Agric Res (September 2013) 2(3):215–221
DOI 10.1007/s40003-013-0065-7

FULL-LENGTH RESEARCH ARTICLE

Optimization of Indole Acetic Acid Production by *Pseudomonas putida* UB1 and its Effect as Plant Growth-Promoting Rhizobacteria on Mustard (*Brassica nigra*)

Umang Bharucha · Kamlesh Patel · Ujjval B. Trivedi

Received: 6 December 2011 / Accepted: 31 May 2013 / Published online: 22 June 2013

Abstract Indole-3-acetic acid (IAA) production was monitored in nine plant growth-promoting rhizobacteria isolated from the rhizospheric soil of alfalfa (*Medicago sativa*). The isolate producing maximum amount of IAA was identified as *Pseudomonas putida* UB1 by 16S rRNA partial gene sequencing. Further investigations were carried out for optimal L-tryptophan concentration and other growth parameters for IAA production by *P. putida*. IAA production increased when the L-tryptophan medium for IAA production was supplemented with sucrose 0.5 %, $(NH_4)_2SO_4$ 10 mg/ml, and tryptophan 0.2 mg/ml at pH 7.5. Maximum IAA production was achieved at 96 h. Production of IAA was confirmed by extraction of crude IAA and subsequent thin-layer chromatography analysis. The specific spot from the extracted IAA was found to correspond with a spot of standard IAA with the same R_f value (0.36). The crude IAA was further purified by passing it through a silica gel column. *P. putida* UB1 and its purified IAA were demonstrated to show stimulatory effect on mustard (*Brassica nigra*) plants in vitro with their incorporation in MS medium as a novel approach.

Keywords Indole-3-acetic acid · *Pseudomonas putida* · Plant growth-promoting rhizobacteria (PGPR) · Mustard (*Brassica nigra*)

Introduction

Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria found in rhizosphere in association with roots and root surfaces or free living in soil and influences plant growth directly or indirectly. A large number of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus,* and *Serratia* have been reported to enhance plant growth [12, 17, 24]. The direct promotion of plant growth involves mechanisms like nitrogen fixation, solubilization of phosphorous and iron from the soil, and production of phytohormones like indole-3-acetic acid (IAA), gibberellins, cytokinins. The indirect mechanisms involve inhibition of phytopathogens [4]. The bacteria that stimulate plant growth are referred to as plant growth-promoting bacteria (PGPB) [3, 8, 13]. Some mycorrhizal fungi and other fungi like *Trichoderma* are also known to stimulate plant growth [21, 37, 39].

Microorganisms inhabiting rhizosphere of plants utilize the rich source of substrates from the roots and are expected to synthesize and release auxins as secondary metabolites [6, 11, 15, 34]. Indole-3-acetic acid is a naturally occurring and main auxin in plants as it controls many important physiological processes like cell enlargement and division, tissue differentiation, and responses to light and gravity [18, 36]. Bacterial auxins have the potential to change any of these processes by altering the plant auxin pool. It depends on the amount of IAA produced and the sensitivity of plant tissue to changing levels of IAA. The roots are the most sensitive organs and respond to the changing levels of IAA by elongation of primary roots, formation of adventitious and lateral roots, or cessation of

U. Bharucha · K. Patel · U. B. Trivedi (✉)
B.R.D. School of Biosciences, Sardar Patel University, Vadtal Road, Vallabh vidyanagar 388 120, Gujarat, India
e-mail: ujjvaltrivedi202@gmail.com; ubt.spu@gmail.com

growth [10]. Indole-3-acetic acid does not function as a hormone in bacterial cells but their ability to produce the same may have evolved as it is important in plant–bacteria relationship [25, 26, 31].

The present study reports optimization of growth parameters for IAA production by *Pseudomonas putida* UB1 isolated from the rhizospheric soils of alfalfa (*Medicago sativa*) plants and its plant growth-promoting effect on Mustard (*Brassica nigra*).

