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Evaluation of rhizobacteria as non-rhizobial inoculants for mung beans

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Abstract

Rhizobacteria associated with natural plant settings were evaluated as non-rhizobial inoculants for *Vigna radiata* (L.). Five bacterial strains of *Providencia*, *Bacillus* and *Alcaligenes* genera were screened for 1-aminocyclopropane-1-carboxylate (ACC) deaminase, auxin production and phosphate solubilization. Highest ACC-deaminse activity was exhibited by *Bacillus pumilus* Sol-1 (430 nmol h^{-1}), *Alcaligenes* sp. Mal-4 (390 nmol h^{-1}) and *Providencia vermicola* Ama-2 (377 nmol h^{-1}). Auxin production by rhizobacteria showed significant positive correlation (up to r = 0.965; P = 0.01) with increasing L-tryptophan concentrations. Bacterial ACC-deaminase activity significantly enhanced root length (up to 50%) and number of roots (up to 47%), over control. On the other hand, L-tryptophan dependent auxin production showed significant negative and positive correlation for root length (up to r = -0.992; P = 0.01) and number of roots (up to r = 0.979; P = 0.01), respectively. In pot trials, *Alcaligenes* sp. Mal-4 recorded maximum increase for shoot length (57%), shoot fresh weight (85%), shoot dry weight (96%), number of pods (64%) and seeds weight (19%). The results showed that *Alcaligenes* sp. Mal-4 could be used as biofertilizer to enhance the vigor and yield of leguminous plants.

Keywords: Bacterial auxin; Natural plant settings; Non-rhizobial inoculants; Rhizobacteria; *Vigna radiate.*
Abbreviations: ACC-1-aminocyclopropane-1-carboxylate; CFU-colony forming units; IAA-indole-3-acetic acid; PGPR-plant growth promoting rhizobacteria; SE-standard error.

Introduction

Plant growth promoting rhizobacteria (PGPR) is a group of free-living bacteria that colonize the rhizosphere and exert a beneficial impact on plant health and soil fertility. PGPR can directly facilitate the plant growth in several ways such as syntheses of phytohormones, siderophores production, solubilization of mineral phosphate and synthesis of the enzyme ACC-deaminase (Raddadi et al., 2008; Ali et al., 2010). The gaseous plant hormone ethylene participates in the regulation of a number of developmental processes in plants. ACC is an immediate precursor of ethylene that may be exuded from plant roots. For many plants a burst of ethylene is required to break seed dormancy. However, after seed germination, a sustained high level of ethylene inhibits root elongation. It has been reported that PGPR contain an enzyme ACC-deaminase that hydrolyses ACC into ammonia and α-ketobutyrate (Penrose and Glick, 2003; Glick, 2005). PGPR have the potential to eliminate the inhibitory effects of higher ethylene concentrations and facilitate the formation of longer roots (Contesto et al., 2008). Several reports have indicated that inoculation of plants with rhizobacteria containing ACC-deaminase increased growth and yield of plants (Shaharoona et al., 2007; Zahir et al., 2009; Siddikee et al., 2011). In recent years, rhizobacteria containing ACC-deaminase activity has been used to alleviate deleterious effects of ethylene under stressed conditions of high salt content, heavy metals, flooding and drought (Grichko and Glick, 2001; Zahir et al., 2008; Koo et al., 2010; Nadeem et al., 2010).

