Isolation and identification of Plant Growth Promoting Rhizobacteria (*Pseudomonas* spp.) and their effect on growth promotion of *Lycopersicon esculentum* L.

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Abstract: Several *Pseudomonas* spp. were isolated from rhizosphere of tomato growing in Punjab. On the basis of morphological, biochemical and physiological characters of isolates only five were selected for further studies. According to Bergey's Manual of Determinative Bacteriology all the five isolates were recognized as *Pseudomonas* spp. All the isolates were positive for catalase, urease, amylase and citrate utilization test. After evaluation for their plant growth promoting attributes, LPN4 were found potential strain for all Plant Growth Promoting activities such as production of IAA, HCN, Ammonia and solubilization of phosphate, Further, *in vitro* studies showed that LPN4 inhibited the growth of phytopathogens such as *Fusarium solani* and enhanced seed germination and all the growth parameter such as shoot and root length etc. significantly. Further, Plant growth promoting and antifungal activities of *Pseudomonas* sp. LPN4 suggest that it may be exploited as a potential bioinoculant agent for *Lycopersicon esculentum.*

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1. Introduction

The region of soil surrounding and including the plant root (rhizosphere) is of crucial importance for plant health and nutrition. *Pseudomonas* is an important component of the rhizosphere, and certain strains have been reported to enhanced plant health of different crop including tomato (**Almaghrabi *et al*., 2013; Goswami *et al*., 2013; Aarab *et al*., 2015; Kalita *et al*., 2015**). Globally considerable research efforts are underway to exploit the potential of pseudomonads as crop protectants and for the maintenance of soil health. They represent dominant bacterial group in the rhizospheric region and are metabolically and functionally most versatile (**Lugtenberg and Dekkers, 1995**). Interaction of soil microorganisms and plant in the rhizosphere can be beneficial, neutral, variable or deleterious for plant growth (**Kukreja *et al*., 2010**). The plant rhizosphere is an important soil ecological environment for plant-microbe interactions. It involves colonization by a variety of microorganisms in and around the roots which may result in symbiotic, associative, neutralistic or parasitic relations within the plant depending on the type of microorganisms, soil nutrient status defence system and soil environment (**Verma *et al*., 2010**).

Tomato (*Lycopersicon esculentum*), one of the most important vegetable in many countries has a worldwide economic and nutritive importance (**Khoso, 1994**). It is one of the most popular and important commercial vegetable crops grown throughout the world; ranking second in importance next to potato. It is rich in vitamins A, B and C and has very high potential for developing value added products like soup, puree, juice, ketchup and powder through processing. It is also economically important for its edible fruits which can be consumed either raw or cooked (**Kirankumar, 2007**).

Pseudomonads are the most diverse and ecologically significant group of bacteria on the planet. Certain strains of *Pseudomonas* promote plant growth by secreting auxins, gibberellins cytokinins, solubilizing phosphate, potassium, zinc, producing siderophores, HCN and lytic enzymes and hence they are also called plant growth promoting rhizobacteria. They are non-pathogenic rhizobacteria and several isolates of *P. fluorescens, P. putida, P. aeruginosa and P. aureofaciens* suppressed the soil borne pathogens by producing secondary metabolites such as siderophore, HCN, protease and antibiotics such as phenazine-1-carboxylic acid (PCA), pyocyanin, 2-acetamidophenol, pyrrolnitrin, pyoluteorin, 2, 4-diacetylphloroglucinol (**Karthikeyan *et al*., 2006; Kumar *et al*., 2005; Hassanein *et al*., 2009; Almaghrabi *et al*., 2013; Patel *et al*., 2015**).

Rhizoctonia solani and *Fusarium* spp. are major soilborne fungal pathogens of both greenhouse and field grown tomatoes in the warm vegetable growing areas of the world, which cause serious diseases as root rots and wilt and finally reduced crop yield and quality (**Saad, 2006; Abdel-Monaim, 2010**). Control

of such diseases mainly depends on various chemical fungicides (**El-Mougy *et al*., 2004**). However, intensive application of these chemical causes hazards to environment as well as human health. Therefore, alternative approaches for the control of plant diseases should be emphasized (**Mandal *et al*., 2009**). Plant growth promoting rhizobacteria having biocontrol potential may be one of the good alternatives. Several workers reported the inhibition of pathogens causing disease in tomato by application of Pseudomonads (**Almaghrabi *et al*., 2013; León *et al*., 2015; Patel *et al*., 2015**). The objectives of the present study was to isolate, identify and evaluate the growth promoting and antagonistic effect of different *Pseudomonas* ssp. applied on tomato plant growing in Punjab, India.

