Acta Physiol Plant (2013) 35:657–665
DOI 10.1007/s11738-012-1106-8

ORIGINAL PAPER

Aeromonas punctata PNS-1: a promising candidate to change the root morphogenesis of *Arabidopsis thaliana* in MS and sand system

Atia Iqbal · Shahida Hasnain

Received: 6 January 2012 / Revised: 7 September 2012 / Accepted: 10 September 2012 / Published online: 26 September 2012

Abstract A model system of sand, comprising Arabidopsis plants inoculated with *Aeromonas punctata* PNS-1 strain, was used to evaluate the bacterial effect in modulation of plant root structure at second-order lateral root level. In MS media, the root morphogenesis was changed only at first-order lateral root level when inoculated with PNS-1 strain. Inoculation with PNS-1 strain was subjected to significant ($P < 0.01$) increase in primary root length and lateral root density in both MS and sand system. However, this strain modulated the root structure in the sand environment in a complex manner that may be helpful for incitation of the plant–microbe interaction close to natural environment. In order to determine whether this change in root morphology was due to bacterial auxin, Arabidopsis transgenic line (DR5:GUS) was used to reveal the change in homeostasis of endogenous auxin. In PNS-1 inoculated transgenic seedlings of Arabidopsis plant (DR5:GUS), endogenous auxin in primary root apices and lateral roots was enhanced. For confirmation, PNS-1 was evaluated for auxin production in vitro, showed an increase in auxin production after supplementation of L-tryptophan. The presence of ACC deaminase activity in PNS-1 showed its possible involvement in primary root elongation. In the present study *Aeromonas punctata* PNS-1 is the potential candidate for triggering the change in root morphogenesis of *Arabidopsis thaliana* with the involvement of auxin and ACC deaminase production.

Keywords *Aeromonas* · *Arabidopsis* · Auxin · ACC · Root

Communicated by H. Li.

A. Iqbal (✉) · S. Hasnain
Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590, Pakistan
e-mail: pisces_dream2000@yahoo.com

S. Hasnain
School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan

Introduction

Lateral root formation is the main determinant of root morphogenesis (Malamy 2005; Lucas et al. 2008). Plant roots interact with variety of bacteria which reside in the soil and are influenced by root exudates. Root exudates, rich in organic compounds, are utilized by some microorganisms (Jones et al. 2003). In response, these microbes exert beneficial effects on root development of the plant by producing different kinds of metabolites (Larcher et al. 2003; Ryu et al. 2004; Pare et al. 2005). Persello-Cartieaux et al. (2003) extracted the plant growth hormones from the supernatant of soil bacterial culture. Among these metabolites, IAA is the widely studied hormone and plays a crucial role in plant productivity by enhancing the root length, elongation of lateral roots and proliferation of root hairs (Vessey 2003; Taghavi et al. 2009). IAA is proved to be a key signal for initiation and proliferation of lateral roots (Casimiro et al. 2003; Laskowski et al. 2006). Plant-exuded tryptophan enters the IAA biosynthesis pathways of bacteria living in the rhizosphere and used in the biosynthesis of IAA. In return, a significant part of the bacterial IAA is delivered to the plant root (Spaepen et al. 2007). It can be synthesized via tryptophan-dependent and tryptophan-independent pathways, although the importance of tryptophan-independent pathways continues to be debated (Cohen et al. 2003). Plant growth promoting rhizobacteria (PGPR) have been shown to release IAA and are assumed

to modify plant auxin content resulting in an elongated and highly branched root system (Barbieri and Galli 1993; Lambrecht et al. 2000; Dobbelaere et al. 2001; Patten and Glick 2002). *Azospirillum brasilense, Aeromonas veronii, Agrobacterium* spp., *Alcaligenes piechaudii, Bradyrhizobium* spp., *Comamonas acidovorans, Enterobacter* spp. and *Rhizobium leguminosarum* are the studied bacteria for IAA production and their effect on plant growth promotion (Saharan and Nehra, 2011). For the evaluation of in situ auxin responses, DR5:GUS transgenic line of *Arabidopsis thaliana (*L.*)* has been used that contains a highly active synthetic auxin-response element (DR5), a minimum promoter and a *β*-glucuronidase (GUS) reporter gene. The GUS activity in reporter line is correlated with concentration of auxin and therefore, can be used to examine the plant response to auxin (Ulmasov et al. 1997). Another important phytohormone, ethylene, plays an important role in different plant developmental processes, i.e. germination of seeds, induction of flowers, morphogenesis and ripening of fruits up to senescence. Further investigation showed its involvement in inhibition of root elongation, lateral root growth and proliferation of root hairs (Mayak et al. 2004). If the level of ethylene after germination is very high, root elongation is inhibited (Glick et al. 1998). Therefore, low level of ethylene indirectly enhances the root length. Bacteria are involved in lowering of ethylene concentration in plants using two mechanisms (Mastretta et al. 2006): (1) producing auxin or (2) 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme. ACC deaminase enzyme decreases the level of ACC which is the precursor for ethylene and successively the ethylene production is reduced, which leads to plant growth promotion by proliferating the plant root system (Penrose et al. 2001; Grichko and Glick 2001; Glick et al. 2007). The occurrence of ACC deaminase is pretty common among soil microorganisms, including *Rhizobium, Agrobacterium, Achromobacter, Enterobacter Burkholderia, Ralstonia, Azospirillum and Pseudomonas* (Wang et al. 2001; Blaha et al. 2006; Duan et al. 2006). The objectives of the present study were to (1) explore the bacterial potential in changing the root morphogenesis of the model plant, (2) design the sand system for better depiction of root structure modulation of *Arabidopsis thaliana*, and (3) evaluate the PGPR traits of isolated *Aeromonas punctata* PNS-1 that are mainly involved in root morphogenesis.

