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Research Article

Relationship between indole acetic acid production by fluorescent *Pseudomonas* and plant growth promotion

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ABSTRACT: Forty fluorescent pseudomonads were quantitatively evaluated for indole acetic acid (IAA) producing ability in the presence (trypt+) or absence (trypt$^-$) of tryptophan and growth promotion in groundnut in response to seed treatment with high IAA producers was analyzed. There were significant differences in the amounts of IAA produced by the isolates and more amounts of IAA were released in trypt+. In trypt$^-$ increased IAA production was noticed for up to 144h and thereafter it stabilized or decreased. Analysis of plant growth promotion showed that the maximum root length (187.5mm) was exhibited by plants treated with BA16(A)2 which was a medium IAA producer whereas the highest IAA producer BA1(E)2 (32.2 µg/ml of IAA) showed a root length of 167.3mm. The isolate OTN7(E)2 which was a low IAA producer showed good root lengths of 148.3mm (sterile soil) and 133.3mm (unsterile soil). When the shoot lengths were compared, highest shoot length of 213.3mm (sterile soil) was by BA4(D) which is a medium to high IAA producer and in unsterile soil highest shoot length of 198.67mm was shown by the isolate ND1which is also a medium to high IAA producer. The highest IAA producer BA1(E)2 showed a low shoot length of 156.7mm (sterile soil) and 115mm (unsterile soil). The results in some way do establish that plant growth is not directly influenced by high IAA producers. Root growth seemed to be more affected by high IAA producers in unsterile soil and maximum vigour index of 32600 was exhibited by BA2(D)1 which is a medium IAA producer and the minimum of 15750 was by BA1(E)2 which is a high IAA producer. Hence IAA producers of the fluorescent *Pseudomonas* group showed significant plant growth when compared with control but plant growth was not greatly influenced by those organisms that produced high amounts of IAA. Antagonistic *Pseudomonas* spp. able to release moderate or even low amounts of IAA may be better growth promoters.

KEY WORDS: *Pseudomonas*, indole acetic acid, shoot length, root length, plant growth promotion, tryptophan, vigour index

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INTRODUCTION

Bacteria that stimulate plant growth by their root colonization are referred to as ‘‘plant growth–promoting rhizobacteria (PGPR)” (Kloepper *et al,* 1990). Increase in plant growth is due to microbial production of plant-growth regulators, including indole-3-acetic acid (IAA) (Kang *et al*, 2006; Patten and Glick, 2002). The IAA produced by microorganisms that colonize the seed or root surface is supposed to act in conjunction with endogenous IAA in plants to stimulate cell proliferation and/or elongation and enhance the host’s uptake of minerals and other nutrients from the soil (Suzuki and Oyaizu, 2003). Reports say that nearly 80% of rhizobacteria can synthesize IAA (Loper and Schroth, 1986). Studies have also shown that root growth promotion by free living PGPR, e.g., *Pseudomonas* as well as by symbionts, such as *Bradyrhizobium japonicum* and *Rhizobium* spp., is related to low levels of IAA secretion (Patten and Glick, 1996). In contrast, some studies have related the inhibitory effect of deleterious rhizobacteria (DRB) to their high amounts of IAA excretion, e.g., *Enterobacter taylorae* (Sarwar and Kremer, 1995) and *P. putida* (Xie *et al*., 1996). Bacteria producing IAA contribute to plant growth promotion and IAA is a part of the complex mechanism that is involved in stimulation of plant growth promotion (Egambardieva *et al.,* 2008). Biosynthesis of IAA is dependent on the presence of tryptophan, which is one of the main compounds present in several plant exudates (Kamilova *et al*., 2006) and a five fold increase in IAA production is observed in the

presence of tryptophan under cultured conditions (Idris *et al.*, 2007).

A study was undertaken to evaluate fluorescent *Pseudomonas* for IAA production and study the role of high IAA producers in plant growth promotion. IAA producing ability was quantitatively evaluated in the presence and absence of tryptophan.

MATERIAL AND METHODS

Isolation of bacteria

Rhizosphere soil samples from groundnut plants were collected from Karnataka and Tamil Nadu. Roots of plants with morphologically healthy aerial parts and without any physiological abnormalities were selected. For the isolation of *Pseudomonas* spp. one gram rhizosphere soil was taken in 100ml of sterile distilled water and mixed well (kept on rotary shaker for 15 to 20 minutes at 200 rpm). Serial dilutions were made and dilutions of 10^{-3} to 10^{-5} were plated on King's medium B (KB medium) using the spread plate technique. The plates were incubated at 28°C for 2 days (Yeole and Dube, 1997). After incubation the colonies which produced water soluble pigments were picked up and sub-cultured to obtain pure cultures. Stock cultures were made in Luria Bertani (LB) broth containing 50% (w/v) glycerol and stored at –80°C (Naik *et al.,* 2008).

Assay for auxin production by qualitative method

The production of IAA was determined as described by Bric (1991). Single colony was streaked onto LB agar amended with 5 mM L-tryptophan, 0.06% sodium dodecyl sulfate (SDS) and 1% glycerol. Plates were overlaid with Whatman no. 1 filter paper (82-mm diameter) and the bacterial isolate was allowed to grow for 3 days. After the incubation period, the paper was removed and treated with Salkowsky's reagent (Gordon and Weber, 1951) having the formulation of 2% of 0.5 M ferric chloride in 35% perchloric acid at room temperature for 60 min and the production of IAA was identified by the formation of a characteristic red halo on the paper immediately surrounding the colony. Three replications were maintained for each organism and the experiment was repeated twice.

Quantitative estimation of IAA

The production of IAA was determined using colorimetric method. Briefly, the tested strains were inoculated in KB broth without and with tryptophan (500µg/ml) and incubated at 28°C at 150 rpm. After 2, 4, 6 and 8 days of cultivation, aliquots of bacterial cultures were centrifuged at 13,000 rpm for 10 min. Two milliliters of supernatant fluid was added to a tube with 100ìl of 10mM ortho-phosphoric acid and 4ml of Salkowsky's reagent (Gordon and Weber, 1951). The mixture was incubated at room temperature for 25 min and the absorbance of the developed pink colour was read at 530 nm. The IAA concentration in culture was determined by using a calibration curve of pure IAA (Sigma) as a standard. The results were statistically analyzed by ANOVA. Three replications were maintained for each organism and the experiment repeated twice. IAA producers were grouped into three *viz.*, 'High', 'Medium' and 'Low' based on the quantity of IAA released in presence or absence of tryptophan. In the presence of tryptophan isolates were grouped as 'High' if >40µg/ml is released, 'Medium' for 25 to 40µg/ml and 'Low' if it released <25 µg/ml of IAA. Similarly since less IAA was released in the absence of tryptophan, isolates were scored as 'High' for >25µg/ml IAA release, 'Medium' for 19-25µg/ml and 'Low' if <19 µg/ml were released.