Materials and Methods

Isolation and Identification of *Pseudomonas putida*

Rhizospheric soil of alfalfa (*M. sativa*) field located at the experimental farm of Anand Agriculture University, Anand, Gujarat, India, was used for isolation of PGPR organisms. After removal of plant from soil, root portion was cut and packed in sterile plastic bags. The rhizosphere soil was collected in a separate bag. The bags were transported to laboratory under cold conditions for immediate processing. Adhering soil was carefully brushed off, and the plant roots were vigorously shaken and washed off with sterile saline solution so as to remove microorganisms closely associated with roots. To prepare soil suspension, approximately 1 gm of soil was suspended in sterile distilled water and vortexed. This was then serially diluted. 100 µl aliquot of dilution was plated in triplicate on nutrient agar and yeast extract mannitol agar medium. Plates were incubated at 28 °C for 3 days. Well-isolated colonies were selected, purified, and maintained on nutrient agar and yeast extract mannitol agar. IAA-producing isolates were selected by growing them in IAA production medium as described below. The isolate producing maximum amount of IAA was further selected for the optimization of IAA production.

The isolate producing maximum IAA was characterized based on the 16S rRNA partial sequencing using universal primer set 16F (5′AGA GTT TGA TCC TGG CTG 3′) and 16R (5′GGT TAC CTT GTT ACG ACT 3′) [27]. Amplification was performed in thermocycler with following PCR conditions: 35 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min with initial denaturation at 95 °C for 5 min and final extension at 72 °C for 10 min. The isolate was identified as *P. putida.*

IAA Production and Optimization of Production Parameters

For IAA production, the culture medium was inoculated with overnight grown *P. putida* UB1 (1 O.D cells/100 ml); the IAA production medium consisted of the following components (g/l): peptone 10, L-tryptophan 1, NaCl 5, yeast extract 6, and pH adjusted to 7.6 [20]. The cultures, in 500 ml flasks containing 200 ml medium each, were incubated at 30 °C, 150 rpm for 72 h. Five ml of the medium was withdrawn regularly at 12-h interval and assayed for IAA production by Salkowski's method [14]. Two ml of supernatant was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski's reagent (50 ml of 35 % perchloric acid and 1 ml 0.5 M $FeCl_3$ solution) and incubated in dark for 1 h. Absorbance was measured at 535 nm using spectrophotometer (Helios).

IAA Production Profile

The fermentation medium with precursor L-tryptophan was prepared and inoculated with culture (1 O.D cells/100 ml) and incubated at 30 °C in an environmental shaker at 150 rpm. Samples of 5 ml each were withdrawn at 12-h intervals for up to 144 h and analyzed for IAA production. Similar incubation conditions were used in other experiments. Concentration of L-tryptophan as a precursor greatly influences the IAA production. The precursor concentration was used in the range of 0.05–0. 25 mg/ml in the medium. To determine the effect of static and shaking condition on IAA production, 2 sets of flasks with similar production media were inoculated. One set was kept in the environmental shaker at 150 rpm, and the other set was maintained in static condition. pH is one of the most important physicochemical parameters, which affects the overall production of IAA. pH range 6–8 was examined for its effect on IAA production. The initial pH of the medium was adjusted using 1 N HCl or 1 N NaOH. Four different sugars viz. sucrose, glucose, lactose, and fructose were used in the fermentation medium at concentration of 0.5 % to analyze their effect on IAA production. The media containing different sugars with the same amount of precursor were inoculated with the organism. After incubation, the supernatants were subjected to IAA estimation. IAA production was studied in the presence of additional five different nitrogen sources like $NaNO_3$, KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, and urea at the concentration of 10 mg/ml in L-tryptophan-supplemented production media.

Extraction, Purification, and Characterization of IAA

Two flasks containing 300 ml of IAA production media with 15 mg/ml of L-tryptophan were inoculated with the organism. After incubation, the media was centrifuged at 5000*g* for 15 min, and the supernatant was acidified to pH 2.5 with 1 N HCl and extracted twice with equal volume of ethyl acetate. The ethyl acetate fraction was evaporated to dryness at 40 °C using rotavapor. The dried extract was dissolved in 3 ml of methanol and kept at 4 °C. The

production of IAA was confirmed by spotting 10 µl of the IAA extract on TLC plate (10 × 10 cm silica gel 60 F_{254}, Merck, Germany) along with 50 µg/ml of IAA (MW 175.19) standard (Hi-Media) in methanol. The solvent system used was n-butanol: ammonia: water (10:1:10), and the plates were developed using Salkowski's reagent.