2. Materials and Methods

Isolation of *Pseudomonas* spp. from Rhizospheric Soil:

Healthy and young seedlings of tomato were gently uprooted from various fields of Punjab and were carried to lab in sterile polythene bags. Each sample was stored in refrigerator at 4°C till further processing (Table 01). The rhizospheric soil of tomato was removed and air dried. 1 gram of soil was weighed and serial dilutions were carried out and plated on King's B plates and incubated at 28°C for 24-36h. Pure cultures were stored on slants on Nutrient agar medium at 4°C for further use.

Gram Staining

A thin smear of test culture was prepared on the clean slide and heat fixed. Few drops of crystal violet were poured on the smear for about 1 min. Washed the slide with running tap water. Flooded the smear with Gram's iodine and kept for 2 min. Decolorised the stain with ethyl alcohol (95%) dropwise. Poured few drops of safranine for 2-4 min. Washed the slide with tap water and mounted in glycerine or oil emulsion and examined under microscope.

Catalase Test

The test culture was performed on clean slide and a drop of hydrogen peroxide (30%) was added and observed for effervescence of bubbles.

Indole Production Test

Test tubes containing 5ml tryptone broth were inoculated with each bacterial culture separately after autoclaving. One test tube was kept as control without inoculation of bacterial culture. After 48 h incubation, 1 ml of Kovac's reagent was added to each tubes including control. Tubes were soaked gently after interval of 10-15 minutes. Tubes were allowed to stand to permit the reagent to come to the top.

Methyl Red (MR) and Voges-Proskauer (VP) Test

MRVP broth was prepared and 5ml broth was poured in each test tube and sterilized by autoclaving. All the test tubes were inoculated with bacterial culture and two test tubes were kept as control. The test tubes were incubated at 28°C for 48 h. Five drops of MR indicator were added to each test tube including the control and observed the change in colour. Similarly, ten drops of VP-I reagent and 2-3 drops of VP-II reagent were added to other incubated test tubes and control also. Observed the test tubes for colour change and compared with control.

Citrate Utilization Test

Slants of Simmon's citrate agar were prepared and streaked with the bacterial culture. The tubes were incubated at 28°C for 48 h. The change in colour of the slants was observed.

Urease Production Test

The slants of urea agar were inoculated by stabbing into the butt (bottom of the tube) with an inoculating loop and then streaked the slants in wavy pattern. Results were observed after 18-24 h of incubation at 27°C. The tubes were compared with the control.

Starch Hydrolysis Test

The actively grown cultures were inoculated on starch agar plates and incubated at 30°C for 48 h. Plates having proper growth of bacteria were flooded with iodine solution with a dropper for 30 sec. Poured off the excess iodine solution. Results were observed by the appearance of clear zones around the line of growth of each isolate, *i.e.* the change in colour of the medium.

Carbohydrate Fermentation Test

The tubes containing broth amended with four different sugars (0.5% of each, *i.e.;* sucrose, mannitol, dextrose and lactose) were taken. One durham tube was immersed in each tube. The tubes with test culture were incubated at 28°C for 24 h. The tubes were examined for the production of acid or gas as shown by the change in colour or appearance of bubbles.

Indole acetic acid (IAA) Production

IAA production was detected as described by **Gordon and Weber (1951).** Bacterial cultures were inoculated in nutrient broth with L-Tryptophan (0.1 g/l). Exponentially grown cultures were centrifuged at 10000 rpm for 15 min. at 4°C. The supernatant (2 ml) was mixed with two drops of Salkowski reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$). Development of pink colour confirmed the production of IAA.

Phosphate solubilization

Phosphate solubilization ability of isolates was detected by spotting them on Pikovskaya's agar plates. The plates were then incubated at 28±1°C for 3 days and then observed for the appearance of clearing zones around the colonies (due to solubilization of inorganic phosphate by producing organic acid by bacteria).