Preparation of bacterial suspension

Forty IAA positive isolates were inoculated in 100 ml KB broth and incubated for 24h at 29°C in shaker. After incubation, the isolate was centrifuged at 5000 rpm for 7-8 minutes and the pellet was resuspended in 10 ml of phosphate buffer. Then the CFU value was enumerated for each isolate.

Assay for seedling growth promotion

The seeds were surface sterilized by immersion in 70% ethanol for five min and subsequently in 0.1% HCl for 1 min. Finally they were soaked in bacterial suspension of 10^7–10^8 CFU ml^{-1} and control seeds were soaked in sterile distilled water. Five seeds per plate were put in Petri dishes containing wet filter paper. Plates were incubated for 7 days at room temperature (Egambardieva *et al.,* 2008). The epicotyl length of seedlings was measured after 7 days and the data were recorded. Forty IAA positive isolates were tested for seedling growth promotion.

Sterilization of soil

The soil used in greenhouse trials was autoclaved twice at 121°C for 15min and used for further treatment. The temperatures and relative humidity (RH) ranged from 20 to 28°C and from 60 to 90% RH, respectively during the glasshouse trials.

Seed treatment

For seed treatment the groundnut seeds were treated with 1% CMC and soaked in bacterial suspension of 10^7–10^8 CFU/ml for overnight. Then the treated seeds were

sown in pots. Five seeds were sown and later thinned to 2 per pot. Three replications were maintained for each treatment.

Greenhouse trials

Forty IAA positive isolates in the form of suspension were tested for their effect on plant growth by using the groundnut seeds. The seeds were coated with the bacteria by dipping the seeds in a bacterial suspension of 10^7–10^8 CFU/ml. To study the effect of bacterial strains on plant weight, shoot and root growth of groundnut, plastic pots containing sterilized soil were used. Non-inoculated control plants and plants inoculated with bacteria were grown on sterile soil for 4 weeks. The inoculation treatments were set up in a randomized design with 3 replications (two plants per pot). Two seeds were sown per pot and after 7 days, germination % was recorded. The plants were harvested after 30 days. The fresh plant weight, shoot length and root length of the harvested plants were measured and recorded. The experiment was replicated thrice and repeated once for confirmation.

RESULTS AND DISCUSSION

Fluorescent pseudomonads are some of the effective candidates for biological control of soil borne plant pathogens owing to their rhizosphere competence (Kloepper and Schroth, 1981). Fluorescent Pseudomonads produce IAA and these IAA producers are implicated in enhanced plant growth (Dubeikovsky *et al.,* 1993; Joseph *et al.,* 2007; Gutierrez *et al*., 2009). Some studies have related the inhibitory effect of deleterious rhizobacteria (DRB) to their high amounts of IAA excretion, e.g., *Enterobacter taylorae* (Sarwar and Kremer, 1995). In the present study fluorescent pseudomonads were evaluated for IAA producing ability in the presence and absence of tryptophan and the effect of high IAA producers on the growth and vigour of groundnut were analyzed.

A total of 79 fluorescent *Pseudomonas* isolates were qualitatively tested for IAA production and it was found that 40 of the isolates showed positive reaction. These 40 isolates were quantitatively analyzed for IAA production in the presence (trypt+) or absence (trypt$^-$) of tryptophan at different intervals. There were significant differences in the amounts of IAA produced by the isolates and more amounts of IAA were released in trypt+ (Table 2). In trypt$^-$ increased IAA production was noticed for up to 144h and thereafter it stabilized or decreased and the isolates namely BA4(D), BA5(D), BA10(D)1, BA14(C)3, BA3(D)1 and BA3(E)2 produced high amounts of IAA (28 to 32 µg ml^{-1}) (Table 1). In trypt+ assay highest IAA was produced by the isolates ND4IART (B) (22.2 µg/ml at 48h), BA14(D)1 (40 µg/ml at 96h), BA10(D)1 (53.6µg ml^{-1} at 144h) and BA5(D) (62.2 µg ml^{-1} at 192h) respectively (Table 2). Biosynthesis of IAA is dependent on the presence of tryptophan, which is one of the main compounds present in several plant exudates (Kamilova *et al*., 2006) and a five fold increase in IAA production is observed in presence of tryptophan under cultured conditions (Idris *et al.*, 2007). Mordukhova *et al.* (1991) had categorized the IAA producing *Pseudomonas* spp.

We tried to analyze whether high IAA production by a bacterium is needed for positive effect on plant growth. The isolates were graded as low (L), medium (M) and high (H) based on the quantity of IAA released (see material and methods) under trypt$^-$ or trypt+ conditions (Tables 1, 2, 3 and 4). There were significant differences in plant growth characters between treated and untreated control and also significant growth differences were observed among the tested IAA producers.

In sterile soil highest root length of 150mm was exhibited by isolate OTN6(E)2 and lowest root length of 120 mm was shown by isolate BA16(2). However highest shoot length of 201mm was exhibited by strain OTN2(D) and lowest shoot length of 143mm was by plants treated with OTN(6)D. The highest vigour index of 25666.7 was by another low IAA producer namely OTN7(E)2 (Table 3). Under unsterile conditions maximum root length of 155 mm was shown by BA8(E)2 and minimum root length of 101.7 mm was shown by BA16(2). Maximum shoot length of 175 mm was by BA8(E)2 and minimum was by OTN7(E)2 . The maximum vigour index of 27166.67 was exhibited by plants treated with BA16(2) (Table 4).

Plant growth characters by Medium-IAA producers under trypt$^-$ conditions

Under sterile soil conditions maximum root length of 185.7 mm was exhibited by isolate BA16(A)2 and minimum root length of 103 mm was shown by isolate BA9(C)2. However maximum shoot length of 208.7 mm was exhibited by strain OTN5(D) and lowest shoot length of 123 mm was by plants treated with OTN7(2). The highest vigour index of 32766.7 was by another medium IAA producer namely BA2(E) (Table 3). In unsterile soil maximum root length of 205 mm was shown by GR7(A) and minimum root length of 87.3 mm was shown by BA2(E). Maximum shoot length of 175 mm was by BA8(E)2 and minimum of 130mm was by CC17(D). Maximum vigour index of 32600 was exhibited by plants treated with BA2(D)1 (Table 4).

