International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; SP4: 05-10

Sangita Sahni
Tirhut College of Agriculture, Dholi, Muzaffarpur, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Bishun Deo Prasad
Bihar Agriculture College, Sabour, Bihar Agriculture University, Sabour, Bhagalpur, Bihar, India

Correspondence
Sangita Sahni
Tirhut College of Agriculture, Dholi, Muzaffarpur, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

(Special Issue -4)
International Conference on Food Security and Sustainable Agriculture
(Thailand on 21-24 December, 2018)

Exploitation of pseudomonads for their plant growth-promoting traits

Sangita Sahni and Bishun Deo Prasad

Abstract
The present study was conducted to characterize the 30 native plant growth promoting (PGP) bacteria from different pulse crops rhizosphere grown in BHU, Varanasi, India. All the 30 isolates were screened *in vitro* for PGP characteristics. Among them, 12 were produced fluorescent pigments on Kings'B medium, P1 and C4 were best in producing IAA (11.37 and 5.75 mg/l, respectively), whereas PSB1 and PSB2 solubilized highest amount of tri-calcium-phosphate (502.39 and 395.45 mg/l, respectively), while C4 and CRM3 were strongly cyanogenic. In addition to it, PUR46 was also found to be positive in other growth promoting traits like phosphorus solubilization and ammonification. The results suggested that solubilization of tri-calcium phosphate might be helpful in enhancement of plant growth.

Keywords: Pseudomonad, Plant growth-promoting traits, Antagonistic activities

Introduction
Over the past few decades, the role of Plant Growth-Promoting Rhizobacteria (PGPR) in plant growth and developmental processes has become firmly entrenched in literature (Deepa *et al*., 2010; Vacheron *et al*., 2013; Majeed *et al*., 2015; Shakeela *et al*., 2017) [10, 45, 29, 41]. PGPR has been in limelight among agriculturists for their benefits on crop. One of important features of PGPR is phosphate solubilizing power. Phosphorus is one of the major plant nutrient limiting plant growth. It plays a key role in nutrition of plants as it promotes development of deeper roots. The average soil is rich in phosphorus as it contains about 0.05% (w/w) phosphorus (Barber, 1984) [7]. But, only one tenth of phosphorus is available to plants and approximately 95–99% is present in the form of insoluble phosphates. Hence insoluble phosphate cannot be utilized by the plants due to its poor solubility and chemical fixation in the soil causing a low efficiency of soluble P fertilizers. Chemical phosphatic fertilizer is widely used to supply phosphorus to the soil–plant system throughout the world. However, the prices, availability, and the environmental concerns of chemical fertilizers especially the P fertilizers are real issues of today's agriculture. Soils microorganisms are involved in a range of processes that affect Phosphate transformation and thus influence the subsequent availability of phosphate to plant roots (Richardson, 2001; Majeed *et al*., 2015) [34, 29].
In recent years application plant growth-promoting rhizobateria (PGPR) for the enhancement of sustainable agricultural production is becoming a more widely accepted practice in intensive agriculture. Plant growth-promoting rhizobacteria are free-living soil bacteria that aggressively colonize the rhizosphere/plant roots, and enhance the growth, and yield of plants when applied to seed or crops (Kumar *et al*., 2014) [26]. The plant growth promoting (PGP) effect of the PGPR is mostly explained by the release of metabolites directly stimulating growth. There are several rhizobacteria are known for PGPR properties. Among the rhizobacteria, *Pseudomonas* spp. are emerged as the largest and most promising group, owing to their potential of rapid and aggressive colonization, rhizosphere abundance, catabolic versatility, and their capacity to produce a diverse array of antifungal compounds (Anuratha and Gnanamanickam 1990; Yeole

and Dube 2000; Sivaprasad 2002; Saharan *et al*. 2011) [1, 50, 43, 36]. Other important mechanisms include production of lytic enzymes such as chitinases and β-1, 3 glucanases which degrade chitin and glucan present in the cell wall of fungi (Frindlender *et al*. 1993; Lim *et al*. 1991; Potgieter and Alexander 1996; Velazhahan *et al*. 1999) [19, 28, 32, 47], HCN production (Defago *et al*. 1990) [14] and degradation of toxin produced by pathogen (Borowitz *et al*. 1992; Duffy and Defago 1997) [9, 14].