Zinc Solubilization

The Zinc solubilization by bacteria were performed following the method of **Saravanan *et al.***

(2003). The bacterial isolates were spotted on Tris-minimal medium plates having zinc phosphate along with bromophenol blue as pH indicator. Inoculated plates were incubated at 28°C for about a week and observed the clear zone around the colonies due to the solubilization of inorganic Zinc by bacteria.

HCN Production

HCN production was determined by modified method of **Bakker and Schippers (1987).** Exponentially grown cultures (10^8 cells/ ml) of isolates were streaked on solid agar plates, supplemented with 4.4 g glycine/ l with simultaneous addition of filter paper soaked in 0.5% of picric acid in 1% Na_2CO_3 in the upper lid of plates. The plates were sealed with parafilm. After incubation at 28±1°C for 48-72 h, the development of colour from yellow to light brown, moderate brown or strong brown was examined for putative HCN production.

Ammonia Production

Bacterial isolates were tested for the production of ammonia in peptone water. 48 h old cultures were inoculated in 10 ml of peptone water in each tube and incubated for 72 h at 28°C. 0.5 ml Nessler's reagent was added on culture broth over slide. After few minutes, yellow to brown precipitation appeared that indicated moderate to strong ammonia production (**Cappuccino and Sherman, 1992**)

Antagonistic Activities

Dual culture technique was used to measure the activity of isolated strains against fungal pathogen *Fusarium solani* (MTCC 3871). Agar blocks (5mm in diameter) from margin of 5 days old culture of fungal pathogen was placed in the centre of the assay plate. One loopful (24 h old) culture of isolated strain was spotted 2 cm apart from the pathogen. Plates were incubated at 28±1°C for 3-7 days. The zone of inhibition was recorded by using formula: Inhibition zone (%) = 100×C-T/C, Where; C = Radial growth in control, T = Radial growth in dual culture

Seed Bacterization

Bacterial strains (LPN1-LPN5) were grown separately in nutrient broth for 48 h at 28±1°C in shaker. The cultures were centrifuged at 8000 rpm for 15 min. at 4°C. The culture supernatants were discarded and pellets were washed and resuspended in sterile distilled water to get final population density of 1× 10^8 cells/ml. The cell suspension of bacterial strains was mixed with 1% CMC solutions separately to form slurry and coated on the surface of seeds. Seeds of tomato coated with 1% CMC slurry were served as control.

Seed Germination

Sterilized garden soil was used for pot assay. Soil was grinded into fine particles and then sterilized in oven at 160°C for 2 h. Seeds of tomato were collected from local market of Chandigarh. Healthy seeds of similar shape and size were selected. Bacterized seeds (separate for each strain) were sown in pots in triplicates. Seeds treated with only 1% CMC were treated as control. Pots were watered when required. Treatments were described as follows: T1; Seeds bacterized with *Pseudomonas* spp. LPN1. T2; Seeds bacterized with *Pseudomonas* spp. LPN2. T3; Seeds bacterized with *Pseudomonas* spp. LPN3. T4; Seeds bacterized with *Pseudomonas* spp. LPN4. T5; Seeds bacterized with *Pseudomonas* spp. LPN5. Germination percentage, root length, shoot length, root weight, shoot weight were all recorded upto 21 DAS.

3. Results

Isolation of *Pseudomonas* spp.

The rhizobacteria were isolated from tomato by serial dilution method using King's medium. Out of several isolates, only five (LPN1, LPN2, LPN3, LPN4 and LPN5) were screened on the basis of preliminary investigation.

Morphological Characterization

Colony Morphology and Gram staining

The colony morphology of isolates was observed as round, yellowish green in colour. All the isolates were found Gram negative and rod shaped.

Biochemical Characterization

Biochemically, all the isolates (LPN1, LPN2, LPN3, LPN4 and LPN5) were found positive for catalase, citrate utilization, urease production and starch hydrolysis whereas negative for indole production, MRVP (Table 01). All the isolate were found to ferment dextrose (Table 01).

