strain CPS63	-	3	-	26	460	645
S24+CPS63	38	54	212	287	1024	1230
S24+CPS63-20	42	57	246	304	908	1036
S24+CPS63-27	28	44	154	263	860	1284
<i>Pseudomonas</i> strain MPS78	-	-	-	-	368	662
S24+MPS78	44	52	263	304	896	1540
S24+MPS78-107	48	58	288	322	842	1508
S24+MPS78-92	43	49	240	296	900	1572
S24+MPS78-166	40	46	206	274	886	1584

producer mutants CPS63-20 and MPS78-107 as well as IAA low producer mutants MPS78-92 increased nodule number and nodule fresh weight. Maximum gains in plant dry weights were observed after coinoculation of strains CPS63 and MPS78 as well as with mutants CPS63-20 and MPS78-92. Plant dry weight ratio of coinoculated plants over control plants at 40 days of growth varied from 2.34 to 2.84, whereas only 1.01 to 1.23 times increase was observed in comparison to those of *Bradyrhizobium*-inoculated plants.

At 50 days of plant growth, coinoculation with the wild-type strains as well as mutants showed nodule-promoting effect (Table 3). IAA over producer mutants CPS63-20 and MPS78-107 caused formation of more nodules by *Bradyrhizobium* strain S24 as compared to parent *Pseudomonas* strains. Significant gains in plant dry weights were observed on coinoculation of wild-type *Pseudomonas* strains and the mutants, viz. CPS63-27, MPS78-92 and MPS78-166. The plant dry weight gains varied from 1.03 to 1.44 times those of *Bradyrhizobium*-inoculated treatment and 2.0 to 3.06 times those of uninoculated control plants.

Coinoculation of legumes with IAA-producing *Pseudomonas* and *Bacillus* species with *Rhizobium* has been reported to significantly increase the number of

nodules, nodule fresh weight and nitrogenase activity compared to inoculation with *Rhizobium* alone (Srinivasan *et al.*, 1996). Mayak *et al.* (1991) showed that an IAA over producing mutant of *P. putida* caused extensive development of adventitious roots on mung bean cuttings. It is possible that inoculation with free-living bacteria will increase the number of infection sites on legume roots for attachment and nodulation by *Rhizobium*. Moreover, enhanced production of flavonoid-like compounds or phytoalexins in roots of several crop plants by inoculation of *Pseudomonas* sp. (Goel *et al.*, 2001) might induce the transcription of rhizobial nodulation (*nod*) genes, leading to increase in nodulation. Similar nodule-promoting effects of *Pseudomonas* sp. on coinoculation with *Rhizobium* strains have been reported in soybean (Nishijima *et al.*, 1988), pea (Bolton *et al.*, 1990), green gram (Sindhu *et al.*, 1999) and chick pea (Sindhu *et al.*, 2002). Better performance of IAA low producer mutants in comparison to IAA over producer mutants in relation to symbiotic effectivity at 50 days of plant growth indicated that overproduction of IAA by bacteria could inhibit plant growth in green gram. These studies suggest that IAA production ability in *Pseudomonas* strains is one of the beneficial traits for selection of PGPR strains from the rhizosphere of plants.