Materials and methods

Isolation and identification

The strain PNS-1 was isolated from wheat plant by following the method of Ali et al. (2009). The strain was identified by 16S rRNA gene sequencing. Bacterial culture was incubated at 37 °C for 24 h. Genomic DNA was isolated with the help of QIAamp DNA mini kit according to instructions (QIAGEN). DNA fragment 1.5 kb was amplified using forward primer 27f (5′-AGAGTTTGATCCTG GCTCAG-3′) and reverse primer 1522r (5′-AAGGAGGTG ATCCA(AG)CCGCA-3′). Total PCR reaction mixture was 50 µl, containing 5 µl 10× buffer, 4 µl (5 mM) dNTPs, 0.1 µl (0.62 units) Taq polymerase, 2 µl (10–20 ng/µl) DNA template, 1 µl of each of the primers (5 pmol) and 36.5 µl water. The PCR program was set on the thermal cycler (Eppendorf, USA) with the following conditions, 2 min at 94 °C, 35 cycles of 1 min at 94 °C, 30 s at 55 °C, 1.5 min at 72 °C, and a final step of 10 min elongation at 72 °C. PCR product was analyzed in 1 % agarose gel, purified by PCR purification kit (QIAGEN) and sent to Cancer Research Centre for sequence facility, University of the Chicago, USA. The resulted sequence was edited and checked for the similarity with already submitted entries using basic sequence alignment BLAST program in NCBI data libraries (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Experimental setup of plant microbe interaction

Autoclaved LB broth was inoculated with bacterial culture in 100 ml flask and incubated for 24 h at 37 °C. After incubation, LB broth was centrifuged (Sigma 2-5; Sigma Laborzentrifugen, Osterode, Germany) at 10,000*g* for 10 min. Pellet was washed with 1 ml of phosphate-buffered saline and suspended in the same buffer. Bacterial cells were adjusted to the cell density of 10^7 CFU ml^{-1}. Arabidopsis Col-0 and DR5:GUS seeds were surface sterilised with 100 % commercial bleach having two drops of Tween 20 for 2 min and then washed with autoclaved distilled water (three times). Sterilized seeds were dipped in bacterial suspension for 30 min, planted in the petriplates containing autoclaved standard Murashige and Skoog media. The MS plates were sealed with Micropore™ tap. Similar procedure was repeated for sand system. Sand system was designed by cutting the 25 ml disposable pipette from one end and filled half of it with quartz sand (Sigma). The MS media was poured on sand to provide nutrients for Arabidopsis growth. This system that contains an opening at the end, filled with cotton, was fitted onto the falcon tube (25 ml) that contains water to keep the environment moist. The whole system was autoclaved. After autoclaving, the seeds incubated in bacterial culture were implanted in the system. The seeds were germinated in the dark at 22 °C and transferred to the environment having 16:8 day:light (200 µE m^{-2} s^{-1}) regime. Root architecture was analyzed after 15 days of seedlings growth. These experiments were repeated three times to check the validity of the results.

Fig. 1 Effect of PNS-1 inoculation on root architecture of *Arabidopsis thaliana* (Col-0) in MS media

Root architecture analysis

For root structure analysis, Arabidopsis plants were removed from MS media plates and the sand system, sand was rinsed away very carefully. The root systems were spread and allowed to dry on the surface of glass plates. After arranging the roots, image was captured by Camera (Olympus). Fifteen plants were selected from MS media and the same from sand system for root measurements. Due to complexity of the root morphology of *Arabidopsis thaliana* grown in sand system, root structure was analyzed using five parameters: the primary root length, total lateral root length, average lateral root length and lateral root density by using the image J software. Root system was divided into first-order lateral root and second-order lateral root and calculated the total lateral root length, average lateral root length and lateral root density for both orders (Lima et al. 2010). Root hairs of inoculated and non-inoculated seedlings in the sand system was observed under dissecting microscope and captured by camera (Olympus).