Characterization for Plant Growth Promoting Attributes

IAA Production

All the five isolates (LPN1, LPN2, LPN3, LPN4 and LPN5) of *Pseudomonas* spp. were found to produce IAA. LPN5 was found to produce the deepest pink colour indicating the maximum IAA production (Table 02).

Phosphate and Zinc Solubilization

All the isolates were able to form clear halos around the spot inoculation on Pikovskaya's agar plate. Such clearing zones around the bacteria showed phosphate solubilization ability (Table 02). None of the isolates were able to solubilize zinc as there were no halo zones around colonies (Table 02).

HCN and Ammonia Production

All the isolates of *Pseudomona*s spp. produced HCN except LPN3 as evidenced by change in colour of filter paper. LPN4 produced the maximum HCN as indicated by strong brown colour of filter paper (Table 02). All the isolates also produced ammonia by forming yellowish brown precipitates in peptone broth (Table 02 and Fig 01).

Table 01: Morphological and Biochemical Characterization of *Pseudomonas* spp. isolated from Tomato rhizosphere:

Parameters	Isolates				
	LPN1	**LPN2**	**LPN3**	**LPN4**	**LPN5**
Cell shape	Rod	Rod	Rod	Rod	Rod
Colony type	Round	Round	Round	Round	Round
Growth of colony	Slow growing	Fast growing	Slow growing	Fast growing	Fast growing
Colour of the colony	Yellowish green	Light green	Yellowish green	Yellowish green	Bright green
Gram staining	-	-	-	-	-
Catalase test	+	+	+	+	+
Indole test	-	-	-	-	-
Methyl Red test	-	-	-	-	-
Voges-Proskauer test	-	-	-	-	-
Citrate utilization test	+	+	+	+	+
Urease test	+	+	+	+	+
Starch hydrolysis test	+	+	+	+	+
Carbohydrate Fermentation					
Sucrose	+	+	+	-	+
Mannitol	+	+	-	-	+
Dextrose	+	+	+	+	+
Lactose	-	-	-	-	+

Abbreviations: (+) positive and (-) negative.

Table 02: Plant growth promoting attributes and Antagonistic activities of *Pseudomonas* spp. isolated from *Lycopersicon esculentum* L.

Isolates	IAA production[A]	Phosphate solubilization[B]	Zinc Solubilization[c]	HCN production[D]	Ammonia Production[E]	Antagonistc against *F. solani*[F]
LPN1	+	+	-	+	++	-
LPN2	+	+	-	+	++	-
LPN3	+	++	-	-	+++	-
LPN4	++	+	-	+++	+++	+
LPN5	+++	+	-	+	+	-

Abbreviations: A -, IAA negative, +, IAA positive; B -, Phosphate solubilization negative; +, phosphate solubilization positive, -, Absence of halo formation; +, small halos <0.5 cm wide surrounding colonies; ++, medium halos > 0.5 cm wide surrounding colonies; +++, large halos >1.0cm wide surrounding colonies; C -, zinc solubilization negative; D -, HCN negative, +, HCN positive; E, +, ++, +++ (Ammonia production in increasing order); F-, do not inbibit pathogens, +, inhibit the growth of pathogens. *Pseudomonas* sp. MTCC-129; All experiments were done in triplicate with three independent trials.

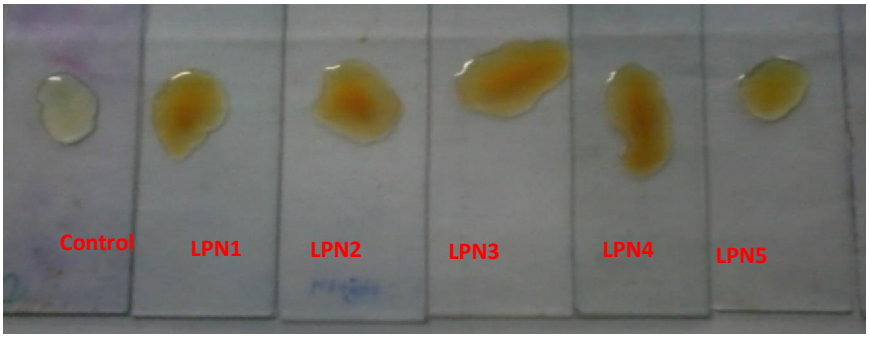

Fig 01. Plant Growth Promoting Attributes of *Pseudomonas* spp.: Ammonia production

Antagonistic Activities

All the *Pseudomonas* isolates were screened for antagonistic activity against *Fusarium solani*. *Pseudomonas* spp. LPN4 inhibited the growth of test pathogens on PDA plate at 28°C. Increase in fungal inhibition corresponded to incubation period. The average radial growth inhibition percentage recorded and found that *Pseudomonas* spp. LPN4 were able to inhibit 41% of *F. solani* after 7 days of incubation (Table 02).