Partial purification of IAA from crude extract was carried out by silica gel column chromatography (22 × 5 cm), and fractions were collected with solvent system, n-butanol: ammonia: water (10:1:10). 10 µl of each fraction was tested for IAA using Salkowski's reagent and thin-layer chromatography as discussed earlier. The fractions showing presence of purified IAA were pooled together and dried. The IAA thus obtained was used for further experiments.

In Vitro Assay for Plant Growth Promotion

Indian mustard (*B. nigra*) was used to check the effect of isolate and its purified IAA for their plant growth-promoting activity. Mustard seeds were sterilized using 0.01 % $HgCl_2$ and washed with sterile distilled water four times. The seeds were then allowed to germinate in sterile petriplates containing filter paper soaked in sterile distilled water for 2 days. These germinated seeds were then transferred to sterile 30 ml of MS [22] medium (Hi-Media) without IAA in the following two different sets.

1. Tubes containing sterile MS medium inoculated with the isolate (100 µl of cell suspension inoculated from a suspension containing 1 O.D cells/100 ml).
2. Tubes containing sterile MS medium containing IAA (16 µg/l) produced by the isolate.

The above tubes were incubated at 30 °C and were exposed to 12-h light/dark period (relative humidity maintained at 40 % and shelves illuminated by cool, white fluorescent lamps (40 W) to obtain the light intensity of 30–50 m.e.m 2 s−1). After 15 days, the seedlings were collected and measured for shoot length, root length, number of leaves, number of adventitious roots, wet weight of plants, shoots: root ratio, and chlorophyll content.

Results and Discussion

All the 9 isolates of *P. putida* UB1, tested for their IAA production showed a significant amount of IAA production in tryptophan-supplemented medium (Table 1), whereas no IAA production was observed in the medium devoid of tryptophan. The production of IAA by the isolates only in the presence of L-tryptophan indicates that the tested strains utilize L-tryptophan as a precursor for IAA production during their growth in the medium. Maximum IAA production was obtained with isolate UB1.

Identification of the Isolate

The isolate was identified by 16S rRNA gene analysis. The 16S rRNA NCBI BLAST analysis showed 99 % similarity with the strain *P. putida* strain NB2011. Further phylogenetic analysis using software MEGA 4 revealed that the strain finds closest relationship with *P. putida* and hence was confirmed to be a putida strain (Fig. 1) (NCBI GenBank accession JF 304108).

IAA Production Profile

To investigate the effect of incubation period on IAA production, the samples were withdrawn from the production media at 12-h interval up to 144 h. The data obtained suggest there was growth-associated IAA production, and maximum production was observed at 96 h (Fig. 2). The results indicated that L-tryptophan from the medium is taken up at more or less constant rate and transform it to IAA. After 96 h, the growth and IAA production gradually decreased. Previous study by Swain et al. [35] showed that IAA production increased linearly from 2 to 8 days and decreased later with a decrease in the growth of organisms in L-tryptophan-supplemented medium. Patten and Glick [25, 26] have also reported increase in IAA production up to 96 h and attributed to the greater availability of the precursor. Datta and Basu [9] studied the IAA-degrading enzymes responsible for decrease in IAA production after 96-h incubation period. The decrease in IAA production might be due to the production of IAA-degrading enzymes.