Histochemical analysis of GUS activity

In order to evaluate the change in endogenous auxin by GUS histochemical staining, 15-days-old non-inoculated PNS-1, inoculated transgenic seedlings from MS media and 3-week-old seedlings from sand system were incubated overnight at 37 °C in a GUS reaction buffer (Malamy and Benfey 1997). The stained seedlings were rinsed in 70 % ethanol for 5 min and then observed under the microscope.

In vitro auxin production

PNS-1 was evaluated for auxin production in the presence and absence of supplemented L-Tryptophan (Sigma Chemical Co., St. Louis MO, USA). For this purpose, L-tryptophan was filter sterilized (Millipore filter, 0.45 µm) and was added in 250-ml Erlenmeyer flasks, containing 100 ml autoclaved LB broth separately. All flasks were inoculated by equal inoculum and incubated for 72 h at 37 °C at 120 rev/min. After incubation, bacterial cells were centrifuged at stationary phase for 15 min. One ml supernatant was added to Salkowski's reagent (2 ml 0.5 M $FeCl_3$ and 98 ml 35 % perchloric acid) and incubated in darkness for 45 min for color development. The intensity of color was measured at 535 nm with the spectrophotometer. Standard solution of synthetic auxin (Oxoid) with different concentrations (µg/ml) was prepared and processed in the same way, and curve of standards was drawn for comparison to determine auxin production (µg/ml) in the bacterial culture supernatant (Akhtar and Ali 2011).

ACC deaminase production

Production of ACC deaminase was determined as described by Glick et al. (1998). LB broth was inoculated with

Table 1 ACC and IAA production (with and without L-tryptophan) by PNS-1 strain in in vitro conditions

Bacterial strain	ACC deaminase production (nanomoles of α-ketobutyrate mg protein^{-1} h^{-1})	Auxin production (µg/l)	
		L-Trp^{-1}	L-Trp
Negative Control	–	1.03 ± 0.05	1.4 ± 0.07
PNS-1	137.92 ± 2.16	13.1 ± 0.233	90.3 ± 0.208

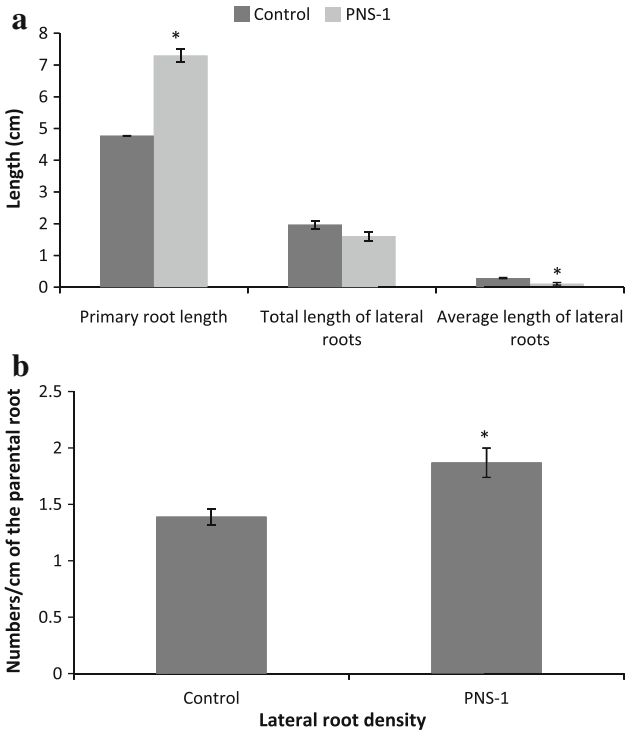

Fig. 2 **a** Effect of PNS-1 inoculation on primary and lateral root length (total and average) of *Arabidopsis thaliana* (Col-0) ($n = 15$) in MS media. Data were expressed as mean values ± standard error of the mean. *Asterisks* designate Student's *t* test ($P < 0.01$). **b** Effect of PNS-1 inoculation on lateral root density of *Arabidopsis thaliana* (Col-0) ($n = 15$) in MS media. Data were expressed as mean values ± standard error of the mean. *Asterisks* designate Student's *t* test ($P < 0.01$)