Pot Trial Studies

Tomato seeds of uniform shape and size were bacterized with the isolates of *Pseudomonas* spp. (LPN1 to LPN5). Seeds bacterized with the above microbial inoculants showed induced vegetative parameters after 21 days of sowing in pots. Maximum seed germination, shoot and root length was observed with *Pseudomonas* sp. LPN4. Similar trends of enhancement were obtained with shoot fresh and dry weight and root fresh and dry weight. The maximum number of plants was noticed in the treatments with *Pseudomonas* spp. LPN1, LPN4 and LPN5. Tomato seeds bacterized with *Pseudomonas* sp. LPN4 showed a significant increase in seed germination percentage (83.3%) followed by LPN5 (76.7%). In the control treatment, seed germination was 40% (Table 03). All the parameters were increased and were significant at 1% and/or 5% as compare to control.

Table 03: Effect of *Pseudomonas* spp. (LPN1 to LPN5) on Seed Germination and Vegetative Growth of *Lycopersicon esculentum* under Pot Assay (21 DAS).

Isolates	Seed Germination (%)	Root length (cm)	Shoot length (cm)	Root weight (g)		Shoot weight (g)	
				Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
LPN1	66.7	4.567*	4.600**	0.020*	0.010**	0.323^{ns}	0.110*
LPN2	63.4	4.233^{ns}	4.467**	0.017^{ns}	0.010**	0.205^{ns}	0.089^{ns}
LPN3	60.0	3.867^{ns}	4.333**	0.013^{ns}	0.007^{ns}	0.187^{ns}	0.104*
LPN4	83.3	5.467**	5.233**	0.023*	0.011**	0.438*	0.118**
LPN5	76.7	4.200^{ns}	4.300 **	0.018^{ns}	0.009*	0.210^{ns}	0.102*
Control	40.0	2.700	2.500	0.009	0.003	0.142	0.075
SEM		0.549	0.231	0.003	0.001	0.074	0.008
CD at 1%		2.461	1.035	0.016	0.007	0.332	0.036
CD at 5%		1.731	0.728	0.011	0.005	0.233	0.025

Abbreviations: SEM = standard error mean; CD = Critical Difference, Values are mean of 3 randomly selected plants from each set, ** significant at 1%, * significant at 5% as compared to control, ns = non-significant as compared to control, Control (Non-bacterized seeds).

4. Discussion:

The present study reveals the preliminary work done to isolate PGPR from rhizospheric soil of tomato growing in Punjab. Rhizosphere is suitable niche for soil microorganisms due to high nutrient availability as root exudates. Community of organisms residing in a particular environment is specific and dependent on the physical and ecological factors of that environment. Similarly, **Suresh *et al.* (2010)** isolated 10 strains of fluorescent pseudomonads from different rhizospheric soil of crop plants *viz.* maize, rice and bajra, using King's medium. **Wahyudi *et al.* (2011)** also isolated a total of 115 isolates of *Pseudomonas* spp. from the rhizosphere of soybean in Plumbon, Cirebon, Indonesia. All the isolates are Gram negative, rod shaped and were found positive for catalase, citrate utilization, urease production and starch hydrolysis whereas negative for indole production, MRVP.