Plant growth characters by High-IAA producers under trypt$^-$ conditions

Under sterile soil conditions maximum root length of 157.3 mm was exhibited by isolate BA1(E)2 and minimum

Table 1. Quantitative estimation of IAA released by fluorescent *Pseudomonas* in the absence of tryptophan

Isolate name	Concentration of IAA/ml released in absence of tryptophan			
	48h	96h	144h	192h
BA1(E)2[H]	8.8	26.6	25.6	32.2
BA5(D) [H]	8.6	15.8	23.2	31.6
BA14(C)3 [H]	12.4	19	24.6	30.4
BA4(D) [H]	8.6	14	22.0	29.2
BA3(E)2 [H]	9.4	14.2	33.8	28.8
BA3(D)1 [H]	9.6	14	24.8	28
BA10(D)1 [H]	9.6	15.8	23.2	27.8
BA14_(D)1 [H]	10.6	15.8	21.6	27
BA3(E)1 [H]	9.8	16	22.4	26.8
BA16(A)1 [H]	10.4	16.2	22.2	25.2
GR4RAUA(B) [M]	10.2	14.2	16.0	24
BA3(D)3 [M]	9.8	16.4	20.6	24
BA2(C) [M]	8.2	13	21.4	23.2
BA9(C)2 [M]	8.8	14.8	21.4	22.6
BA2(D)2 [M]	10.2	16.4	20.4	22.6
BA9(C)1 [M]	11.4	18	18.2	22
OTN7(2) [M]	14.0	22.6	24	22
GR7(A) [M]	11.8	14	17.4	22
BA2(D)1 [M]	11.6	16.2	21.4	21.6
BA16(A)2 [M]	9.6	17.4	20.8	21.6
ND3IART(A) [M]	9.6	18	18.2	21.6
OTN5(D) [M]	9.4	18	19	21.6
NDI [M]	10.0	14.2	16.2	20.8
GR4RAUA(A) [M]	9.4	14.6	18.4	20.6
BA2(E) [M]	13.8	17	21.2	20.6
BA14(C)2 [M]	11.2	14.8	19.6	20.6
OTN7(D)1 [M]	10.6	16	18	20.6
BA11(D) [M]	8.4	13	21.8	20.6
CC17(D) [M]	9.2	14.6	15.8	20.4
ND4IART(B) [M]	9.8	16.8	22.4	20
BA2(C)(3-1) [M]	9.4	18.6	19.6	20
OTN3(E) [M]	9.0	11	15.6	19.8
OTN7(E)1 [M]	8.2	18.2	18.6	19.2
BA6(E)2 [M]	8.6	14.2	19.6	19.2
BA8(E)2 [L]	9.6	14.8	20.0	18.8
OTN6(E)2 [L]	10.4	16.2	17.2	18.8
OTN2(D) [L]	8.6	17	17.2	18
OTN6(D) [L]	11.2	14	16.0	18
BA16(2) [L]	4.6	14.2	14.6	18
OTN7(E)2 [L]	8.6	14.6	16.0	16.4
$CD_{p=0.05}$	3.07	3.70	3.01	4.0

Tryp- = without tryptophan; Tryp+ = with tryptophan; [H] = high IAA producer; [M]= medium IAA producer; [L]= Low IAA producer

Table 2. Quantitative estimation of IAA released by fluorescent *Pseudomonas* in the presence of tryptophan

Isolate name	Concentration of IAA/ml released in absence of tryptophan			
	48h	96h	144h	192h
BA5(D) [H]	16.2	21.6	52.8	62.2
BA10(D)1 [H]	14.6	38.4	53.6	58
BA14_(D)1 [H]	16.2	40	48.8	56.2
BA2(D)2 [H]	19.4	34.8	42.6	51
BA1(E)2 [H]	11.6	38.2	46.4	48.4
ND3IART(A) [H]	14.2	23.6	33.2	47.6
ND 1 [H]	22.0	34.4	44.4	46.4
BA3(E)1 [H]	13.8	22	35.6	46
BA3(E)2 [H]	14.2	30.4	36.0	40
OTN7(2) [H]	18.4	30	33.8	40
BA4(D) [M]	15.6	27.8	37.2	38.2
BA3(D)1 [M]	13.6	28.6	35.2	38
BA8(E)2 [M]	14.6	24.8	32.0	35.2
BA14(C)3 [M]	13.8	29	33.2	34.4
BA16(A)2 [M]	15.4	21	25.8	32.4
BA16(A)1 [M]	13.4	19.4	24.0	30.4
BA2(D)1 [M]	14.2	18.6	22.8	29.2
BA2(C) [M]	11.8	21.6	24.0	29.2
BA3(D)3 [M]	13.2	22.8	27.2	28
ND4IART(B) [M]	22.2	22.2	24.8	28
GR4RAUA(B) [M]	19.4	18.4	20.4	28
CC17(D) [M]	15.8	20.2	16.8	26
BA11(D) [M]	12.4	18	22.0	26
BA2(E) [M]	14.6	18.6	23.4	25.2
OTN5(D) [M]	19.2	20.8	24.4	25.2
OTN7(E)1 [L]	17.0	18.6	20.2	24.8
BA2(C)(3-1) [L]	21.2	22	23.0	24.4
BA9(C)1 [L]	17.2	18	20.2	24.4
BA14(C)2 [L]	13.2	16.4	22.4	24.4
BA9(C)2 [L]	18.8	20	22.8	23.6
OTN2(D) [L]	18.2	19	19.6	23.6
GR4RAUA(A) [L]	17.8	16.4	19.2	22.8
OTN6(E)2 [L]	19.4	19.6	20.4	22.8
GR7(A) [L]	18.0	20	18.6	22.8
BA6(E)2 [L]	15.2	17.8	21.8	22.6
OTN3(E) [L]	17.0	18.6	19.8	22.4
OTN7(E)2 [L]	16.0	19.6	16.2	21.6
OTN6(D) [L]	18.0	18.4	19.8	21.6
OTN7(D)1 [L]	18.8	18	20.2	20.8
BA16(2) [L]	16.0	17.2	18.4	20.8
$CD_{p=0.05}$	3.03	5.30	4.40	4.70

Tryp- = without tryptophan; Tryp+ = with tryptophan; [H] = high IAA producer; [M]= medium IAA producer; [L]= Low IAA producer

Table 3. Groundnut plant growth in response to inoculation with IAA producing bacteria in sterile soil