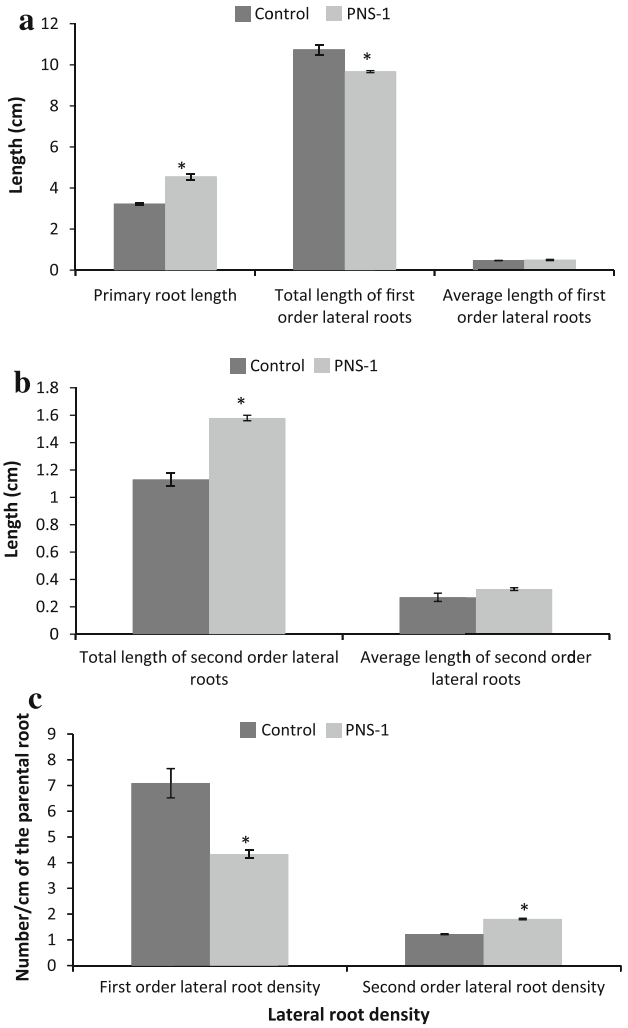

Fig. 3 **a** Effect of PNS-1 inoculation on primary and first-order lateral root length (total and average) of *Arabidopsis thaliana* (Col-0) ($n = 15$) in sand system. Data were expressed as mean values ± standard error of the mean. *Asterisks* designate Student's *t* test ($P < 0.01$). **b** Effect of PNS-1 inoculation on second-order lateral root length (total and average) of *Arabidopsis thaliana* (Col-0) ($n = 15$) in sand system. Data were expressed as mean values ± standard error of the mean. *Asterisks* designate Student's *t* test ($P < 0.01$). **c** Effect of PNS-1 inoculation on first and second-order lateral root density of *Arabidopsis thaliana* (Col-0) ($n = 15$) in sand system. Data were expressed as mean values ± standard error of the mean. *Asterisks* designate *t* test ($P < 0.01$)

bacterial strains at 37 °C for 24 h at 120 rev/min. Bacterial culture was centrifuged at 12,000 g for 10 min at 4 °C. Cells were washed with 5 ml DF salt minimal media (Dworkin and Foster, 1958) and centrifuged at 12,000*g* for 10 min. Then, cultures were incubated in DF salt minimal media containing 3 mM ACC at 28 °C for 24 h at 120 rpm. After incubation, harvested cells were washed again with 5 ml DF salt minimal media and suspended in 7.5 ml DF salt minimal media containing 0.5 M ACC and incubated for 1 h at 28 °C at 120 rev/min shaking condition. After 1 h, media was centrifuged at 12,000*g* for 10 min and liberated ammonia was measured following the method of Nagatsu and Yagi (1966). All the above experiments were repeated three times for the validity of the results.

Statistical analysis

The values were analyzed as the mean of replicates (mean ± SE). The data obtained were statistically evaluated using Student's *t* test.

Results

Isolation and identification

On the basis of 16s rRNA sequence comparison, the isolated PNS-1 strain showed 99 % homology with *Aeromonas punctata*. Sequence was submitted to the GENBANK and was assigned the accession number JF320797.

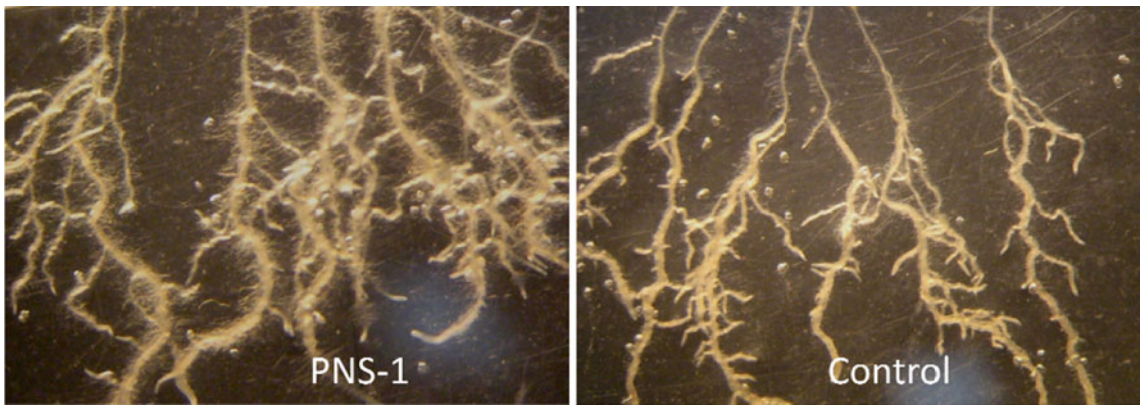

Fig. 4 Root hair proliferation of *Arabidopsis thaliana* (Col-0) in sand system caused by PNS-1 strain inoculation

Effect of PNS-1 inoculation on root morphogenesis in MS media

PNS-1 inoculation changed the root morphogenesis of Arabidopsis plant (Col-0) by increasing the primary root length (Fig. 1), (Table 1). Figure 2a shows that PNS-1 strain exhibited 53 % increase in primary root length as compared to the non-inoculated plant. On the other hand, the total length of lateral roots was decreased non significantly over control. PNS-1 inoculation led significant decrease in average root length rather than increasing it. While lateral root density increased 34.53 % upon inoculation when compared to the non-inoculated plant, confirming that PNS-1 had a positive effect on increasing lateral root density (Fig. 2b).