Indole-3-acetic acid (IAA) represents one of the most extensively studied and abundant type of auxin in plants. Like plants, IAA is also quantitatively the most abundant phytohormone secreted by rhizobacteria. Synthesis of IAA by plant associated bacteria is probably one of the most important cause for improving growth and yields of various crops (Ali et al., 2009a, b). For instance, in case of *Azospirillum*, it is generally agreed that auxin production rather than nitrogen fixation, is the major factor responsible for the stimulation of rooting and enhanced plant growth (Bloemberg and Lugtenberg, 2001). A bacterium may directly affect plant growth and development using any one or more of these mechanisms. Since many PGPR possess several of these traits, a bacterium may utilize different traits to facilitate plant growth (Raddadi et al., 2008; Gulati et al., 2009; Mehnaz et al., 2010). Effect of rhizobacteria with auxin production, ACC-deaminase activity and phosphate solubilization is well documented in literature. However, the rhizobacteria exhibiting these growth promoting traits simultaneously have not been evaluated in detail. Moreover, little is known about the plant growth promoting potential of *Alcaligenes* and *Providencia* strains associated with the rhizosphere of natural plant settings. Especially, the strains of *Alcaligenes* and *Providencia* genera have not been evaluated as non-rhizobial inoculants for leguminous plants. Therefore, in present work we compared *Alcaligenes*, *Providencia* and *Bacillus* strains for their growth promoting effects on *Vigna radiata* (L.) Wilczek.

Results

16S rRNA gene sequencing

The sequences of 16S rRNA gene were analyzed by comparison with sequences in GenBank through BLAST (http://www.ncbi.nlm.nih.gov/BLAST). After comparison,

strains Ama-2, Ama-6 associated with the rhizosphere of *A. viridis* showed 98 and 99% similarity with *Providencia vermicola* and *Bacillus pumilus*, respectively. Strains Mal-4 and Nic-2 isolated from the rhizosphere of *M. tricuspedatum* and *N. plumbaginifolia*, respectively, had maximum similarity of 99% with genus *Alcaligenes*. Strain isolated from *S. nigrum* (Sol-1) showed homology of 99% with *B. pumilus*. The sequences from strains Ama-2, Ama-6, Mal-4, Nic-2 and Sol-1 have been deposited in the GenBank under HQ161774, HQ161775, HQ161776, HQ161777 and HQ161778 accession numbers, respectively (Table 1).

ACC-deaminase Activity

Strains showed variable potential for ACC-deaminase activity (Fig 1). *B. pumilus* Sol-1 isolated from the rhizosphere of *S. nigrum* showed highest ACC-deaminase activity (430 nmol h^{-1}). On the other hand, *P. vermicola* Ama-2, *B. pumilus* Ama-6, *Alcaligenes* sp. Mal-4 and *A. faecalis* Nic-2 expressed 377, 234, 390 and 160 nmol h^{-1} ACC-deaminase activity, respectively.

Auxin production by rhizobacteria

Analysis of culture supernatants revealed that bacterial strains produced variable amounts of auxin when grown in the absence and presence of different concentrations of L-tryptophan (Fig 2). In the absence of L-tryptophan, *P. vermicola* Ama-2, *Alcaligenes* sp. Mal-4 and *A. faecalis* Nic-2 did not showed auxin production. However, the addition of L-tryptophan (50 to 500 µg ml^{-1}) to L-broth medium enhanced the auxin production several folds compared to non-amended medium. For instance, *Providencia vermicola* Ama-2 (r = 0.965; P = 0.01), *Bacillus pumilus* Ama-6 (r = 0.814; P = 0.05) and *Alcaligenes* sp. Mal-4 (r = 0.947; P = 0.01), *A. faecalis* Nic-2 (r = 0.907; P = 0.01) and *B. pumilus* Sol-1 (r = 0.931; P = 0.01) showed significant positive correlation with increasing L-tryptophan concentrations. The most active IAA producers were *Providencia vermicola* Ama-2, *Bacillus pumilus* Ama-6 and *Alcaligenes* sp. Mal-4 that produced 46, 45 and 75 µg IAA ml^{-1} respectively at 500 µg ml^{-1} L-tryptophan.

Phosphate solubilization

Phosphate solubilization ability of rhizobacteria was determined qualitatively by streaking bacteria on Pikovskaya agar medium. Out of five bacterial strains, *P. vermicola* Ama-2, *B. pumilus* Ama-6 and *B. pumilus* Sol-1 were found positive for phosphate solubilization (Fig 3).