Isolate Name	Concentration of IAA/ml released at 192h		Seedling growth test	*In vivo* evaluation of plant growth under potted conditions				
	Trypt⁻	Tryp+	Epicotyl length of seeds (mm)	Fresh plant weight (g)	Root length (mm)	Shoot length (mm)	% Germination	Vigour Index
BA5(D)	31.6 H	62.2 H	59.6	4.1	122.7	208.3	83 (75.00)	28216.7
BA10(D)1	27.8 H	58.0 H	63.8	4.2	131.7	166.7	83 (75.00)	24333.3
BA14_(D)1	27.0 H	56.2 H	62.6	4.7	120.0	173.3	66.7 (60.00)	19500.0
BA2(D)2	22.6 M	51.0 H	71.4	4.9	160.0	165.0	83 (75.00)	27500.0
BA1(E)2	32.2 H	48.4 H	63.6	4.3	167.3	156.7	66.7 (60.00)	21866.7
ND3IART(A)	21.6 M	47.6 H	29.6	3.3	115.0	151.7	83 (75.00)	22200.0
ND 1	20.8 M	46.4 H	75.6	4.0	160.0	168.3	83 (75.00)	27500.0
BA3(E)1	26.8 H	46.0 H	58.8	3.7	116.3	174.0	83 (75.00)	24316.7
BA3(E)2	28.8 H	40.0 H	20.1	4.8	124.0	150.0	66.7 (60.00)	17866.7
OTN7(2)	22.0 M	40.0 H	37.8	3.6	126.7	123.0	66.7 (60.00)	16316.7
BA4(D)	29.2 H	38.2 M	62.0	3.6	130.0	213.3	83 (75.00)	28666.7
BA3(D)1	28.0 H	38.0M	53.2	3.9	131.7	172.3	66.7 (60.00)	19983.3
BA8(E)2	18.8 L	35.2 M	36.8	4.7	141.3	195.0	66.7 (60.00)	22216.7
BA14(C)3	30.4 H	34.4 M	52.2	4.6	129.0	178.3	66.7 (60.00)	20116.7
BA16(A)2	21.6 M	32.4 M	20.4	4.7	185.7	189.3	83 (75.00)	31466.7
BA16(A)1	25.2 H	30.4 M	37.2	4.8	161.7	203.3	100 (90.00)	36500.0
BA2(D)1	21.6 M	29.2 M	45.6	3.6	160.0	190.0	66.7 (60.00)	23666.7
BA2(C)	23.2 M	29.2 M	16.8	4.0	131.0	162.7	100 (90.00)	29366.7
BA3(D)3	24.0 M	28.0 M	20.2	4.9	130.3	161.0	100 (90.00)	29133.3
ND4IART(B)	20.0 M	28.0 M	36.8	5.1	146.7	203.3	83 (75.00)	29833.3
GR4RAUA(B)	24.0 M	28.0 M	44.4	3.8	133.3	154.7	66.7 (60.00)	19766.7
CC17(D)	20.4 M	26.0 M	18.2	4.7	128.3	160.0	83 (75.00)	24166.7
BA11(D)	20.6 M	26.0 M	34.4	3.7	126.7	210.0	83 (75.00)	28083.3
BA2(E)	20.6 M	25.2 M	33.4	4.4	153.3	174.3	100 (90.00)	32766.7
OTN5(D)	21.6 M	25.2 M	33.6	4.8	130.0	208.7	100 (90.00)	32666.7
OTN7(E)1	19.2 M	24.8 L	50.0	4.1	180.0	180.7	83 (75.00)	30433.3
BA2(C)(3-1)	20.0 M	24.4 L	53.0	3.4	116.7	128.3	83 (75.00)	20666.7
BA9(C)1	22.0 M	24.4 L	39.8	4.8	140.0	170.0	66.7 (60.00)	21500.0
BA14(C)2	20.6 M	24.4 L	27.9	5.4	115.7	186.7	66.7 (60.00)	19816.7
BA9(C)2	22.6 M	23.6 L	39.6	3.5	103.3	181.7	83 (75.00)	24000.0
OTN2(D)	18.0 L	23.6 L	29.2	4.5	135.0	201.0	66.7 (60.00)	22766.7
GR4RAUA(A)	20.6 M	22.8 L	35.0	3.8	130.7	161.7	83 (75.00)	24316.7
OTN6(E)2	18.8 L	22.8 L	52.2	4.2	150.0	171.7	66.7 (60.00)	20166.7
GR7(A)	22.0 M	22.8 L	36.4	4.1	128.3	173.0	100 (90.00)	30133.3
BA6(E)2	19.2 M	22.6 L	32.0	4.6	161.7	179.0	83 (75.00)	26633.3
OTN3(E)	19.8 M	22.4 L	44.4	4.3	170.0	160.0	83 (75.00)	28000.0
OTN7(E)2	16.4 L	21.6 L	36.2	4.2	148.3	161.7	83 (75.00)	25666.7
OTN6(D)	18.0 L	21.6 L	34.0	3.7	121.7	143.3	66.7 (60.00)	17583.3
OTN7(D)1	20.6 M	20.8 L	25.8	3.7	148.3	158.3	83 (75.00)	25083.3
BA16(2)	18.0 L	20.8 L	20.2	4.2	120.0	181.7	83 (75.00)	24916.7
Control	–	–	9.6	2.2	49.0	94.3	50 (45.00)	7166.7
$CD_{p=0.05}$	4.00	4.70	10.53	0.58	29.05	32.37	8.18	3070.64

Tryp- = Without tryptophan; Tryp+ = With tryptophan, Figures in parenthesis are angular transformed values; H=high, M=medium, L=low

root length of 120 mm was shown by isolate BA14(D)1. But maximum shoot length of 213.3 mm was exhibited by strain BA4(D) and lowest shoot length of 150mm was by plants treated with BA3(E)2. However highest vigour index of 36500 was by the high IAA producer namely BA16(A)1 (Table 3). In unsterile soil highest root length of 167.7 mm was shown by BA4(D) and lowest root length of 96.7 mm was shown by BA16(E)1. Maximum shoot length of 184 mm was by BA3(E)1 and minimum of 115 mm was by BA1(E)2 . Maximum vigour index of 29866.67 was exhibited by plants treated with BA14(D)1 (Table 4).

Plant growth response to treatment with Low-IAA producers under trypt+ conditions

Under sterile soil conditions maximum root length of 180 mm was exhibited by isolate OTN7(E)1 and minimum root length of 103.3 mm was shown by isolate BA9(C)2. But maximum shoot length of 201 mm was exhibited by strain OTN2(D) and lowest shoot length of 128.3mm was by plants treated with BA2(C)(3-1). However highest vigour index of 30433.3 was by the low IAA producer namely OTN7(E)1 (Table 3). In unsterile soil highest root length of 205 mm was shown by GR(7)A and lowest root length of 101.7 mm was shown by BA16(2). Maximum shoot length of 180 mm was by BA6(E)2 and minimum of 146.67 mm was by GR(7)A. Maximum vigour index of 31166.67 was exhibited by plants treated with BA9(C)1 (Table 4).