Effect of L-tryptophan Concentration on IAA Production

L-tryptophan is considered as a precursor for IAA production because its addition to medium increases IAA production [1, 29, 32]. Different concentrations of L-tryptophan between 0.05 and 0.25 mg/ml were selected to see

Table 1 Indole acetic acid production by cultures isolated from the rhizospheric soil of alfalfa

Name of the isolate	Production of IAA (µg/ml)
UB1	591.8
UB2	480.02
UB3	428.8
UB4	35.2
UB5	26.4
UB6	96.8
UB7	29.7
UB8	152.9
UB9	78.9

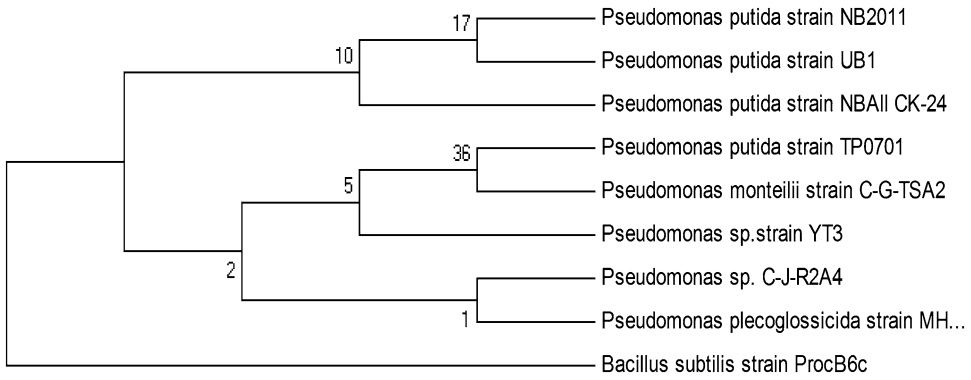

Fig. 1 Phylogenetic analysis based on 16S rRNA gene sequences available from National Center for Biotechnology information data library constructed after multiple alignments of data by ClustalX. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Phylogenetic analyses were conducted in MEGA software version 4.0

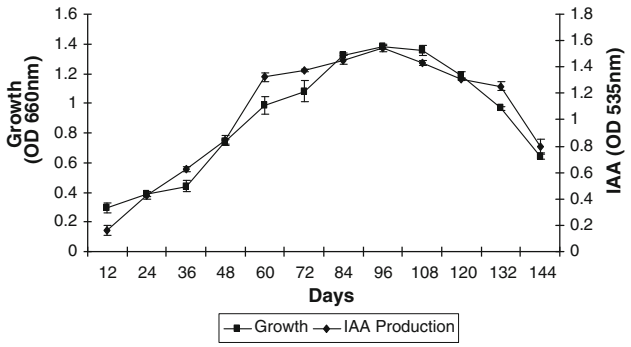

Fig. 2 Effect of incubation period on IAA production

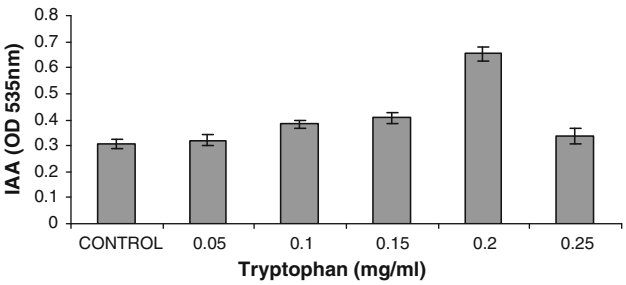

Fig. 3 Effect of L-tryptophan concentration on IAA Production

the effect on IAA production. The spectrophometric analysis showed gradual increase in the IAA production with the increase in L-tryptophan concentration. 0.2 mg/ml of L-tryptophan concentration in the medium showed maximum IAA production (Fig. 3). Our results are in agreement with the work of Khalid et al. [16] who studied the effect of L-tryptophan concentration for the production of IAA and observed that L-tryptophan-derived auxin biosynthesis was enhanced several folds. Khalid et al. [16] showed variable amount of auxins produced by the rhizobacteria in vitro, and amendment of the culture media with L-tryptophan (l-TRP) further stimulated auxin biosynthesis. As per our studies, L-tryptophan concentration used (ranging from 0.05 to 0.25 mg/ml) which resulted in an increase in IAA production where 0.2 mg/ml tryptophan concentration gave maximum IAA production.