Bioassays

Inoculation of seeds with rhizobacteria exhibiting ACC-deaminase activity stimulated root growth in majority of the treatments (Fig 4). For root length, significant increases of 50%, 40%, 40% and 30%, respectively, were recorded with *P. vermicola* Ama-2, *Alcaligenes* sp. Mal-4, *B. pumilus* Sol-1 and *A. faecalis* Nic-2. Increases in number of roots were also observed with *P. vermicola* (33%) and *Alcaligenes* sp. (47%). Significant positive correlation (r = 0.903; P = 0.05) was observed between bacterial ACC-deaminase activity and root length. However, non-significant correlation was observed with number of roots. Effect of bacterial auxin on root growth of *V. radiata* was also evaluated in the presence of different concentrations of L-tryptophan (Fig 5). Generally, strains showed inhibitory effect on root length with increasing concentrations of L-tryptophan (Fig 5a). For instance, *P. vermicola* Ama-2 and *Alcaligenes* sp. Mal-4 showed 50% reduction in root length. Maximum root inhibition (62%) was recorded with *A. faecalis* Nic-2 at 500 µg ml^{-1} L-tryptophan. However, bacterial strains significantly stimulated number of roots at 100 (122%), 200 (136%), 300 (122%), 400 (205%) and 500 (220%) µg ml^{-1} L-tryptophan (Fig 5b). *Alcaligenes* sp. Mal-4 was the most effective to enhance number of roots and showed 122%, 136% and 205% increases at 100, 200, and 400 µg ml^{-1} L-tryptophan, respectively, over control. Highly significant negative correlations between L-tryptophan concentrations and root length were recorded with *P. vermicola* Ama-2 (r = -0.942; P = 0.01), *B. pumilus* Ama-6 (r = -0.896; P = 0.05), *A. faecalis* Nic-2 (r = -0.992; P = 0.01) and *B. pumilus* Sol-1 (r = -0.888; P = 0.05). On the other hand, number of roots showed highly significant positive correlations with *P. vermicola* Ama-2 (r = 0.915; P = 0.05), *B. pumilus* Ama-6 (r = 0.979; P = 0.01), *Alcaligenes* sp. Mal-4 (r = 0.892; P = 0.05), *A. faecalis* Nic-2 (r = 0.927; P = 0.01) and *B. pumilus* Sol-1 (r = 0.982; P = 0.01).

Plant growth

Bacterial inoculations showed variable responses for different vegetative and yield parameters at full maturity (Table 2). Significant increases in shoot length were observed with *Alcaligenes* sp. Mal-4 (57%), *B. pumilus* Sol-1 (28%) and *B. pumilus* Ama-6 (25%) as compared to water treated control. Maximum increases in shoot fresh weight were recorded with *Alcaligenes* sp. Mal-4 (85%), *B. pumilus* Sol-1 (67%) and *B. pumilus* Ama-6 (44%). On the other hand, shoot dry weight showed 96% increase with *Alcaligenes* sp. Mal-4. In case of number of pods, significant increases of 64%, 43% and 35% were recorded with *Alcaligenes* sp. Mal-4, *A. faecalis* Nic-2 and *B. pumilus* Sol-1, respectively, over control. Similarly, *Alcaligenes* sp. Mal-4 and *B. pumilus* Ama-6 showed 19% and 8% increases in seeds weight, respectively. However, marginal improvements in seed weight were observed with *P. vermicola* Ama-2 and *A. faecalis* Nic-2. Significant positive correlation between bacterial auxin production (at 500 µg ml^{-1} L-tryptophan) and seed weight (r = 0.857; P = 0.05) was observed. Shoot length also showed highly significant positive correlation with shoot fresh weight (r = 0.941; P = 0.01), shoot dry weight (r = 0.989; P = 0.01) and number of pods (r = 0.424; P = 0.05).