All the isolates were found to produce IAA. **Kamble and Galerao (2015)** reported the production of IAA from *Pseudomonas* species isolated from rhizosphere of garden plants in Amravati (Maharashtra). IAA producing bacteria are known to promote root elongation and plant growth (**Patten and Glick, 2002**). Phosphate solubilization by bacterial isolates is related to the production of organic acids as gluconic, acetic, lactic, fumaric and succinic acids. The production of organic acids result in decrease in soil pH, producing H^+ which replace the Ca^{2+} and release HPO_4^{2-} to the soil solution. **Tilak *et al.* (2005)** reported *Pseudomonas* and *Bacillus* to be the main phosphate solubilizers. **Yazdani *et al*. (2009)** reported that inoculation with phosphate solubilizing bacteria improve growth and grain yield of corn, reduced fertilizer costs and emission of the greenhouse gas. **Kaur and Sharma (2013)** reported that 70 % of their isolates have

ability to solubilize phosphate in the range of 5.08 to 13.45 mg/100 ml and enhance the growth of chickpea. All these findings support our result.

Except LPN3 all the isolate changed the colour of filter paper from yellow to orange-brown was considered to be the HCN producer (**Chen *et al*., 2015**). **Blumer and Hass (2000)** reported the HCN production by *Pseudomonas* spp. which can inhibit the growth of phytopathogens. **Ramette *et al.* (2003)** reported that HCN is a broad spectrum antimicrobial compound involved in biological control of root diseases by many plant associated fluorescent pseudomonads. In this study all the isolated were found good producer of Ammonia. Similarly, **Joseph *et al*. (2007)** reported ammonia production in 95% of isolates of *Bacillus* followed by *Pseudomonas* (94.2%) which support our finding. Development of brown to yellow colour was a positive test for ammonia production (**Cappuccino and Sherman, 1992**).

In our study only LPN4 were found to inhibit (*in vitro*) the growth of *Fusarum solani.* Similarly, **Wahyudi *et al.* (2011)** reported the antifungal activity of *Pseudomonas* spp. against *Fusarium solani*. **Mansoor *et al.* (2007)** found significant control of *F. solani* by the application of *P. aeruginosa* and *P. lilacinus* alone and by the combined use of them. Tomato seeds bacterized with *Pseudomonas* sp. LPN5 showed a significant increase in root length, shoot length, fresh and dry weight of root and shoot in pot trial study. Seed germination percentage 83.3% and 76.7% were recorded in LPN4 and LPN5 treated seeds respectively. Where as only 40% seeds germinated in control (uninoculated seeds). These results are in close conformation to those of **Sayyed *et al.* (2005)** who reported 10% increase in the rate of germination of wheat seed when inoculated with *P. fluorescens* NCIM 5096. Similar finding was also recorded by **Ashrafuzzaman *et al.* (2009)** who reported the increase in seed germination when seeds were pretreated with PGPR isolates in rice. **Dey *et al.* (2004)** also suggested that PGPR enhanced growth and seed emergence in peanut. Thus it might be concluded that the bacterial strains of *Pseudomonas* spp. with their multifunctional properties will attract more attention in the field of biofertilization and biological control. Present investigation revealed the ability of *Pseudomonas* spp. (LPN4), having good plant growth promoting attributes such as IAA production, phosphate solubilization, HCN production, ammonia production and biocontrol, could be used as bioinoculants for tomato and other crops also.

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References:

1. Aarab, S., Ollero, F.J., Megías, M., Laglaoui, A., Bakkali, M., and Arakrak, A. (2015) Isolation and screening of bacteria from rhizospheric soils of rice fields in Northwestern Morocco for different plant growth promotion (PGP) activities: An *in vitro* study Int. J. Curr. Microbiol. App.Sci. 4(1), 260-269.
2. Abdel-Monaim, M.F. (2010) Induced systemic resistance in tomato plants against Fusarium wilt disease. Pages 253-263. In Proceedings of the 2nd Minia Conference for Agriculture and Environmental Science, 22-25 March, 2010, Minia, Egypt.
3. Almaghrabi, O.A., Massoud, S.I., and Abdelmoneim, T.S. (2013) Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. Saudi J. Biol. Sci. 20(1), 57–61.
4. Ashrafuzzaman, M., Hossen, F.A., Ismail, M.R., Hoque Md, A., Islam, M. Z., Shahidullah, S. M. and Mcon, S. (2009) Efficiency of plant growth promoting rhizobacteria (PGPR) for the enhancement of rice growth. Afr. J. Biotechnol. 8, 1247-1252.
5. Bakker, A.W. and Schippers, B. (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annu. Rev. Phytopathol. 25, 339–358.
6. Blumer, C. and Hass, D. (2000) Mechanism, regulation and ecological role of bacterial cyanide biosynthesis. Arch. Microbiol. 173(3), 170-177.
7. Cappuccino, J.C. and Sherman, N. (1992) Microbiology: A Laboratory Manual, third ed. Benjamin/ cummings Pub. Co. New York, pp 125-179.
8. Chen, W., Shen, X., Peng, H., Hu, H, Wang, W. and Zhang, X. (2015) Comparative genomic analysis and phenazine production of *Pseudomonas chlororaphis*, a plant growth-promoting rhizobacterium, Genomics Data 4, 33–42.
9. Dey, R., Pal, K.K., Bhatt, D. M. and Chauhan, S.M. (2004) Growth promotion and yield enhancement of peanut by application of plant growth promoting rhizobacteria. Microbiol. Res. 159, 371-394.