Effect of Static and Shaking Conditions on IAA Production

Analysis after incubation showed higher production in shaking condition compared to the production in the static

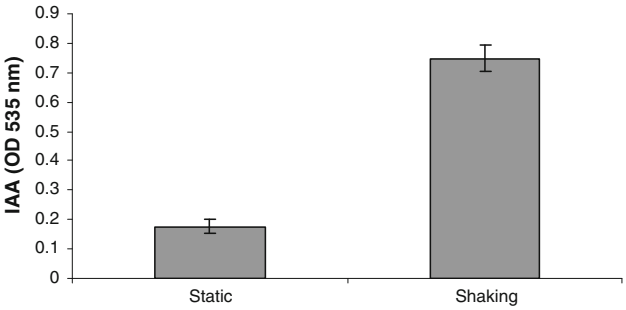

Fig. 4 Effect of agitatic and static condition on IAA production

condition (Fig. 4). Higher production under shaking condition might be due to good oxygen transfer rate as oxygen availability influences the activity of enzymatic transformations of tryptophan to auxins. Reinecke and Bandurski [28] showed the importance of oxygen concentration for IAA production where shaking resulted in better levels of IAA excretion. Sarwar et al. [30] also demonstrates that IAA production in shaking conditions increases up to twofold as compared with static incubation under the optimal assay conditions.

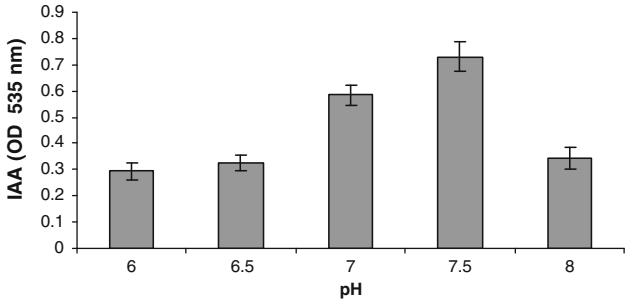

Fig. 5 Effect of pH on IAA production on IAA production

Effect of pH on IAA Production

Physicochemical conditions of the media used are always specific for the organisms to biosynthesize the products. One of the most important parameter for the growth of IAA-producing organisms and their metabolic activity is the pH of the IAA production media [40]. In our investigation, maximum IAA production was observed at pH 7.5 (Fig. 5). Previous studies by Santi et al. [29] also suggest that *Rhizobium* sp isolated from the root nodules of *Vigna mungo* (L.) also varied with the IAA production in different pH ranging from 6.4 to 7.8 where maximum IAA production was found at pH 7.2. It has also been demonstrated by Sarwar et al. [30] that soil pH has a significant effect on L-tryptophan-derived IAA production. Yuan et al. [40] studied the effect of different fertilization treatment on soil leads to change in the pH of soils which in turn affects the IAA production by soil bacteria.

Effect of Carbon Source on IAA Production

The carbon sources that are used in the biosynthesis of plant hormone during their growth in liquid culture media contribute to the overall efficiency of biosynthesis. Four different sugars (sucrose, fructose, lactose, and glucose) were studied for their effect on IAA production. Sucrose in the medium gave maximum production as compared to other carbon sources (Fig. 6). Studies on IAA production by bacteria suggest that individual carbon sources effects the IAA production by bacteria [29, 33, 38]. Studies by

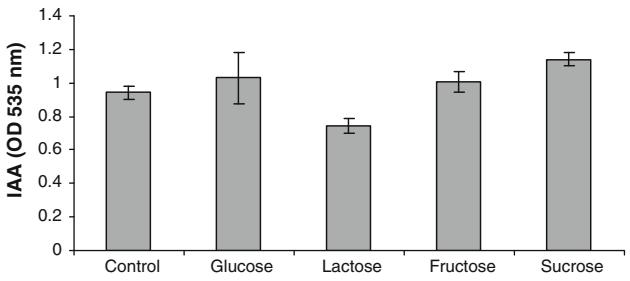

Fig. 6 Effect of carbon source on IAA production

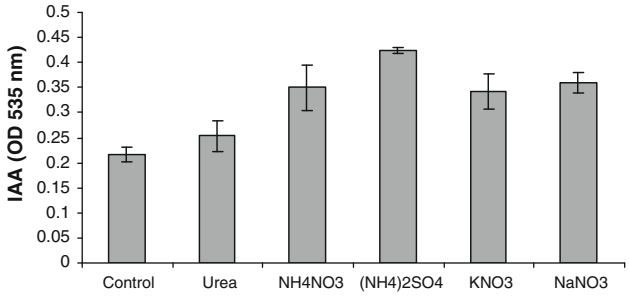

Fig. 7 Effect of nitrogen source on IAA production

Sridevi et al. [33] revealed that mannitol and L-glutamic acid were the best promoters for IAA production by *Rhizobium* isolates. Basu and Ghosh [5] have reported biomass to carbon source ratio playing very important role in cell yield and related IAA production where the preferred carbon source by *Rhizobium* species was glucose at the rate of one percent.