Discussion

Present work demonstrated the ability of newly isolated strains of *Alcaligenes*, *Providencia* and *Bacillus* genera from the rhizosphere of natural plant settings. The results showed that strains exhibited auxin production, ACC-deaminase and phosphate solubilization simultaneously. Colorimetric analysis confirmed that rhizobacteria varied in their ability for auxin production in the absence and presence of L-tryptophan. However, bacterial efficiency for auxin production enhanced several folds when medium was amended with L-tryptophan. It is evident from highly significant positive correlation (r = 0.814; P = 0.05 to r = 0.965; P = 0.01) between bacterial auxin production and L-tryptophan concentrations. Our results are in agreement with previous findings that increasing concentrations of L-tryptophan also stimulated bacterial auxin production (Ahmad et al., 2008; Ali et al., 2009b). Treatment of *V. radiata* seeds with rhizobacteria exhibiting ACC-deaminase activity significantly enhanced the root length (up to 50%)

Table 1. Identification of rhizobacteria by 16S rRNA gene sequencing.

Strains	Plant	Identified as	Accessions
Ama-2	*Amaranthus viridis* L.	*Providencia vermicola* Ama-2	HQ161774
Ama-6	*A. viridis* L.	*Bacillus pumilus* Ama-6	HQ161775
Mal-4	*Malvastrum tricuspedatum* A. Gray	*Alcaligenes* sp. Mal-4	HQ161776
Nic-2	*Nicotiana plumbaginifolia* Viv.	*A. faecalis* Nic-2	HQ161777
Sol-1	*Solanum nigrum* L.	*B. pumilus* Sol-1	HQ161778

Table 2. Effect of bacterial inoculations on vegetative and yield parameters of *V. radiata* (L.).

Strains	Vegetative growth parameters			Yield parameters	
	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Number of pods/ plant	Weight of 100 seeds (g)
Control	16.1 a	10.8 a	2.7 a	12.6 a	3.7 a
P. vermicola Ama-2	17.0 a	13.0 ab	2.8 a	12.7 a	3.9 ab
B. pumilus Ama-6	20.2 b	15.6 bc	3.5 a	17.0 ab	4.0 b
Alcaligenes sp. Mal-4	25.3 c	20.0 d	5.3 b	20.7 b	4.4 c
A. faecalis Nic-2	17.2 a	14.3 abc	3.0 a	18.0 ab	3.9 ab
B. pumilus Sol-1	20.7 b	18.1 cd	4.0 a	17.0 ab	3.6 a

Mean of six replicates (30 plants). Different letters within same column indicate significant difference between treatments using Duncan's multiple range test (P=0.05).

and number of roots (up to 47%), over water treated control. Root length showed positive correlation (r = 0.903; P = 0.05) with bacterial ACC-deaminase activity. The biological activity of bacterial IAA was demonstrated by its inhibitory effect on root length and increase in lateral root numbers. Dobbelaere et al. (2002) reported the root proliferation and increase in root dry weight upon inoculation with *Azospirillum brasilense* as compared to control plants. Similarly, inoculation of seeds with increasing inoculum concentration of wild type *A. brasilense* Sp245 resulted in a strong inhibition of root length and increase in root hair formation (Spaepen et al., 2008). Inoculation of wheat and mung beans with auxin producing rhizobacteria has been shown to result in the reduction of root length and increase in lateral root number (Ali et al., 2009a, b). Statistical analysis of data revealed that majority of the bacterial inoculations significantly enhanced vegetative and yield parameters under natural environmental conditions. The percentage increases in shoot length, shoot fresh weight, shoot dry weight, number of pods and seed weight were up to 57%, 85%, 96%, 64% and 19%, respectively. PGPR inoculated mung bean plants have shown to improve shoot length, fresh weight, number of pods and seed weight (Ali et al., 2009b). It has also been reported that inoculation of *V. radiata* with rhizospheric isolates significantly enhanced the plant growth under natural environmental conditions in pot trials (Shahroona et al., 2006; Ali et al., 2010). The improved plant growth due to PGPR inoculation on different crop plants has also been reported by several workers. Faisal and Hasnain (2006) demonstrated that *B. cereus* inoculations significantly enhanced growth and yield of *V. radiata* under *in vitro* and wire house conditions. In another study, PGPR strains when co-inoculated with *Rhizobium* increases the vegetative growth and grain yield of *V. radiata* (Raza et al., 2004).