Plant growth characters by Medium-IAA producers under trypt+ conditions

In sterile soil maximum root length of 185.7 mm was exhibited by isolate BA16(A)2 and minimum root length of 126.7 mm was shown by isolate BA11(D). Maximum shoot length of 213.3mm was exhibited by strain BA4(D) and lowest shoot length of 154.7 mm was by plants treated with GR4RAUA(B). However highest vigour index of 36500 was by another medium IAA producer namely BA16(A)1 (Table 3). In unsterile soil maximum root length of 157.7 mm was shown by BA4(D) and minimum root length of 87.3 mm was shown by BA2(E). Maximum shoot length of 176.67mm was exhibited by two isolates namely BA2(C) and BA3(D)3 and minimum 130mm was by CC17(D). Maximum vigour index of 32600 was exhibited by plants treated with BA2(D)1 (Table 4).

Plant growth characters by High-IAA producers under trypt+ conditions

Under sterile soil conditions maximum root length of 167.3 mm was exhibited by isolate BA1(E)2 and minimum root length of 120 mm was shown by isolate BA14(D)1. But maximum shoot length of 208.3 mm was exhibited by strain BA5(D) and lowest shoot length of 123mm was by plants treated with OTN(7)2. However highest vigour index of 28216.7 was by the high IAA producer namely BA5(D) (Table 3). In unsterile soil highest root length of 190 mm was shown by ND1 and lowest root length of 115 mm was shown by BA2(D)2. Maximum shoot length of 198.67 mm was by ND1 and minimum of 115mm was by BA1(E)2. Maximum vigour index of 29866.67 was exhibited by plants treated with BA14(D)1 (Table 4).

The above results establish that the response of treated plants varied between treatments and there is no clear cut evidence to establish that high IAA producers are invariably involved in better plant growth. When all the isolates were compared the maximum root length of 187.5 mm was exhibited by plants treated with BA16(A)2 which was a medium IAA producer whereas the highest IAA producer BA1(E)2 (32.2 µg/ml of IAA) showed a root length of 167.3 mm. The isolate OTN7(E)2 which was low IAA producer showed good root lengths of 148.3 mm (sterile soil) and 133.3 mm (unsterile soil). When the shoot lengths were compared, highest shoot length of 213.3 mm (sterile soil) was by BA4(D) which is a medium to high IAA producer and in unsterile soil highest shoot length of 198.67 mm was shown by the isolate ND1which is also a medium to high IAA producer. But the medium IAA producer BA11(D) also showed high shoot length of 210 mm (sterile soil) and 168.67 mm (unsterile soil). The highest IAA producer BA1(E)2 showed a low shoot length of 156.7 mm (sterile soil) and 115 mm (unsterile soil) .The results in some way do establish that plant growth is not directly influenced by high IAA producers.

The lowest IAA producer BA16(2) had a positive effect on shoot length (181.7 mm) under sterile conditions and 170 mm under unsterile conditions but the root growth was not greatly influenced. The highest vigour index of 36500 (sterile soil) was exhibited by a medium to high IAA producer namely BA16(A)1. Lowest vigour index 16316.7 was observed in OTN7(2) which is also a medium to high IAA producer. In unsterile soil maximum vigour index of 32600 was exhibited BA2(D)1 which is a medium IAA producer and the minimum of 15750 was by BA1(E)2 which is a high IAA producer. Hence treatment with high IAA producers does not necessarily translate into enhanced plant growth (Fig. 1). The present studies indicate that IAA producers have positive effect on shoot length and that selection of plant growth promoting bacteria need not be based on high IAA producing ability but rather on the bacterium having IAA producing trait that has an enhanced effect on plant growth. One point that was consistent is that all IAA

Table 4. Groundnut plant growth in response to inoculation with IAA producing bacteria in unsterile soil

Isolate Name	Concentration of IAA/ml released at 192h		Seedling growth test	*In vivo* evaluation of plant growth under potted conditions			
	Trypt⁻	Tryp+	Fresh plant weight (g)	Root length (mm)	Shoot length (mm)	% Germination	Vigour Index
BA5(D)	31.6 H	62.2 H	3.4	118.7	166.67	66.7 (60.00)	18533.33
BA10(D)1	27.8 H	58.0 H	4.2	158.3	155	83 (75.00)	25166.67
BA14_(D)1	27.0 H	56.2 H	4.2	143.3	155.33	100 (90.00)	29866.67
BA2(D)2	22.6 M	51.0 H	3.9	115	169.67	83 (75.00)	23633.34
BA1(E)2	32.2 H	48.4 H	3.4	123.3	115	66.7 (60.00)	15750.00
ND3IART(A)	21.6 M	47.6 H	4.0	126.7	188.33	100 (90.00)	28166.67
NDI	20.8 M	46.4 H	3.8	190	198.67	66.7 (60.00)	26200.00
BA3(E)1	26.8 H	46.0 H	3.4	155	184	83 (75.00)	28166.67
BA3(E)2	28.8 H	40.0 H	4.2	158	169.67	66.7 (60.00)	20800.00
OTN7(2)	22.0 M	40.0 H	4.4	121.7	170.67	83 (75.00)	21233.34
BA4(D)	29.2 H	38.2 M	5.2	167.7	157.33	83 (75.00)	26366.67
BA3(D)1	28.0 H	38.0M	3.3	108.7	165	83 (75.00)	22600.00
BA8(E)2	18.8 L	35.2 M	4.1	155	175	83 (75.00)	27000.00
BA14(C)3	30.4 H	34.4 M	4.1	160	158	66.7 (60.00)	20833.33
BA16(A)2	21.6 M	32.4 M	4.2	125	170	66.7 (60.00)	19250.00
BA16(A)1	25.2 H	30.4 M	3.3	96.7	141.67	83 (75.00)	19666.67
BA2(D)1	21.6 M	29.2 M	5.3	153.3	172.67	100 (90.00)	32600.00
BA2(C)	23.2 M	29.2 M	5.1	145	176.67	100 (90.00)	32166.67
BA3(D)3	24.0 M	28.0 M	3.5	136.7	176.67	83 (75.00)	26333.34
ND4IART(B)	20.0 M	28.0 M	3.6	132.7	163.33	83 (75.00)	24600.00
GR4RAUA(B)	24.0 M	28.0 M	3.4	132.3	131.67	100 (90.00)	26400.00
CC17(D)	20.4 M	26.0 M	3.5	104.3	130	83 (75.00)	18933.33
BA11(D)	20.6 M	26.0 M	4.8	138.3	168.67	100 (90.00)	30700.00
BA2(E)	20.6 M	25.2 M	4.2	87.3	171.67	83 (75.00)	20966.67
OTN5(D)	21.6 M	25.2 M	4.7	156.7	168.33	83 (75.00)	27250.00
OTN7(E)1	19.2 M	24.8 L	4.2	159	165	83 (75.00)	26650.00
BA2(C)(3-1)	20.0 M	24.4 L	4.1	137.3	163	83 (75.00)	24950.00
BA9(C)1	22.0 M	24.4 L	4.5	143.3	168.33	100 (90.00)	31166.67
BA14(C)2	20.6 M	24.4 L	4.6	133.3	158.33	100 (90.00)	29166.67
BA9(C)2	22.6 M	23.6 L	4.5	156.7	155	83 (75.00)	25833.34
OTN2(D)	18.0 L	23.6 L	4.6	140	156.67	83 (75.00)	25666.67
GR4RAUA(A)	20.6 M	22.8 L	4.1	113.3	168.33	66.7 (60.00)	18083.33
OTN6(E)2	18.8 L	22.8 L	3.5	115	148.33	83 (75.00)	21916.67
GR7(A)	22.0 M	22.8 L	4.6	205	146.67	83 (75.00)	30250.00
BA6(E)2	19.2 M	22.6 L	3.8	126.7	180	83 (75.00)	26666.67
OTN3(E)	19.8 M	22.4 L	4.4	166	164.67	66.7 (60.00)	23283.34
OTN7(E)2	16.4 L	21.6 L	5.0	133.3	152.33	83 (75.00)	24316.67
OTN6(D)	18.0 L	21.6 L	4.4	135	170	66.7 (60.00)	20166.67
OTN7(D)1	20.6 M	20.8 L	4.1	138.3	169.33	83 (75.00)	25633.34
BA16(2)	18.0 L	20.8 L	3.8	101.7	170	100 (90.00)	27166.67
Control	–	–	2.9	70.3	120.33	33 (30.00)	6291.00
CD $P = 0.05$	4.00	4.70	0.62	42.34	37.89	8.72	4402.26