Knowledge of the native bacterial population and their characterization is required for understanding the distribution and diversity of indigenous bacteria in the rhizosphere of specific crops (Keating *et al*., 1995; Chahboune *et al*., 2011; Majeed *et al*., 2015) [23, 10, 29]. With increasing awareness about the-chemical-fertilizers-based agricultural practices, it is important to search for region-specific microbial strains which can be used as a growth promoting/enhancing inoculum to achieve desired crop production (Deepa *et al*., 2010; Majeed *et al*., 2015) [10, 29].

Materials and methods

Rhizobacteria

Soil isolates of *Pseudomonas* spp. were obtained from the Department of Botany; B. H. U., Varanasi, where as rhizosphere isolates of pseudomonads were isolated from soil around the roots of different pulse crops grown in the campus of Banaras Hindu University. For isolation of fluorescent Pseudomonads, the roots with the tightly adhering soil particles were cut into small pieces and one gram of these root pieces were vigorously shaken in 100ml of sterilized distilled water for 10-20 minutes to get the bacterial suspension. The bacterial suspensions thus obtained were used for the isolation of *Pseudomonas* rhizobacteria by dilution plate technique (Johnson and Curl 1972) in king's B medium (KMB) (King *et al*. 1954); supplemented with benomyl, 20 mg/ml to avoid the growth of fungi. The organisms were allowed to grow at 28 ± 2 ºC. Dilutions of 10^{-4} and 10^{-6} were used and the typical different colonies of *Pseudomonas* spp. were further purified and maintained on KMB slants at 4 ºC in a refrigerator.

Plant growth-promoting attributes

Estimation of Indole Acetic Acid (IAA)

IAA produced by bacteria was assayed colorimetrically using ferric chloride-perchloric acid reagent ($FeCl_3$-$HClO_4$) (Saxena *et al*. 2003) [37]. This method estimated the quantities of indole compounds produced by bacteria in LB (Luria Bertani medium) amended with 50 µg/ml tryptophan. The LB medium contained (in 1000 ml distilled water), 5.0 g Tryptone, 3.0 g Yeast extract and, 5.0 g NaCl. The pH of LB medium was adjusted to 7.0 before autoclaving. All the isolates were grown in LB broth for 24 hours on rotary shaker (240 rpm) at 28 ± 2 ºC. After overnight incubation, each bacterial broth culture was centrifuged at 10,000 rpm for 15 minutes. Two milliliter of the supernatant was added to 4 ml $FeCl_3$-$HClO_4$ reagent. After 25 minutes (after color density reaches its maximum), the mixture was read in a spectrophotometer at 530 nm absorbance. The amount of IAA produced per milliliter culture was estimated using a standard curve. The experiment was conducted with three replications for each isolate.

Phosphorus-solubilization

Solubilization of tri-calcium phosphate was quantified in Pikovskaya's broth (Pikovskaya 1948). The medium consisted of 10.0 g glucose, 5.0 g tri-calcium phosphate, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7\ H_2O$, trace of $MnSO_4$ and $FeSO_4$ and 0.5 g Yeast extract, in 1,000 ml distilled water. Each flask containing 100 ml of Pikovskaya broth having 500 mg of tri-calcium phosphate was inoculated with 0.5 ml of 24 h broth culture of each isolate in triplicate and incubated in a rotary shaker (240 rpm) at 28 ± 2 ºC for 4 day. The culture was centrifuged at 15,000 rpm for 10 min. One milliliter of supernatant was added to 10 ml of ammonium molybdate and shaken well then after diluted the contents of the flask to 45 ml followed by addition of 0.25 ml of chlorostannous acid and immediately the volume was made to 50 ml with distilled water. Spectrophotometeric measurement of blue colored intensity was taken at 600 nm absorbance. The amount of soluble phosphorus was estimated using a standard curve. The experiment was conducted with three replications for each isolate.