10. El-Mougy, N.S., Abd-El-Karem, F., El-Gamal, N.G., and Fotouh Y.O. (2004) Application of fungicides alternatives for controlling cowpea root rot diseases under greenhouse and field conditions. Egypt. J. Phytopathol. 32, 23-35.
11. Gordon, A.S. and R.P. Weber (1951) Colorimetric estimation of indole acetic acid. Plant Physiol. 26, 192.195.
12. Goswami, D., Vaghela, H., Parmar, S., Dhandhukia P., and Thakker, J.N. (2013) Plant growth promoting potentials of *Pseudomonas* spp. strain OG isolated from marine water. J. Plant Interac. 8(4), 281-290.
13. Hassanein, W. A., Awny, N. M., E1-Mougith, A. A. and Salah E1-Dien, S. H. (2009) The antagonistic activities of some metabolites produced by *P. aeruginosa*. J. Appl. Sc. Res. INSInet Publication.
14. Joseph, B., Patra, R. and Lawrence, R. (2007) Characterization of plant growth promoting Rhizobacteria associated with chickpea (*Cicer arietinum L.).* Int. J. Plant Prod., 1(2), 141-152.
15. Kalita, M., Bharadwaz, M., Dey, T., Gogoi, D.P., Unni, B.G., Ozah, D. and Saikia, I. (2015) Developing novel bacterial based bioformulation having PGPR properties for enhanced production of agricultural crops. Ind. J. Exp. Biol. 53, 56-60.
16. Kamble, K.D. and Galerao D.K. (2015) Indole acetic acid production from *Pseudomonas* species isolated from rhizosphere of garden plants in Amravati. Int. J. Adv. Pharmacy. Biol. Chem. 4(1),23-31.
17. Karthikeyan, M., Radhika, K., Mathiyazhagan, S., Bhaskaran, R., Samiyappan, R. and Velazharan, R. (2006) Induction of phenolics and defense-related enzymes in coconut (*Cocos nucifera L.)* roots treated with biological agents. Br. J. Plant Physiol. 18(3), 367-377.
18. Kaur, N. and Sharma, P. (2013) Screening and characterization of native *Pseudomonas* sp. as plant growth promoting rhizobacteria in chickpea (*Cicer arietinum* L.) rhizosphere. Afr. J. Microbiol. Res. 7(16), 1465-1474.
19. Khoso, A. W. (1994) Growing vegetable in Sindh. Allied Printing Corporation, Hyderabad, pp 136.
20. Kirankumar, R. (2007) Evaluation of Plant Growth Promoting rhizobacterial strains against TMV on tomato. M.Sc. dissertation submitted to Department of Agricultural Microbiology, University of Agri. Sci., dharwad. pp 26-98.
21. Kukreja, G. P., Bhute, S. S., Mangate, S. A. and Dhawale, M. N. (2010) Exploring the potential of *Pseudomonas* spp. as phosphate solubilizer, plant growth promoter, bio-control agent and pesticide degrader. Asian J. Expt. Biological Sci. Special, 40-44.
22. Kumar, R. S., Ayyadurai, N., Pandiaraja, P., Reddy, A. V., Venkatesvarlu, Y., Prakash, O. and Sakthivel, N. (2005) Multifarious antagonistic potentials of rhizosphere associated bacterial isolates against soil-borne diseases of Tomato. J. Appl. Microbiol. 98, 145-154.
23. León, RH, Solís,DR, Pérez, MC., Mosqueda, MCO., Rodríguez, LIM., Cruz, HR., Cantero, EV., Santoyo, G. (2015) Characterization of the antifungal and plant growth-promoting effects of diffusible and volatile organic compounds produced by *Pseudomonas fluorescens* strains Biological Control 81, 83-92.
24. Lugtenberg, B.J.J. and Dekkers, L.C. (1995) What makes *Pseudomonas* bacteria rhizosphere competent? Environ. Microbiol. 1, 9-13.
25. Mandal, S., Mallick, N., and Mitra, A. (2009) Salicylic acid- induced resistance to *Fusarium oxysporum* f. sp. lycopersici in tomato. Plant Physiol Biochem. 47, 642-649.
26. Mansoor, F., Sultana, V. and Ehteshamul-Haque, S. (2007) Enhancement of biocontrol potential of *Pseudomonas aeroginosa* and *Paecilomyces lilacinus* against root rot of mungbean by a medicinal plant *Launaea nudicaulis* L. Pak. J. Bot. 39, 2113-2119.
27. Patel, R.R., Thakkar, V.R., Subramanian, B.R. (2015) A *Pseudomonas guariconensis* strain capable of promoting growth and controlling collar rot disease in *Arachis hypogaea* L. DOI 10.1007/s11104-015-2436-2.
28. Patten, C.L. and Glick, B.R. (2002) Role of *Pseudomonas putida* indole acetic acid in the development of host plant root system. Can. J. Microbiol. 59, 220-224.
29. Ramette, A., Frapolli, M., Defago, G., Monenne, Y. (2003) Phylogeny of HCN synthase-encoding *hcnBC* genes in biocontrol fluorescent pseudomonas and its relationship with host plant species and HCN synthesis ability. Mol. Plant Microbe Interact.16, 525–535.
30. Saad, M.M. (2006) Destruction of *Rhizoctonia solani* and *Phytophthora capsici* causing tomato root-rot by *Pseudomonas fluorescens* lytic enzymes. Res. J. Agric. Biol. Sci. 2, 274-281.
31. Saravanan, V.S., Subramoniam, S.R., Raj, S.A. (2003) Assessing in vitro solubilization of different zinc solubilizing bacterial (ZBS) isolates. Brazil J. Microbiol. 34, 121-125.
32. Sayyed, R., Badgujar, M. D., Sonawane, H. M., Mhaske, M. M. and Chincholkar, S. B. (2005) Production of microbial iron chelators