Tryp- = Without tryptophan; Tryp+ = With tryptophan, Figures in parenthesis are angular transformed values; H=high, M=medium, L=low

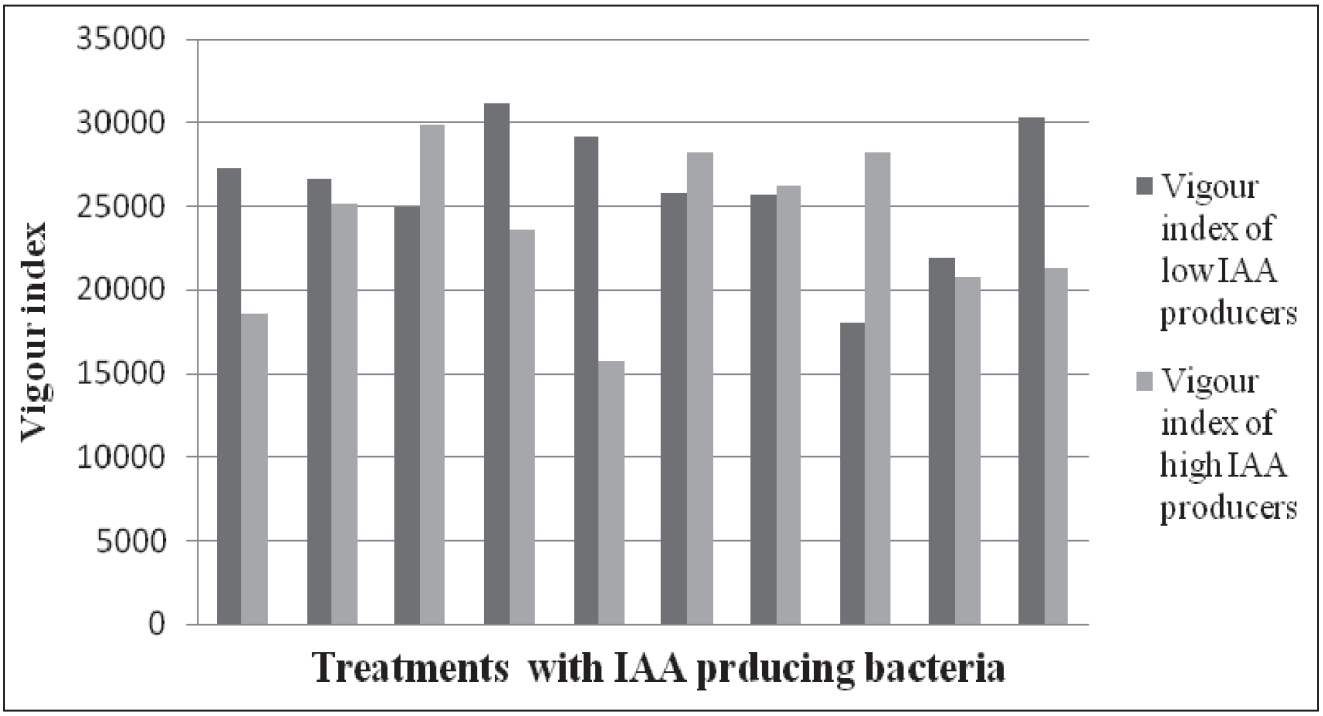

Fig. 1. Vigour index of groundnut in response to seed treatment with low and high IAA producing flourescent *Pseudomonas*