Effect of bacterial inoculation on root morphogenesis in sand system

The inoculation with PNS-1 led a significant increase ($P < 0.01$) in the primary root length as compared to control whereas decrease in the first-order total root length was observed with PNS-1 inoculation over non-inoculated plant. Any significant change in the first-order average root length of the both inoculated and non-inoculated plants was not observed (Fig. 3a). A clear-cut increase in second-order total lateral root length was observed in PNS-1 inoculated plant over non-inoculated plant while there was no change in the second-order average root length of both PNS-1 inoculated and the control plants (Fig. 3b). PNS-1 inoculation caused the significant decrease in the first-order lateral root density over non-inoculated plant but on the other hand, significant increase (48.36 %) in second-order lateral root density was observed in PNS-1 inoculated plant (Fig. 3c). These results showed that PNS-1 inoculation had the potential to change the root morphogenesis positively by increasing the lateral root density at second-order lateral root. PNS-1 inoculations showed a tremendous proliferation of root hairs in inoculated plant under the microscope (Fig. 4).

Histochemical GUS analysis

Microscopic observations showed that the GUS staining was stronger in the pericycle region of the roots and apices of the lateral roots of PNS-1 inoculated transgenic seedlings as compared to non-inoculated control plant (Fig. 5a, b). PNS-1 inoculated seedlings, grown in sand system, also showed the increased level of GUS staining in primary root apices and vasculatures of the lateral roots as well over non-inoculated seedlings (Fig. 5c, d).

In vitro auxin and ACC deaminase production

The amount of auxin production was 13.1 μg/l, which increased up to 90.3 μg/l by the amendment of the L-tryptophan. Calorimetric determination of ACC deaminase showed the deep blue color for PNS-1 strain and showed 137.92 nmol of α-ketobutyrate mg protein^{-1} h^{-1} ACC deaminase activity.

Discussion

The use of soil in growing plants under laboratory conditions does not depict the true structure of roots due to soil's opacity and problems in extricating the root that damages the root structure (Contesto et al. 2010). Gamalero et al. (2002, 2004) used the system comprising mixture of soil and sand or sand alone for analyzing the root architecture of tomato plant inoculated with fluorescent pseudomonades. Similarly, Chapman et al. (2011) studied the root morphogenesis of *Arabidopsis thaliana* by using the sand system. We also aimed to develop a system which might give the true picture of root modulation without damaging roots and close to natural environment. In this work, we