(siderophores) by fluorescent pseudomonads. Ind. J. Biotechnol. 4, 484-490.
33. Suresh, A., Pallavi, P., Srinivas, P., Kumar, P. V., Chandran, J.S. and Reddy, R. S. (2010) Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants. Afr. J. Microbiol. Res. 4(14), 1491-1494.
34. Tilak, K.V.B.R., Ranganayaki, N., Pal, K.K., De, R., Saxena, A.K., Nautiyal, C.S., Mittal, S., Tripathi, A. K. and Johri, B. N. (2005) Diversity of plant growth and soil health supporting bacteria. Curr. Sci. 89, 136-150.
35. Verma, J. P., Yadav, J. and Tiwari, K.N. (2010) Application of *Rhizobium* spp. BHURCO1 and plant growth promoting rhizobacteria on nodulation, plant biomass and yields of Chickpea (*Cicer arietinum L.).* Int. J. Agric. Res., 5, 148-156.
36. Wahyudi, A. T., Astuti, R. I. and Giyanto, N. (2011) Screening of Pseudomonas spp. isolated from rhizosphere of soybean plant as plant growth promoter and biocontrol agent. Amer. J. Agri. Biological Sci. 6 (1), 134-141.
37. Yazdani, M., Bahmanyar, M. A., Pirdashti, H. and Esmaili, M. A. (2009) Effect of Phosphate Solubilization Microorganisms (PSM) and Plant Growth Promoting Rhizobacteria (PGPR) on Yield and Yield Components of Corn (*Zea mays L.).* World Acad. Sc., Engi. Technol. 49, 90-92.

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