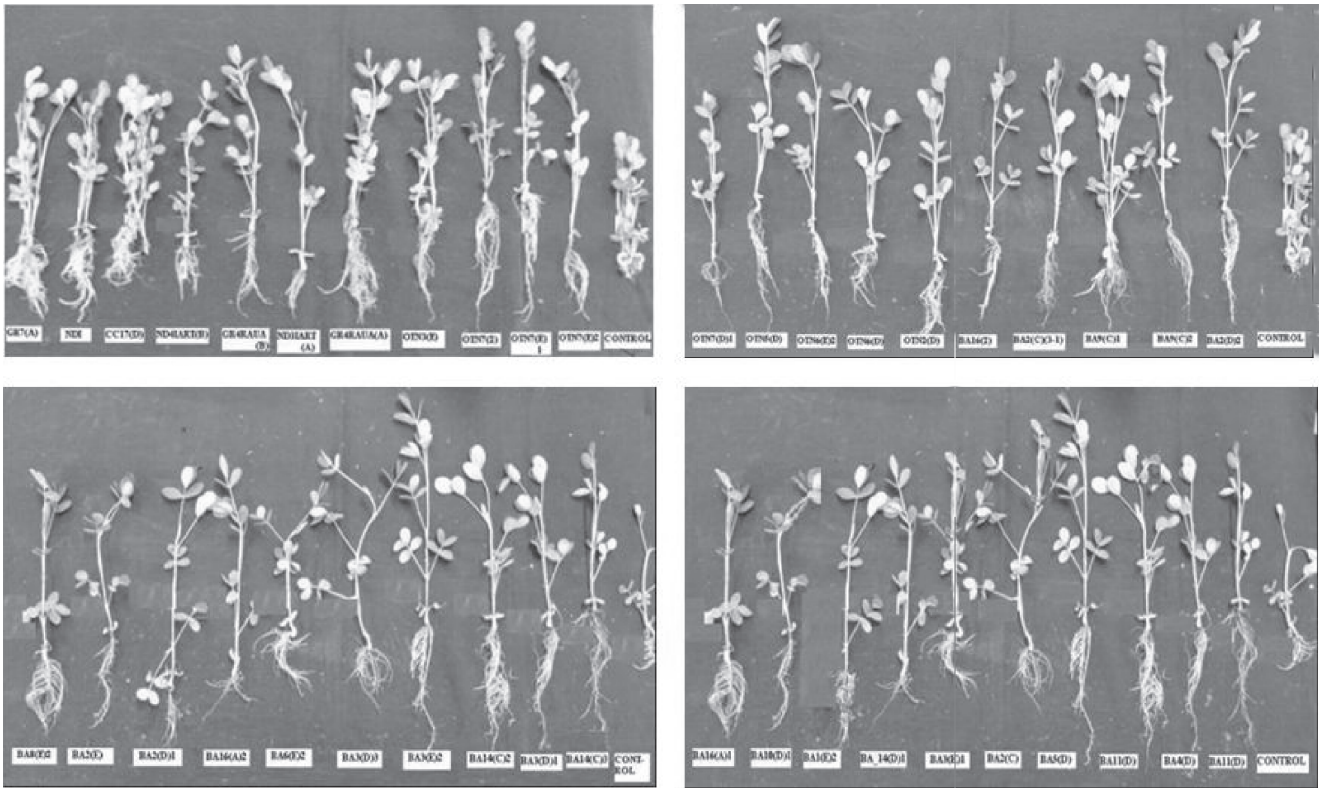

Fig. 2. Evidence of plant growth promotion in groundnut by IAA producing fluorescent *Pseudomonas* (top=sterile soil, down=unsterile soil)

positive isolates had significant effect on plant growth when compared with untreated control. Even when the seed growth studies were analyzed (Table 3), the isolate NDI showed maximum epicotyl growth (75.6 mm) compared to that of the control (9.6) and minimum growth was produced by the isolate BA2(C) (16.8mm). The NDI isolate is a medium to high IAA producer and the BA2(C) is a medium IAA producer. These results show that there is less correlation between the amounts of IAA produced and its contribution to plant growth promotion. However IAA producing ability is important in selecting an antagonist. Rameshkumar *et al.,* (2005) reported the diversity of fluorescent pseudomonads isolated from sugarcane and rice rhizosphere and observed the prevalent IAA production in strains isolated from sugarcane. At least 66% of banana isolates reported in the study produced IAA. Meyer and Hornsperger (1978) reported that the synthesis under certain growth of yellow green, fluorescent, water soluble pigments is a characteristic property of some *Pseudomonas* spp. These species are all members of the some intrageneric homology group. They included *P. aeruginosa*, *P. putida* and *P. fluorescens*. Shinde *et al*. (2003) reported that among ten tested, nine isolates gave positive test for Auxin production (IAA). Jayakumar *et al*. (2004) showed increased plant growth of cotton by *P. fluorescens* due to production of gibberellins, cytokinin and IAA. Bhatia *et al.* (2005) confirmed that all the isolates of *P. fluorescens* from sunflower, potato, maize and groundnut produced fluorescent pigment in succinate broth and displayed IAA production. Other organisms, including *Azospirillum brasilense* and *P. putida* GR12-2, have proven beneficial to plants, and many IAA producers have been shown to stimulate increases in root mass and/or length (Gutierrez *et al*., 2009).

Many have reported that plant beneficial bacteria stimulate increases in root mass and/or length (Patten and Glick, 2002; Holguin *et al*., 1999; Tien *et al*., 1979). Low IAA producers OTN6(D), OTN2(D) and BA16(2) did show good root and shoot growth (Tables 3 & 4). The highest vigour index of 32600 (Table 4) was exhibited by medium IAA producer BA2(D)1. Under unsterile conditions plant growth promotion can be attributed to several factors like IAA, gibberellic acid, cytokinins, and ethylene; nitrogen fixation, suppression of pathogens, siderophores, â-1,3-glucanases, chitinases, antibiotics, cyanide and solubilization of mineral nutrients (Cattalan *et al*., 1999). Bazarani and Friedman (1999) said that bacterial metabolites other than IAA are also involved in root growth and that the nature of these growth promoters is as yet unknown. It has been suggested that succinic and lactic acids eluted from *Pseudomonas putida* are involved in the promotion of root elongation (Yoshikawa *et al.,* 1993). Different concentrations of IAA have different effects on the roots and shoots of a plant. Very low concentrations of IAA stimulate root growth but have no effect on the shoot, whereas higher concentrations of IAA stimulate shoot growth but inhibit root growth. Very high concentrations of IAA inhibit both root and shoot growth (Sarwar and Frankenberger, 1994). Reports on the exact nature of action by IAA produced by microbes on plant growth are still unclear. However, studies using IAA mutant strains of IAA producing isolates are needed to explore the exact contribution of IAA production in the promotion of plant growth (Shahab *et al.,* 2009). Our results indicate that IAA producers of the fluorescent *Pseudomonas* group enhanced plant growth when compared (Fig. 2) with control but plant growth was not greatly influenced by those organisms that produced high amounts of IAA. Antagonistic *Pseudomonas* spp. able to release moderate or even low amounts of IAA may be better growth promoters. The studied isolates are currently being characterized for desirable biochemical traits, antagonistic ability and stress tolerance.

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REFERENCES

Barazani, O. and Friedman, J. 1999. Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? *Journal of Chemical Ecology,* **25**: 2397–2406.