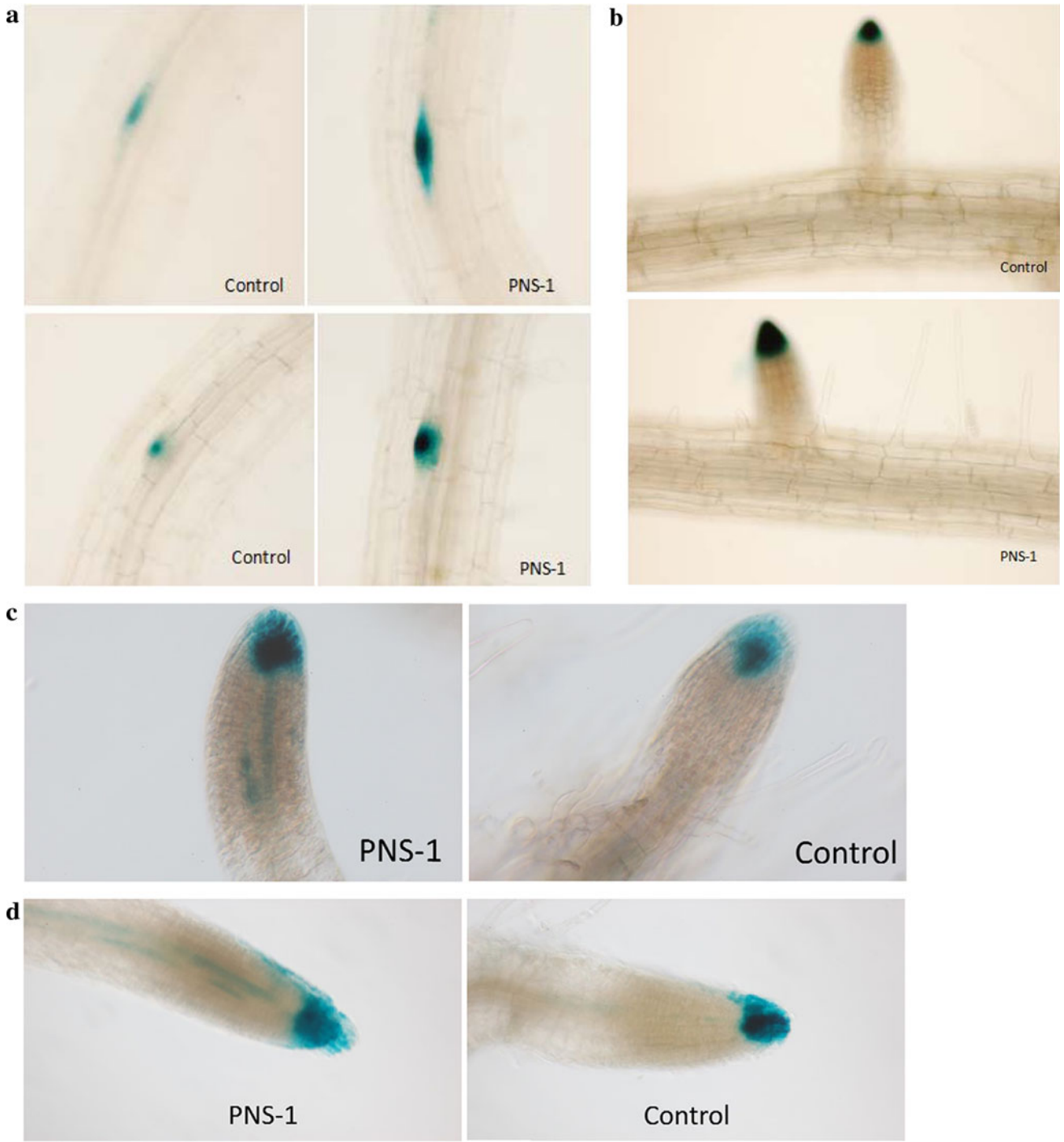

Fig. 5 **a** Effect of PNS-1 inoculation in increasing the GUS staining in the pericycle region of the roots in MS media. **b** Effect of PNS-1 inoculation in increasing the GUS staining on the tips of lateral roots in MS media **c** Expression of DR5:GUS marker in lateral root tip when transgenic seedling *Arabidopsis thaliana* (DR5:GUS) was inoculated with PNS-1 strain in the sand system. **d** Expression of DR5:GUS marker in primary root tip when transgenic seedling *Arabidopsis thaliana* (DR5:GUS) was inoculated with PNS-1 strain in sand system. There is an increase in vascular auxin of Arabidopsis plant

showed how the change in root morphogenesis of *Arabidopsis thaliana* induced by PNS-1 in sand system is different from MS medium.

The amount of auxin produced by PNS-1 strain varied greatly with the addition of L-tryptophan ranging from 13.1 to 90.3 μg/ml. Bacterial IAA production was

comprehensively reviewed recently by Spaepen et al. (2007) with at least five independent identified pathways. Usually, PGPR produce IAA through the indole-3-pyruvate pathway, exploiting the enzyme indole-3-pyruvate decarboxylase encoded by *ipdC*. The *ipdC* gene expression and production of IAA, occurs in stationary phase and induced by amendment of tryptophan (Ryu and Patten 2008).When inoculated with PNS-1, Arabidopsis showed great variation, both in MS and sand system. In MS media, it increased the number of lateral roots and lateral root density whereas Contesto et al. (2010) found the results with *Phyllobacterium brassicacearum* STM 196 inoculations with Arabidopsis plant, showed an increase in lateral root length. *Azospirillum brasilense* showed proliferation in root numbers rather than increasing root length (Kapulnik et al. 1985). These findings indicated that different microbes had different and diverse effects on plant growth. To confirm the involvement of bacterial auxin in changing the endogenous auxin, transgenic line (DR5:GUS) of *Arabidopsis thaliana* was inoculated with PNS-1 strain, which showed the increase in staining, mainly in root aspects of primary and lateral roots. This GUS activity pattern indicated that change in endogenous auxin in the vascular regions of both primary and lateral roots is related to bacterial IAA. The GUS activity pattern, therefore, may reflect that changes in IAA distribution within plant root showed that the root enhancement provoked by PNS-1 is auxin dependent. This increase in endogenous auxin resulted due to the released bacterial auxin in rhizospheric region and finally in plant roots (Contesto et al. 2010). The illustration of change in Arabidopsis root system with bacterial inoculation was very similar to previous results (Lopez-Bucio et al. 2002; Stepanova et al. 2005). Our findings showed significant increase in primary root length both in MS and sand system. This growth promotion triggered by selected strain indicated that PNS-1 has the ability to promote the plant growth by increasing the primary root length and lateral root proliferation. Gamalero et al. (2003) also evaluated that two pseudomonas strains increased the total root length of the cucumber seedlings by producing auxin, although most finding showed that auxin produced by rhizobacteria may inhibit the primary root length depending upon the auxin concentration (Lopez-Bucio et al. 2007). Lot of studies has shown that elongation of primary root length and root hair development was also controlled by ethylene, which is generally considered to be synthesized by auxin dependent mechanism (Stepanova et al. 2005). Inoculation of plants with PNS-1 altered the endogenous level of ethylene by producing ACC deaminase (Glick et al. 1998). Certain rhizobacterial strains produce ACC deaminase that lowers the ethylene level by breaking down the ethylene precursor and in turn elongate the root length (Mayak et al. 2004), which is inhibited by high ethylene level. Glick et al. (1998) claimed that ACC deaminase containing rhizobacteria have the ability to elongate plant root while colonizing the plant roots and hence considered as PGPR. The dual function of ACC deaminase for plant growth, i.e., as plant growth promotion and defense against plant pathogens, puts this enzyme as one of the important traits among various beneficial characters of plant growth-promoting bacteria (Cattelan et al. 1999; Shaharoona et al. 2006). The enhancement in root number and surface caused better improvement in the length of the primary root, fabrication of adventitious roots, and increasing number and length of root hairs. Alternatively, the coordinated existence of several rhizobacterial mechanisms, inducing physiological changes in the plant, make it complicated to recognize the expression of a particular character. In this sense, it is difficult to determine whether the development of roots was a direct consequence of PNS-1 IAA, or by IAA-induced plant ethylene, or both. Different rhizobacterial strains have the combination of variety of modes of action. By utilizing these different mechanisms they promote the plant growth. PNS-1 is one of those bacteria which modulated the Arabidopsis root by utilizing different mechanisms and without using the mutants of PNS-1 that are deficient in IAA and ACC deaminase. We cannot rule out the possibility that other produced metabolites may be responsible for plant root modulation. Thus, PNS-1 has the ability to modulate the root architecture for the sand system by elongation and proliferation of primary and lateral roots, respectively.