Ammonia production

For the detection of ammonia production, all the isolates were grown in test tube containing peptone water: 10.0 g peptone, 5.0 g NaCl, 1,000 ml distilled water, 7.0 pH (Dye 1962). The tubes were inoculated with 100 µl of 24 h grown cultures in broth and incubated at 28 ± 2 ºC for 4 days. The accumulation of ammonia was detected by adding Nessler's reagent (0.5 ml $tube^{-1}$). An uninoculated control was kept for comparison of results. The experiment was conducted with three replications for each isolate. A faint yellow color indicated a small amount of ammonia, and deep yellow to brownish color indicated maximum production of ammonia.

HCN production

HCN production by isolates was detected qualitatively by the method of Bakker and Schipper (1987) [3]. King's B medium was prepared and amended with 4.4 g l^{-1} glycine and sterilized. 25 ml of this medium was poured into each plate. After solidification, the isolates were streaked separately in plates. Whatman no. 1 filter paper disc (9 cm in dia.) was soaked in 0.5 % picric acid in 2 % sodium carbonate. A soaked disc was placed in the lid of each Petri plate. The Petri plate was sealed with parafilm and incubated at 28 ± 2 ºC for 4 days. An uninoculated control was kept for comparison of results. The experiment was conducted with three replications for each isolate.

Results

Growth-promoting traits of pseudomonad isolates

Pseudomonads were isolated from rhizosphere of different pulse crops and soil, were tested for a wide array of traits associated with biocontrol as well as growth promoting attributes, like HCN production, fluorescence pigmentation, ammonification, IAA production and solubilization of tri-calcium phosphate *in vitro*. Results indicated that all isolates except L3 were exhibited the ammonification property, twelve isolates produced fluorescence pigment, eight isolates produced HCN (Cyanide), eleven isolates produced IAA and twenty six isolates positive for phosphorus solubilization out of thirty isolates (Table 1). After 24 h of inoculation of pseudomonad isolates in KBM, the center of the some isolates showed reddish tinge with slight green colouration in the medium. Later on the intensity of green colour intensified. The culture having fluorescence under UV light of wavelength 260 nm with excretion of diffusible yellow-green pigment was identified as *Pseudomonas fluorescence*. Among 30 pseudomonad isolates, 12 isolates viz., R1, L2, L3, C1, C3, C5, C6, CRM2, CRM3, PUR46, PSB1 and PSB2 were found

to be positive for fluorescence pigment. Out of twelve, six isolates (R1, C1, C3, C5, C6, CRM3, and PSB1) were strongly fluorescent pigment producers (Table 1, Figure 1A).

Out of 30 isolates, eight isolates viz., C4, CRM3, C6, C3, A1, A2, C5 and P4 were found to produce volatile cyanide, among this C4 and CRM3 were strongly cyanogenic, A1, C3 and C6 were moderately cyanogenic whereas A2, P4 and C5 were weakly cyanogenic (Table1, Figure 1C).

Our experimental result revealed that all pseudomonad isolates except L3 were exhibited the ammonification property. P1 and PUR46 were found to produce maximum amount of ammonia, 14 isolates produced medium amount of ammonia whereas L3 showed negative response for ammonification activity (Table 1, Figure 1B).

Among the eleven isolates for IAA producers, P1 and C4 were the best in producing IAA (11.37 and 5.75 mg/l, respectively). However, among the twenty six isolates positive for phosphorus solubilization, five of the isolates viz., PSB1, PSB2, R3, A2 and R1 solubilized highest amount of tri-calcium phosphate (502.39, 395.45, 385.55, 346.34 and 312.23 mg/l, respectively) (Table1, Figure 1D).

Ten of the 30 pseudomonad isolates were positive for two traits, while C3, C5 and CRM3 were best in exhibiting multiple PGPR traits like ammonification, IAA production (0.25, 0.63 and 0.13 mg/l, respectively), and phosphate solubilization (11.42, 66.98 and 217.32 mg/l, respectively). The data clearly suggested the wide spectrum of biological activities performed by these strains under *in vitro* conditions.