Materials and methods

Isolation of bacterial strains

Bacterial strains were isolated from the rhizosphere of *Amaranthus viridis*, *Malvastrum tricuspedatum*, *Nicotiana plumbaginifolia* and *Solanum nigrum*. One gram rhizosphere soil was thoroughly mixed in 99 ml of autoclaved glass-distilled water to make suspensions (Cappuccino and Sherman, 2002). Suspensions were serially diluted (10^{-2}, 10^{-4} and 10^{-6}) and 100 µl plated on L-agar plates. Plates were incubated at 37°C for 24 h. After incubation, thirty bacterial colonies showing prolific growth were picked and purified by many rounds of restreaking on L-agar plates. Finally, five rhizobacterial strains showing plant growth promoting traits were selected (Table 1) and maintained by transferring them to L-agar slants.

DNA extraction, PCR amplification and 16S rRNA gene sequencing

Genomic DNA was obtained from bacterial cultures grown in L-broth for 24 h, at 37^{o}C, using AquaPure genomic DNA isolation kit (BIO-RAD) in accordance with the instructions of manufacturer. The 1.5 kb DNA fragment containing 16S rRNA gene was amplified using forward primer 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1522r (5'-AAGGAGGTGATCCA(AG)CCGCA-3') (Johnson, 1994). PCR amplification was performed by using 50 µl of Dream Taq^{TM} Green PCR Master Mix (Fermentas) with 0.5 µg of chromosomal DNA template and 0.5 µM of each primer. The reaction mixtures were incubated in a thermocycler Primus 96 (PeQLab, Erlangen, Germany) at 94^{o}C for 5 min and passed through 30 cycles: denaturation for 20 s at 94^{o}C, primer annealing for 20 s at 50^{o}C and extension at 72^{o}C for 2 min. Final extension was carried out at 72^{o}C for 5 min. The amplified products were purified using QIAquick Gel Extraction Kit (QIAGEN) and sequenced using 27f and 1522r primers by ABI PRISM-3100 Genetic Analyzer (Applied Biosystems, USA).

ACC-deaminase assay

ACC-deaminase activity of rhizobacteria was assessed by quantifying ammonia liberated by the hydrolysis of ACC. Induction of ACC-deaminase activity in rhizobacteria was accomplished following the method of (Penrose and Glick, 2003). Bacterial cultures were grown in 25 ml L-broth by incubating overnight in an orbital shaker at 120 rev min^{-1} at 37^{o}C. Bacterial biomass was harvested by centrifugation (8000 *g*) for 10 min at 4^{o}C. Cells were washed with 5 ml DF salts minimal medium and re-suspended in 7.5 ml DF salts minimal medium supplemented with 3 mM ACC to induce

the ACC-deaminase activity. The tubes were incubated in an orbital shaker at 120 rev min^{-1} at 30°C for 24 h. After incubation, supernatant was removed and the cells were washed with 5 ml DF salts minimal medium. Finally, bacterial cells were suspended in 7.5 ml DF salts minimal medium in a fresh culture tube containing 3 mM ACC as mentioned above. The bacterial cultures were incubated for 1 h with shaking at 120 rev min^{-1} at 30°C. After 1 h, cultures were centrifuged at 8000 *g* and liberated ammonia was measured in supernatant as described in Nagatsu and Yagi (1966).