Author contribution A. Iqbal performed the experiments, interpreted the data and prepared the first draft. S. Hasnain analyzed the data, revised and corrected the manuscript.

Acknowledgments The Higher Education Commission of Pakistan is acknowledged for providing funds to Atia Iqbal (IRSIP No.1-8/HEC/HRD/2009/514) to visit (Department of Molecular Genetics and Cell Biology, university of Chicago) to carry out this study. We are highly obliged to Jocelyn E. Malamy for her assistance and providing lab facilities.

References

Akhtar S, Ali B (2011) Evaluation of rhizobacteria as non-rhizobial inoculants for mung beans. AJCS 5(13):1723–1729

Ali B, Sabri AN, Ljung K, Hasnain S (2009) Quantification of indole-3-acetic acid from plant associated *Bacillus* spp. and their phytostimulatory effect on *Vigna radiata* (L.). World J Microbiol Biotechnol 25:519–526

Barbieri P, Galli E (1993) Effect on wheat root development of inoculation with an *Azospirillum brasilense* mutant with altered indole-3-acetic-acid production. Res Microbiol 144:69–75

Blaha D, Prigent-Combaret C, Mirza MS, Moenne-Loccoz Y (2006) Phylogeny of the 1-aminocyclopropane-1-carboxylic acid deaminase-encoding gene acdS inphytobeneficial and pathogenic