REFERENCES

- Ahmad F, Ahmad I and Khan MS (2008). Screening of free-living rhizosphere bacteria for their multiple plant growth promoting activities. *Microbiol. Res.*, 163: 173-181.
- Arshad M and Frankenberger WT Jr (1992). Microbial production of plant growth regulators. In: *Soil Microbial Ecology, Applications in Agricultural and Environmental Management* (Ed. Metting FB). Marcel Dekker, Inc., New York, pp. 27- 32.
- Astrom B, Gustafsson A and Gerhardsson B (1993). Characteristics of a plant deleterious rhizosphere pseudomonad and its inhibitory metabolite(s). *J. Appl. Bacteriol.*, 74: 20-28.
- Astrom S and Burns RG (1989). Cyanide production by rhizosphere bacteria as a possible mechanism of plant growth inhibition. *Biol. Fertil. Soils*, 7: 232-238.
- Bolton Jr H and Elliott LF (1989). Toxin production by a rhizobacterial *Pseudomonas* sp. that inhibits wheat root growth. *Plant Soil*, 114: 269-278.
- Bolton Jr H, Elliott LF, Turco RF and Kennedy AC (1990). Rhizoplane colonization of pea seedlings by *Rhizobium leguminosarum* and a deleterious root colonizing *Pseudomonas* sp. and effects on plant growth. *Plant Soil*, 123: 121-124.
- Dey R, Pal KK, Bhatt DM and Chauhan SM (2004). Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth promoting rhizobacteria. *Microbiol. Res.*, 159: 371-394.
- Garg RP and Dadarwal KR (1991). *Ex planta* nitrogenase expression associated with *Hup* character in cowpea miscellany *Rhizobium*. *Indian J. Exp. Biol.*, 29: 371-374.
- Goel AK, Sindhu SS and Dadarwal KR (2001). Seed bacterization with fluorescent *Pseudomonas* enhances the synthesis of flavonoid-like compounds in chickpea (*Cicer arietinum* L.). *Physiol. Mol. Biol. Plants*, 6: 195-198.
- Goel AK, Sindhu SS and Dadarwal KR (2006). Production and partial characterization of antifungal and root growth stimulating metabolites from *Pseudomonas* sp. *Physiol. Mol. Biol. Plants*, 12: 201-205.
- Gordon SA and Weber RP (1951). Colorimetric estimation of indole acetic acid. *Plant Physiol.*, 26: 192-195.
- Lifshitz R, Kloepper JW and Kozlowski M (1987). Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. *Can. J. Microbiol.* 33: 390-395.
- Loper JE and Schroth MN (1986). Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. *Plant Pathol.*, 76: 386-389.
- Mayak S, Tirosh T and Glick BR (1999). Effect of wild-type and mutant plant growth-promoting rhizobacteria on the rooting of mung bean cuttings. *J. Plant Growth Regul.*, 18: 49-53.
- Mordukhova EA, Skovortsova NP, Kochetkov VV, Dubeikovskii AN and Boronin M (1991). Synthesis of the phytohormone indole acetic acid by rhizosphere bacteria of the genus *Pseudomonas*. *Mikrobiologiya*, 60: 494-500.
- Nishijima F, Evans WR and Vesper SJ (1988). Enhanced nodulation of soybean by *Bradyrhizobium* in the presence of *Pseudomonas fluorescens*. *Plant Soil*, 111: 149-150.
- Patten CL and Glick BR (1996). Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol.*, 42: 207-220.
- Rajkumar M and Freitas H (2008). Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard. *Bioresour. Technol.*, 99: 3491-3498.
- Sambrook J, Fritsch EF and Maniatis T (1989). Molecular cloning, A Laboratory manual. Cold Spring Harbor, New York.
- Sarwar M and Kremmer RJ (1995). Enhanced suppression of plant growth through production of L-tryptophan compounds by deleterious rhizobacteria. *Plant Soil*, 172: 261-269.
- Sindhu SS, Gupta SK and Dadarwal KR (1999). Antagonistic effect of *Pseudomonas* spp. on pathogenic fungi and enhancement of plant growth in green gram (*Vigna radiata*). *Biol. Fertil. Soils*, 29: 62-68.
- Sindhu SS, Suneja S, Goel AK, Parmar N and Dadarwal KR (2002). Plant growth promoting effect of *pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *Cicer* strain under sterile and "wilt sick" soil conditions. *Appl. Soil Ecol.*, 14: 57-64.
- Sloger C (1969). Symbiotic effectiveness and nitrogen fixation in nodulated soybean. *Plant Physiol.*, 44: 1666-1668.
- Srinivasan M, Peterson DJ and Holl EB (1996). Influence of indole acetic acid producing *Bacillus* isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under genotobiotic conditions. *Can. J. Microbiol.*, 42: 1006-1014.
- Suzuki S, Yuxi Hi, Oyaizu H and He Y (2003). Indole-3-acetic acid production in *Pseudomonas fluorescens* HP72 and its association with suppression of creeping bentgrass brown patch. *Curr. Microbiol.*, 47: 138-143.
- Weller DM (2007). *Pseudomonas* biological control agents of soilborne pathogens: Looking back over 30 years. *Phytopathology*, 97: 250-256.
- Xie H, Pasternack JJ and Glick BR (1996). Isolation and characterization of mutants of plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2 that over-produce indole acetic acid. *Curr. Microbiol.*, 32: 67-71.
- Yang T, Law DM and Davies PJ (1993). Magnitude and kinetics of stem elongation induced by exogenous indole-3-acetic acid in intact light-grown pea seedlings. *Plant Physiol.*, 102: 717-724.