Effect of Nitrogen Source on IAA Production

Impact of nitrogen sources on IAA production was studied by addition of various nitrogenous compounds like urea, $NaNO_3$, KNO_3, NH_4NO_3, and $(NH_4)_2SO_4$ to the tryptophan-supplemented medium. Among all the nitrogen sources used, $(NH_4)_2SO_4$ was found to be the best nitrogen source for IAA production (Fig. 7). The nitrogen source of the production medium affects IAA production [19, 29, 33]. Studies on IAA biosynthesis by root nodule bacteria of various leguminous plants revealed that L-asparagine and glutamic acid when used as nitrogen source stimulate the IAA production.

Chromatographic Detection and Partial Purification of IAA

Indole-3-acetic acid produced was subject to detection by thin-layer chromatography using the solvent system n-butanol: ammonia: water (10:1:10). The chromatograms were observed under visible and UV light (254 nm). Upon spraying with the developing agent (Salkowski's reagent), pink-colored spots were observed with the R_f values 0.36 similar to the authentic IAA. Thin-layer chromatography and comparison with R_f value of the standard revealed purified compound to be IAA. Ahmad et al. [1] have used the extract of the cultures and standard IAA and tested them in the solvent system hexane: ethyl acetate (80:20).

In Vitro *Assay of Pseudomonas putida* UB1 for its Plant Growth-Promoting Activity

Mustard (*B. nigra*) seeds were selected to check the plant growth-promoting activity of *P. putida* UB1. The germinated

Table 2 Changes in various growth parameters during growth of *B. nigra* inoculated with a) *P. putida* UB1 b) purified IAA from *P. putida* UB1

Treatment parameters	Inoculation with *P. putida* UB1		Purified IAA from *P. putida* UB1	
	Control	Experiment	Control	Experiment
Average chlorophyll content	0.44 ± 0.015	2.916 ± 0.03	0.55 ± 0.02	1.5 ± 0.02
Average shoot length	3.26 ± 0.030	4.23 ± 0.035	3.56 ± 0.026	3.8 ± 0.005
Average root length	2.4 ± 0.015	2.4 ± 0.0152	2.4 ± 0.025	2.5 ± 0.005
Average no. of Adventitious roots	2.4 ± 1.154	13.66 ± 0.036	4.33 ± 0.577	8 ± 0.577
Average no. of leaves	5.33 ± 0.577	5.6 ± 0.152	4 ± 0.577	6 ± 0.577

seeds of mustard were placed in tubes containing MS medium supplemented with (a) *P. putida* UB1 and (b) IAA purified from the broth inoculated with *P. putida* UB1 as a novel approach of incorporating the organism and its product in tissue culture media (Table 2). Uninoculated tubes were kept as control for each set of experiment. Analysis of the seedlings after 15 day's growth showed that both the treatments helped in increasing the chlorophyll content, shoot length, and in the number of leaves as compared to control; the increase was more pronounced in seedlings grown in the medium containing *P. putida* compared to the medium supplemented with IAA. The root length was not much affected but an increase in the number of adventitious roots was observed which provides more surface area to the roots for better utilization of nutrients. Microorganisms in the rhizosphere utilize the root exudates and synthesize various metabolites, which are utilized by plants. Presence of *P. putida* UB1 in the medium showed an increase in the shoot length, root length, and number of adventitious roots; hence, it can be concluded that *P. putida* UB1 is able to utilize the root exudates and synthesize plant growth regulator like IAA [6, 11].