proteobacteria and relation with strain biogeography. FEMS Microbiol Ecol 56:455–470
Casimiro I, Beeckman T, Graham N, Bhalerao R, Zhang H, Casero P, Sandberg G, Bennett M (2003) Dissecting Arabidopsis lateral root development. Trends Plant Sci 8:165–171
Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680
Chapman N, Whalley WR, Lindsey K, Miller AJ (2011) Water supply and not nitrate concentration determines primary root growth in Arabidopsis. Plant Cell Environ 34:1630–1638
Cohen JD, Slovin JP, Hendrickson AM (2003) Two genetically discrete pathways convert tryptophan to auxin: more redundancy in auxin biosynthesis. Trends Plant Sci 8:197–199
Contesto C, Milesi S, Mantelin S, Zancarini A, Desbrosses G, Varoquaux F, Bellini C, Kowalczyk M, Touraine B (2010) The auxin-signaling pathway is required for the lateral root response of Arabidopsis to the rhizobacterium *Phyllobacterium brassicacearum*. Planta 232:1455–1470
Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Aguirre J, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S, Okon Y (2001) Responses of agronomically important crops to inoculation with Azospirillum. Austr J Plant Physiol 28:871–879
Duan J, Muller KM, Charles TC, Vesely S, Glick BR (2006) 1-Aminocyclopropane-1-carboxylate (ACC)deaminase genes in Rhizobia: Isolation, characterization and regulation. Proceedings of the 7th International PGPRWorkshop, Amsterdam
Dworkin M, Foster J (1958) Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol 75:592–601
Gamalero E, Martinotti MG, Trotta A, Lemanceau P, Berta G (2002) Morphogenetic modifications induced by *Pseudomonas fluorescens* A6RI and *Glomus mosseae* BEG12 in the root system of tomato differ according to plant growth conditions. New Phytol 155:293–300
Gamalero E, Fracchia L, Cavaletto M, Garbaye J, Frey-Klett P, Varese GC, Martinotti MG (2003) Characterization of functional traits of two fluorescent pseudomonads isolated from basidiomes of ectomycorrhizal fungi. Soil Biol Biochem 35:55–65
Gamalero E, Trotta A, Massa N, Copetta A, Martinotti MG, Berta G (2004) Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. Mycorrhiza 14:185–192
Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68
Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, Mcconkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26(5):227–242
Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol Biochem 39:11–17
Jones DL, Dennis PG, Owen AG, Paw VH (2003) Organic acid behavior in soils-misconceptions and knowledge gaps. Plant Soil 248:31–41
Kapulnik Y, Okon Y, Henis Y (1985) Changes in root morphology of wheat caused by *Azospirillum* inoculation. Can J Microbiol 31:881–887
Lambrecht M, Okon Y, Vande Broek A, Vanderleyden J (2000) Indole-3-acetic acid: a reciprocal signaling molecule in bacteria–plant interactions. Trends Microbiol 8:298–300
Larcher M, Muller B, Mantelin S, Rapior S, Cleyet-Marel J-C (2003) Early modifications of *Brassica napus* root system architecture induced by a plant growth-promoting *Phyllobacterium* strain. New Phytol 160:119–125
Laskowski M, Biller S, Stanley K, Kajstura T, Prusty R (2006) Expression profiling of auxin-treated Arabidopsis roots: toward a molecular analysis of lateral root emergence. Plant Cell Physiol 47:788–792
Lima JE, Kojima S, Takahashi H, von-Wiren N (2010) Ammonium triggers lateral root branching in *Arabidopsis* in an Ammonium Transporter1;3-dependent manner. Plant Cell 22:3621–3633
Lopez-Bucio J, Hernandez-Abreu E, Sanchez-Calderon L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. Plant Physiol 129:244–256
Lopez-Bucio J, Campos-Cuevas JC, Hernandez-Calderon E, Velasquez-Becerra C, Farias-Rodriguez R, Macias-Rodriguez LI, Valencia-Cantero E (2007) *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in Arabidopsis thaliana. Mol Plant Microbe Interact 20:207–217
Lucas M, Guedon Y, Jay-Allemand C, Godin C, Laplaze L (2008) An auxin transport-based model of root branching in *Arabidopsis thaliana*. PLoS ONE 3(11):1–13
Malamy JE (2005) Intrinsic and environmental response pathways that regulate root system architecture. Plant Cell Eviron 28:67–77
Malamy J, Benfey P (1997) Down and out in Arabidopsis: the formation of lateral roots. Trends Plant Sci 2:390–396
Mastretta C et al (2006) Endophytic bacteria and their potential application to improve the phytoremediation of contaminated environments. Biotechnol Gen Eng Rev 23:175–207
Mayak S et al (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572
Nagatsu T, Yagi K (1966) A simple assay of monoamine oxidase and D-amino acid oxidase by measuring ammonia. J Biochem 60:219–221
Pare PW, Farag MA, Krishnamachari V, Zhang H, Ryu CM, Kloepper JW (2005) Elicitors and priming agents initiate plant defense responses. Photosynth Res 85:149–159
Patten C, Glick B (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801
Penrose DM, Moffatt BA, Glick BR (2001) Determination of 1-aminocyclopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. Cnd J Microbiol 47:77–80
Persello-Cartieaux F, Nussaume L, Robaglia C (2003) Tales from the underground: molecular plant-rhizobia interactions. Plant, Cell Environ 26:189–199
Ryu RJ, Patten CL (2008) Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by TyrR in *Enterobacter cloacae* UW5. J Bacteriol 190:7200–7208
Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW (2004) Bacterial volatiles induce systemic resistance in Arabidopsis. Plant Physiol 134:1017–1026
Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. LSMR 21:1–30
Shaharoona B, Arshad M, Zahir ZA (2006) Effect of growth-promoting rhizobacteria containing ACCdeaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (Vigna radiata L.). Lett Appl Microbiol 42:155–159
Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448
Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM (2005) A link between ethylene and auxin uncovered by the characterization of

two root-specific ethylene-insensitive mutants in Arabidopsis. Plant Cell 17:2230–2242

Taghavi S et al (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar. Appl Environ Microbiol 75:748–757

Ulmasov T, Murfett J, Hagen G, Guilfoyle T (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9:1963–1971

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Wang C, Ramette A, Punjasamarnwong P, Zala M, Natsch A, Moenne-Loccoz Y, Defago G (2001) Cosmopolitan distribution of phlD-containing dicotyledonous crop-associated pseudomonads of worldwide origin. FEMS Microbiol Ecol 37:105–116