Auxin production

Auxin production by bacterial strains was determined in the presence and absence of precursor L-tryptophan. Different concentrations of L-tryptophan were used to evaluate the *in vitro* auxin production by plant associated bacteria. Strains were grown in 100 ml L-broth medium in 250 ml Erlenmeyer flasks supplemented with filter sterilized solution of L-tryptophan in different concentrations such as 0, 50, 100, 200, 300, 400 and 500 µg ml^{-1}. The flasks were inoculated in triplicate with 50 µl of bacterial cell suspension adjusted to 10^7 CFU ml^{-1}. All inoculated flasks were incubated at 37°C for 72 h at 120 rev min^{-1}. After incubation, cells were removed by centrifugation at 2300 *g* for 15 min (Sigma 2-5). One ml of supernatant was taken and mixed with 2 ml of Salkowski reagent as mentioned previously (Ali and Hasnain, 2007). The contents in test tubes were allowed to stand for half an hour for color development. The intensity of color was measured at 535 nm by Aquarius UV/Visible Double Beam Spectrophotometer (CECIL CE 7200). Standard curve was drawn for comparison to determine auxin production in bacterial culture supernatant.

Phosphate solubilization

Phosphate solubilization ability of plant associated bacteria was determined qualitatively by streaking strains on Pikovskaya agar plates (Pikovskaya, 1948). The presence of clearing zone around bacterial growth after one week incubation period at 30°C was used as indicator for positive phosphate solubilization.

Seed sterilization and preparation of inoculum

Certified seeds of *V. radiata* were obtained from Punjab Seed Corporation, Lahore, Pakistan. Healthy seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes, with continuous shaking followed by repeated washing for 3-4 times in sterilized glass-distilled water. For preparation of bacterial suspensions, bacterial strains were grown in 100 ml L-broth overnight at 37°C. Bacterial cells from cultures were harvested by centrifugation at 2300 *g* as mentioned above. Cells were washed and re-suspended in sterilized glass-distilled water. To ensure the equal cell population of each bacterial strain in the suspension, optical density was measured by spectrophotometer at 600 nm. Optical density of cultures was adjusted with sterilized glass-distilled water, to a final concentration of 10^7 CFU ml^{-1}.

Petri dish bioassays

Effect of bacterial ACC-deaminase activity and auxin production on root growth of *V. radiata* was demonstrated in the absence and presence of L-tryptophan, respectively. Petri dishes with two filter papers were autoclaved and soaked

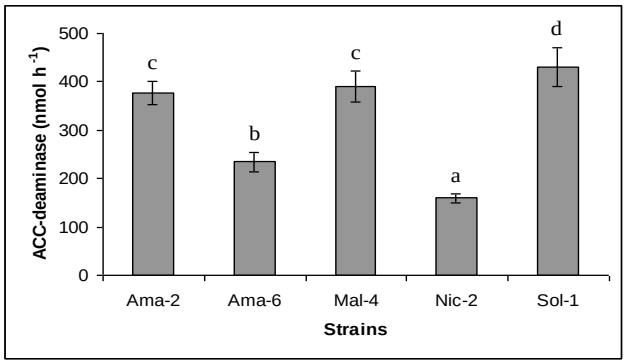


Fig 1. ACC-deaminase activity of rhizobacteria. Bars represents mean ± SE of three replicates. Different letters on bars indicate significant difference between treatments, using Duncan's multiple range test (P=0.05).

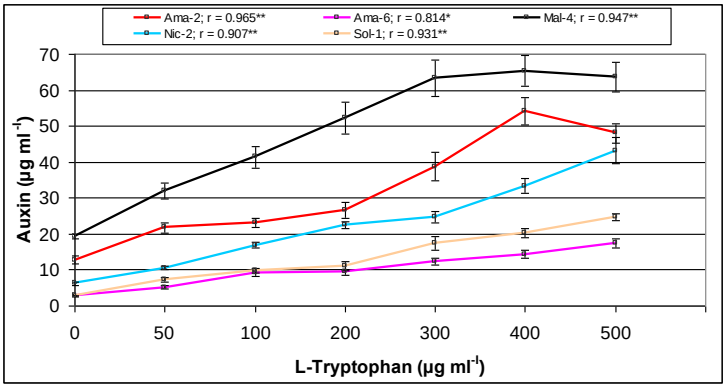


Fig 2. Effect of different L-tryptophan concentrations on auxin production by rhizobacteria. The results shown are representative of three repetitions of the experiment. Bars at different points indicate SE for each treatment. Value "*r*" indicates highly significant positive correlation between L-tryptophan and bacterial auxin production. ** (P = 0.01), * (P = 0.05).