Bhatia, S., Dubey, S. C. and Maheshwari, R. C. 2005. Enhancement of plant growth and suppression of collar rot of sunflower caused by *Sclerotium rolfsii* through fluorescent *Pseudomonas*. *Indian Phytopath*ology, **58**: 17–24.

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

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

Dubeikovsky, A. N., Mordukhova, E. A., Kochethov, V. V., Polikarpova, F. Y. and Boronin, A. M. 1993. Growth promotion of black currant soft wood cuttings by recombinant strain, *Pseudomonas fluorescens*, BSP 53 a synthesizing an increased amount indole-3-acetic acid. *Soil Biology and Biochemistry,* **25**: 1277–1281.

Egamberdieva, D. 2008. Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loamy sand soil. *Turkish Journal of Biology,* **32**: 9–15.

Egamberdieva, D., Kamilova, F., Validov, S., Gafurova, L., Kucharova, Z. and Lugtenberg, B. 2008. High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. *Environmental Microbiology,* **10**: 1–9.

Gordon, S. A. and Weber, R. P. 1951. Colorimetric estimation of indole acetic acid. *Plant Physiol*iology, **26**: 192–195.

Gutierrez, C. K., Matsui, G. Y., Lincoln, D. E. and Lovell, C. R. 2009. Production of the phytohormone indole-3-acetic acid by estuarine species of the genus *Vibrio*. *Applied and Environmental Microbiology,* **75**: 2253–2258.

Holguin, G., Patten, C. L. and Glick, B. R. 1999. Genetics and molecular biology of *Azospirillum*. *Biology and Fertility of Soils,* **29**: 10–23.

Idris, E. E., Iglesias, D. J., Talon, M. and Borriss, R. 2007. Tryptophan-dependent production of indole-3-acetic acid (iaa) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Molecular Plant-Microbe Interactions*, **20**: 619–626.

Jayakumar, J., Ramakrishnan, S. and Rajendran, G. 2004. Evaluation of *Pseudomonas fluorescens* strains isolated from cotton rhizosphere against *Rotylenchulus reniformis*. *Indian Journal of Nematology,* **34**: 206–207.

Joseph, B., Patra, R. R. and Lawrence, R. 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *International Journal of Plant Production,* **2**: 141–152.

Kamilova, F., Kravchenko, L. V., Shaposhnikov, A. I., Azarova, T., Makarova, N. and Lugtenberg, B. 2006. Organic acids, sugars, and L-tryptophan in exudates of vegetables growing on stone wool and their effects on activities of rhizosphere bacteria. *Molecular Plant- Microbe Interactions*, **19**: 250–256.

Kang, B. R., Yang, K. Y., Cho, B. H., Han, T. H., Kim, I. S., Lee, M. C., Anderson, A. J. and Kim, Y. C. 2006. Production of indole-3-acetic acid in the plant-beneficial strain *Pseudomonas chlororaphis* O6 is negatively regulated by the global sensor kinase gacs. *Current Microbiology*, **52**: 473–476.

Kloepper, J. W., Zablotowivz, R. M., Tipping, E. M. and Lifshitz, R. 1990. Plant growth promotion mediated by bacterial rhizosphere colonizers, pp. 315-326. In: D. L. Keister and P. B. Cregan (eds.). *The Rhizosphere and Plant Growth.* Kluwer Academic, Dordrecht.

Kloepper, J. W. and Schroth, M. N. 1981. Relationship of *in vitro* antibiosis of plant growth promoting rhizobacteria and the displacement of root microflora. *Phytopathology,* **71**: 1020–1024.

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

Meyer, J. M., and Hornsperger. 1978. Role of Pyoverdinepf, the iron-binding fluorescent pigment of *Pseudomonas fluorescens,* in iron transport. *Journal of General Microbiology,* **107:** 329–331.

Mordukhova, E. A., Skvortsova, N. P., Kochetkov, V. V., Dubeikovskii, A. N. and Boronin, A.M. 1991. Synthesis of the phytohormone indole-3-acetic acid by rhizosphere bacteria of the genus *Pseudomonas*. *Mikrobiologiya,* **60**: 494–500.

Naik, P. R., Sahoo, N., Goswami, D., Ayyadurai, N. and Sakthivel, N. 2008. Genetic and functional diversity among fluorescent pseudomonads isolated from the rhizosphere of banana. *Microbial Ecology*, **56**: 492–504.

Patten, C. and Glick, B. R. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology,* **42**: 207–220.

Patten, C. L. and Glick, B. R. 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, **68**: 3795–3801.

Rameshkumar, N., ThirumalaiaArasu, V. and Gunasekaran, P. 2005. Biodiversity of rice (*Oryza sativa* L.) and sugarcane (*Saccharum officinarum* L.) rhizosphere pseudomonads. *Indian Journal of Experimental Biology,* **43**: 84–89.

Sarwar, M. and Frankenberger, W. T. 1994. Tryptophan dependent biosynthesis of auxins in soil. *Plant and Soil*, **160**: 97–104.

Sarwar, M. and Kremer, R. J. 1995. Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. *Plant and Soil,* **172**: 261–269.

Shahab, S., Ahmed, N. and Khan, N. S. 2009. Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs**.** *African Journal of Agricultural Research*, **4**: 1312–1316.

Shinde, U., Ashish, K. Singh., Pavan, K. P., Smita, D. V. Sundareswaran, V. R. and Shivaji, S. 2003. Importance of *trmE* for growth of the psychrophile *Pseudomonas syringae* at low temperatures. *Applied and Environmental Microbiology*, **75:** 4419–4426.

Suzuki, S., He, Y. and Oyaizu, H. 2003. Indole-3-acetic acid production in *Pseudomonas fluorescens* HP72 and its association with suppression of creeping bentgrass brown patch. *Current Microbiology*, **47**: 138–143.

Tien, T. M., Gaskins, M. H. and Hubbell, D. H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum*). *Applied and Environmental Microbiology*, **37**: 1016–1024.

Xie, H., Pasternak, J. J. and Glick, B.R. 1996. Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indoleacetic acid. *Curent Microbiology*, **32**: 67–71.

Yeole, R. D. and Dube, H. C. 1997. Increased plant growth and yield through seed bacterization. *Indian Phytopathology,* **50**: 316–319.

Yoshikawa, M., Hirai, N., Wakabayashi, K., Sugizaki, H. and Jwamura, H. 1993. Succinic and lactic acids as plant growth promoting compounds produced by rhizospheric *Pseudomonas putida. Canadian Journal of Microbiology,* **39**: 1150–1154.