Table 1: Growth-promoting traits of pseudomonad isolates

Pseudomo-nad isolates	Habitat (Host rhizosphere)	(Fluoresc-ence)a	(NH_4 producti-on)b	(HCN producti-on)c	IAA production (mgl^{-1}) ‡	P solubilization (mgl^{-1}) $
A1	Arhar	-	+	++	-	82.59 ± 1.21
A2	Arhar	-	+	+	-	346.34 ± 2.71
A3	Arhar	-	++	-	-	-
R1	Rajma	++	++	-	1.00 ± 0.08	228.36 ± 1.82
R2	Rajma	-	+	-	-	312.23 ± 2.38
R3	Rajma	-	+	-	-	385.55 ± 2.79
P1	Pea	-	+++	-	11.37 ± 0.38	271.37 ± 2.26
P2	Pea	-	++	-	1.75 ± 0.22	-
P3	Pea	-	++	-	2.12 ± 0.15	23.98 ± 1.53
P4	Pea	-	+	+	0.88 ± 0.06	109.23 ± 1.98
M1	Mungbean	-	++	-	-	-
L1	Lentil	-	++	-	-	216.18 ± 2.54
L2	Lentil	+	+	-	-	88.30 ± 1.68
L3	Lentil	+	-	-	1.75 ± 0.09	71.93 ± 1.61
L4	Lentil	-	++	-	-	133.57 ± 2.42
C1	Chickpea	++	++	-	-	145.40 ± 2.10
C2	Chickpea	-	+	-	-	-
C3	Chickpea	++	+	++	0.25 ± 0.02	11.42 ± 0.42
C4	Chickpea	-	++	+++	5.75 ± 0.45	191.82 ± 2.10
C5	Chickpea	++	+	+	0.63 ± 0.03	66.98 ± 1.87
C6	Chickpea	+	++	++	-	131.31 ± 2.07
C7	Chickpea	-	+	-	0.75 ± 0.07	161.37 ± 1.81
CRM1	Soil	-	++	-	-	274.03 ± 2.48
CRM2	Soil	+	++	-	-	194.87 ± 2.03
CRM3	Soil	++	++	+++	0.13 ± 0.01	217.32 ± 2.26
KB133	Soil	-	+	-	-	93.25 ± 1.92
PUR46	Soil	+	+++	-	-	50.24 ± 1.32
PUR171	Soil	-	+	-	-	76.12 ± 1.19
PSB1	Soil	++	+	-	-	395.45 ± 3.62
PSB2	Soil	+	++	-	-	502.39 ± 4.17

a± indicates degree of fluorescence pigment production on KBM; - indicates no fluorescent pigment production, + indicates mild fluorescent pigment production, ++ indicates strong fluorescent pigment production. b± indicates degree of HCN production; - indicates no HCN production, + indicates small amount of HCN production, ++ indicates medium amount of HCN production., +++ indicates maximum amount of HCN production. c± indicates degree of reaction for ammonia production in peptone water broth; - indicates no ammonia production, + indicates small amount of ammonia production, ++ indicates medium amount of ammonia production, +++ indicates maximum amount of ammonia production. ‡ IAA= indole acetic acid; - indicates no IAA production. $ P solubilization = phosphorus solubilization; - indicates no P solubilization.

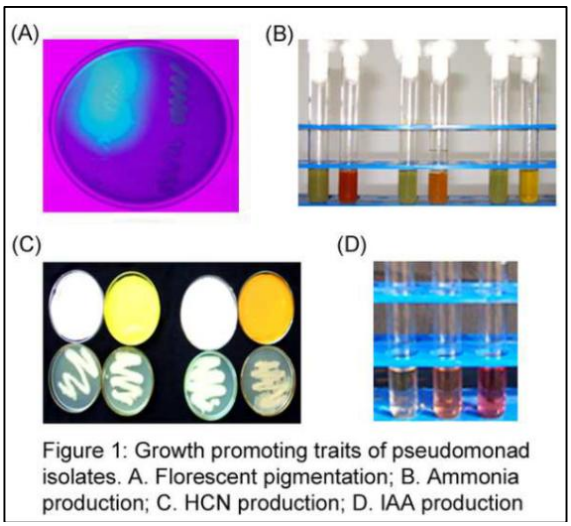

Figure 1: Growth promoting traits of pseudomonad isolates. A. Florescent pigmentation; B. Ammonia production; C. HCN production; D. IAA production

Discussion

Plant growth-promoting attributes

Fluorescent *Pseudomonas* spp. are important for biological control. Certain strains can suppress diseases caused by phytopathogenic fungi (Salman *et al*. 2013; Weller 1988) and are candidates as hosts for the delivery of genes, such as biocontrol toxin, to the plant rhizosphere (Obukowicz *et al*. 1986; Van Elsas *et al*. 1991; Araujo *et al*.1994) [31, 2, 4].