Conclusions

In this study, nine isolates were tested for their ability to produce IAA. The isolate producing maximum amount of IAA was identified as *P. putida* UB1. Studies on optimization of IAA production suggest that maximum amount of IAA is produced at 96 h of incubation in IAA production media. The isolate produces maximum IAA at 0.2 mg/ml of tryptophan concentration, using sucrose and ammonium sulfate as carbon and nitrogen source, respectively. The presence of IAA in the rhizosphere influences the growth of the plant. Results of the plants harvested from the tubes indicated that the treatments helped in increasing the chlorophyll content, shoot length, and in the number of leaves as compared to control. The root length was not much affected but an increase in number of adventitious roots was observed which provides more surface area to the roots for better utilization of nutrients. Microorganisms in the rhizosphere utilize the root exudates and synthesize various metabolites utilizable by plants. Presence of *P. putida* UB1 in the medium showed an increase in the shoot length, root length and number of adventitious roots, hence it can be said that *P. putida* UB1 is able to utilize the root exudates and synthesize plant growth regulator like IAA. *P. putida* has been used for bioremediation as soil inoculant, as biocontrol agent, and for plant growth promotion [2, 7, 23]. We have demonstrated the incorporation of the organism and its product during the growth of the plant in tissue culture media as a novel approach resulting in better growth of the plant. Similar approach may help in obtaining healthy and better plants with tissue culture technique resulting in better survival of the plants when transferred to fields from laboratory. Thus, besides many other applications of *Pseudomonas* in biotechnology, it may find its importance in agricultural biotechnology as well.

Acknowledgments Umang Bharucha is thankful to University Grants Commission (Delhi, India) for Teacher Fellowship.

References

1. Ahmad F, Ahmad I, Khan MS (2005) Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turk J Biol 29:29–34
2. Akhtar MS, Siddiqui ZA (2009) Use of plant growth promoting rhizobacteria for the biocontrol of root rot disease complex of chickpea. Australs Plant Pathol 38:44–50
3. Arshad M, Frankenberger WTJ (1998) Plant growth-regulating substances in the rhizosphere: microbial production and functions. Adv Agron 62:145–151
4. Bashan Y, Holguin G (1998) A proposal for the division of "plant growth promoting rhizobacteria" into two classifications: biocontrol-PGPB and PGPB. Soil Biol Biochem 30:1225–1228
5. Basu PS, Ghosh AC (2001) Production of indole acetic acid in cultures by a *Rhizobium* species from the root nodules of a monocotyledonous tree, *Roystonea regia*. Acta Biotechnol 21:65–72
6. Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486
7. Bhattacharya PN, Jha DK (2012) Plant growth promoting rhizobacteria(PGPR): emergence in agriculture. World J Microbiol Biotechnol 28(1327):1350
8. Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant associated bacteria. Crit Rev Microbiol 21: 2001–2013
9. Datta C, Basu PS (2000) Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. Microbiol Res 155:123–127