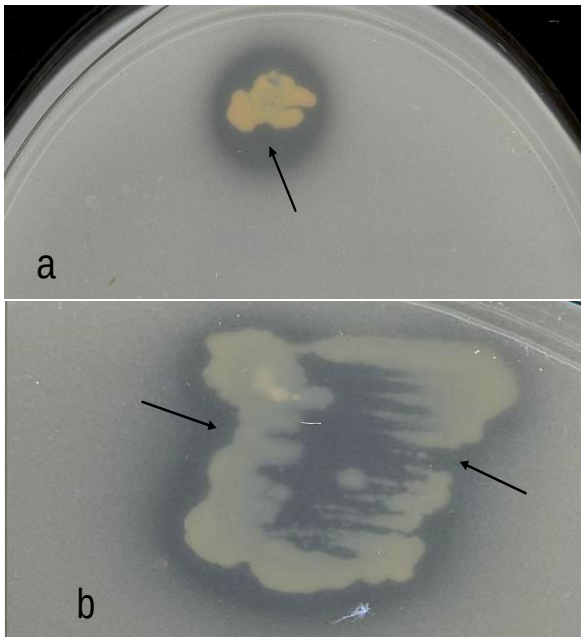


Fig 3. Phosphate solubilization by rhizobacteria. Arrows indicate the clearing zones around bacterial growth. (a)- *P. vermicola* Ama-2; (b) - *B. pumilus* Sol-1.

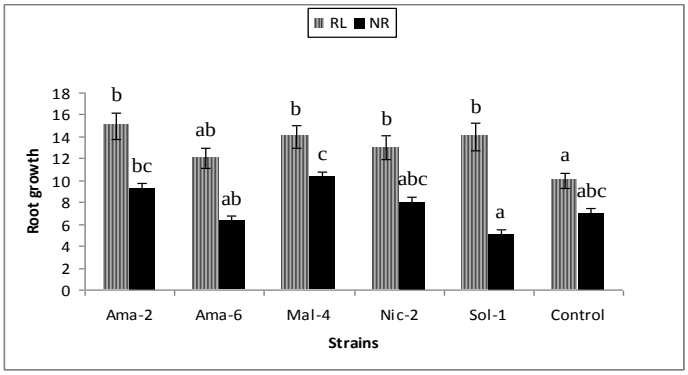
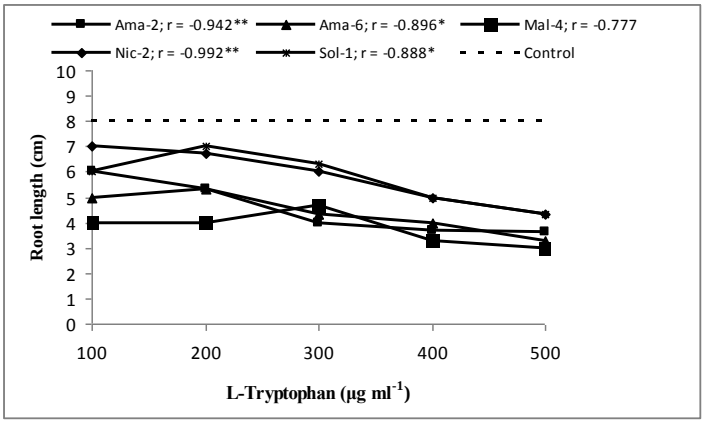
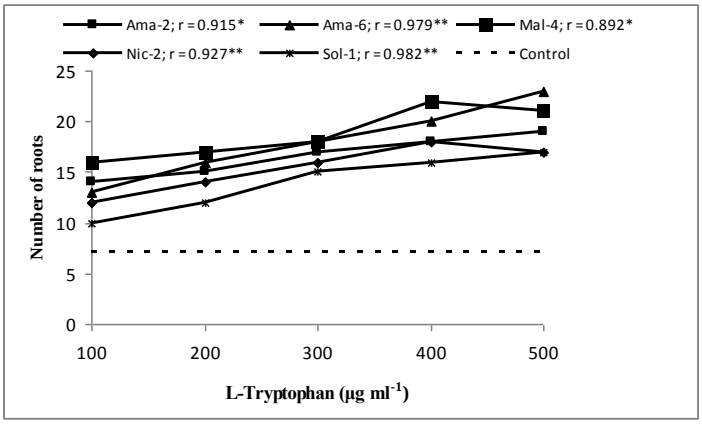


Fig 4. Effect of bacterial ACC-deaminase activity on root growth of *V. radiata*. Bars represents mean ± SE of three replicates (15 plants). Different letters on bars indicate significant difference between treatments, using Duncan's multiple range test (P=0.05). Abbreviations: RL, root length; NR, number of roots.



(A)



(B)

Fig 5. Effect of different L-tryptophan concentrations on root growth of *V. radiata*. (a)- Root length, (b)- Number of roots. Value "*r*" in each figure indicates significant correlation between L-tryptophan concentrations and root growth. ** (P = 0.01), * (P = 0.05).

with 10 ml sterilized distilled water. Five sterilized seeds were placed in each petri dish and experiment was repeated three times under axenic conditions. Two ml stationary phase cultures grown in the presence of 0, 50, 100, 200, 300, 400 and 500 µg ml^{-1} L-tryptophan in L-broth were poured. To observe the effect of bacterial ACC-deaminase on root growth, two ml bacterial cultures grown in the absence of L-tryptophan were applied. Water treated seeds were used as control and all petri plates were incubated in Versatile Environmental Test Chamber (MRL-350H; Sanyo, Osaka, Japan). After 10 days, effect of bacterial cultures on root length and number of roots was observed.

Pot trials

Sterilized seeds of *V. radiata* were inoculated with single bacterial culture suspensions adjusted to the final concentration of 10^7 CFU ml^{-1} as described above. For control treatment, seeds were dipped in sterilized glass-distilled water for 15 minutes. Seeds were sown to a depth of 1cm in earthen pots (30×30 cm) filled with 10 Kg of garden soil. Six pots for each bacterial strain as well as control were used. Initially, 15 seeds in each pot were planted. Pots were moistened immediately after sowing. Arrangement of the pots was made in a completely randomized design in the wire house under ambient light and temperature. After complete emergence of seedlings, plantlets were thinned out to 10 plants per pot. Plants were irrigated regularly and growth of plants was observed daily. After six weeks, 5 plants were removed from each pot to record fresh and dry weight of plants. After measurements of fresh weight, plants were dried in electric oven at 80ºC for 24 h and dry weight of inoculated and non-inoculated plants was taken. Harvesting of plants was carried out at full maturity to record vegetative and yield parameters.

Statistical analysis

For all experiments, the data were subjected to statistical analysis using software SPSS 16 program (SPSS lnc., Chicago, IL). Data were subjected to analysis of variance (ANOVA) and means separated using Duncan's multiple range test (P=0.05). The correlation coefficients between bacterial auxin production and L-tryptophan concentrations as well as between bacterial growth traits and plant growth parameters were also calculated (P = 0.01 or P = 0.05).

Conclusion

In conclusion, rhizobacteria exhibiting multiple plant growth promoting traits enhanced vegetative and yield parameters of *V. radiata* under natural environmental conditions. Bacterial ACC-deaminase activity significantly enhanced root growth under axenic conditions. Increasing L-tryptophan concentrations showed inhibitory effect on root length but enhanced later root numbers that indicated the production of high concentrations of auxin by rhizobacteria.

The present study suggested that *Alcaligenes* sp. Mal-4, *B. pumilus* Ama-2 and *B. pumilus* Sol-1 can be used as a crop enhancer and biofertilizers.

Hence, PGPR strains from natural plant settings harbor beneficial traits that can be applied as non-rhizobial inoculants to enhance the growth and yield of leguminous plants.

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