10. Davies PJ (1995) The plant hormone concept: concentration, sensitivity and transport. In: Davies PJ (ed) Plant hormones: physiology, biochemistry, and molecular biology. Kluwer Academic Publishers, Dordrecht, pp 13–18
11. Drake J, Darby B, Giasson M, Kramer M, Phillips R, Finzi A (2013) Stoichiometry constrains microbial response to root exudation–insights from a model and a field experiment in a temperate forest. Biogeosciences 10:821–838
12. Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–114
13. Glick BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth-promoting bacteria. Imperial College Press, London
14. Glickmann E, Dessauxm Y (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61: 793–796
15. Kampert M, Strzelczyk E, Pokojska A (1975) Production of auxins by bacteria isolated from pine roots (*Pinus syivestris* L.). Acta Micro-biol Poll 7:135–143
16. Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96:473–480
17. Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–43
18. Lambrecht M, Okon Y, Broek AV, Vanderleyden J (2000) Indole-3-acetic acid: a reciprocal signaling molecule in bacteria-plant interactions. Trends Microbiol 8:298–300
19. Leelahawonge C, Pongsilp N (2009) Factors influencing indole-3-acetic acid biosynthesis of root nodule bacteria isolated from various leguminous plants. Thamasat Int J Sci Tech 14(2):1–12
20. Loper JE, Schroth MN (1986) Influence of bacterial source of indole –3-acetic acid of root elongation of sugar beet. Phytopathol 76:386–389
21. Molla AL, Haque M, Haque A, Ilias GNM (2012) Trichoderma enriched biofertilizers enhances production and nutritional quality of tomato (Lycopersicon esculentum Mill) and minimizes NPK fertilizer use. Agric Res 1:265–272
22. Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497
23. Nigam A, Phale PS, Wangikar PP (2012) Assessment of the metabolic capacity and adaptability of aromatic hydrocarbon degrading strain *Pseudomonas putida* CSV86 in aerobic chemostat culture. Bioresours Technol 114:484–491
24. Okon Y, Labandera-Gonzalez CA (1994) Agronomic applications of *Azospirillum*. In: Ryder MH, Stephens PM, Bowen GD (eds) Improving plant productivity with rhizosphere bacteria. Commonwealth Scientific and Industrial Research Organization, Adelaide, pp 274–278
25. Patten CL, Glick BR (2002) Regulation of indole acetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and the stationary phase sigma factor RpoS. Can J Microbiol 48:635–642
26. Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801
27. Pidiyar VJ, Jangid K, Dayananda KM, Kanznowski A, Gonzalez JM, Patole MS (2003) Phylogenetic affiliation of *Aeromonas culicicola* MTCC 3249 (T) based on gyrB gene sequence and PCR-amplicon sequence analysis of cytolytic enterotoxin gene. Syst Appl Microbiol 26:197–202
28. Reinecke DM, Bandurski RS (1987) Auxin biosynthesis and metabolism pp. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Kluwer Academic Publishers, Dordrecht, pp 24–42
29. Santi M, Keshab C, Dey S, Pati BR (2007) Optimization of cultural and nutritional conditions for indole acetic acid production by a *Rhizobium* sp. isolated from root nodules of Vigna *mungo* (L.) Hepper. Res J Microbiol 2:239–246
30. Sarwar M, Arshad M, Martens DA, Frankenberger WT (1992) Tryptophan-dependant biosynthesis of auxins in soil. Plant Soil 147:207–215
31. Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism plant signaling. FEMS Microbiol Rev 31:425–448
32. Sridevi M, Mallaiah KV (2007) Bioproduction of indole acetic acid by *Rhizobium* strains isolated from root nodules of green manure crop, *Sesbania sesban* (L.). Merr Iran J Biotechnol 5: 178–182
33. Sridevi M, Yadav NCS, Mallaiah KV (2008) Production of indole acetic acid by *Rhizobium* isolates from *Crolataria* species. Res J Microbiol 3(4):276–281
34. Strzelczyk E, Pokojska-Burdziej A (1984) Production of auxins and gibberlin like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and mycorhizosphere of pine (*Pinus silvestris* L.). Plant Soil 81:185–194
35. Swain M, Naskar S, Ray R (2007) Indole-3-acetic acid production and effect on sprouting of yam (*Dioscorea rotundata* L.) minisets by *Bacillus subtilis* isolated from culturable cow dung microflora. Pol J Microbiol 56:103–110
36. Taiz L, Zeiger E (1998) Plant Physiology, 2nd edn. Sinauer Associates, Sunderland
37. Varma A, Bakshi M, Lou B, Hartmann a, Oelmueller R (2012) Piriformospora indica: a novel plant growth-promoting mycorrhizal fungus. Agric Res 1:117–131
38. Wang MS, Zapaa FJ, De Castro DC (1987) Plant regeneration through somatic embryogenesis from mature seed and young inflorescence of wild rice (*Oryza perennis*). Plant Cell Rep 6: 294–296
39. Yadav K, Aggarwal A (2013) Arbuscular mycorrhizal fungi induced acclimatization and growth enhancement of *Glycyrrhiza glabra* L: a potential medicinal plant. Agric Res 2(1):43–47
40. Yuan CL, Mou CX, Wu WL, Guo YB (2011) Effect of different fertilization treatments on indole-3-acetic acid producing bacteria in soil. J Soils Sediments 11:322–329