Production of volatile ammonia was also implicated as a possible mechanism to control soil borne pathogens (Baligh *et al*. 1990, 1991 and 1996; Conway *et al*. 1989) [6, 4, 5]. In our experiment, all pseudomonad isolates except L3 produced ammonia. Among these P1 and PUR46 were found to produce maximum amount of ammonia, where PUR46 showed strong antibiosis against DL2, while P1 did not show strong antibiosis.

The production of volatile cyanide is very common among the rhizosphere pseudomonad (Bakker and Schippers 1987; Dowling and O'Gara 1994) [3]. HCN production has been postulated to play an important role in biocontrol of pathogens (Defago *et al*. 1990) [14]. Other workers have also reported HCN production by antagonistic bacteria (Saxena *et al*. 1996; Rangeswaran and Prasad 1998) [37, 33] and are known to inhibit the electron transport, disturbing the energy supply to the cells, ultimately leading to the death of the pathogen (Knowels 1976). Production of HCN by pseudomonad is associated with biological control of the black root of tobacco (Beauchamp *et al*. 1991) [8]. Flaishman *et al*. (1996) [18] also reported that overproduction of cyanide may control fungal diseases in wheat seedlings. However, some other workers observed that it can have a detrimental effect on plant growth (O'Sullivan and O'Gara 1992) [30]. In this investigation, eight HCN producing strains viz., C4, CRM3, C6, C3, A1, A2, C5 and P4 were found to be deleterious and caused differential lysis and deformation of mycelia.

Indole acetic acid is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR (Lee *et al*. 2010; Lynch 1985: Frankenberger and Brunner 1983) [27,]. Among the 30 isolates, 11 isolates were able to produce IAA in the range 0.13 to 11.37 mg/l. Out of eleven, two isolates P1 and C4 showed high level (11.37 and 5.75 mg/l, respectively) production of IAA. This observation revealed that IAA production varies in different isolates of pseudomonad. Tien *et al*. (1979) [44] established that rhizobacteria that produce IAA could responsible for increasing the number of lateral roots and root hairs in pearl millet. But there are some reports that rhizobacteria that overproduce IAA inhibit root elongation, and this was attributed to the stimulation of ethylene synthesis by IAA (Xie *et al*. 1996, Glick *et al*.1998) [49, 20]. Rhizospheric bacteria are known to play a very significant role in plant growth promotion by different mechanisms, one of them being the ability to solubilize phosphorus in soil and making it available for plant uptake (Selvakuma *et al*. 2011; Sharma *et al*. 2011; Kucey *et al*. 1989; Gyaneshwar *et al*. 2002) [39, 37]. The mineral phosphate solubilizing property is associated primarily with the production of low molecular weight organic acids which forms complexes with the metal ions such as Fe, Al, and Ca of the phosphate ore. The metabolic and genetic basis for the high efficiency solubilization of P by majority of the gram-negative PSB, studied so far, has been attributed to the production of gluconic acid by direct oxidation of glucose via, membrane bound quinoprotein glucose dehydrogenases (Gcd) enzyme. This enzyme is known to act not only on glucose but also on several other aldo sugars such as xylose, arabinose, maltose, etc. and oxidizes them to their corresponding acids. Most of the pseudomonads used in this study were found to solubilize complex form of P to the plant available form in the *in vitro* studies conducted. In the screening of the 30 pseudomonad isolates, 26 isolates showed phosphorus solubilization activity. Among these, five isolates viz., PSB1, PSB2, R3, A2 and R1 were the most effective solubilizers *in vitro* and solubilized (502.39, 395.45, 385.55, 346.34 and 312.23 mg/l, respectively) highest amount of tri-calcium phosphate. These results indicate that the solubilization of phosphorus varies in different isolates of the same species. Some P solubilizing organisms have been reported as plant growth promoters (Selvakuma *et al*. 2011; Sharma *et al*. 2011; De Freitas *et al*. 1997; Whitelaw *et al*. 1997) [39, 37, 12]. The ability to convert insoluble P to an accessible form like orthophosphate is an important trait for a PGPR for increasing plant yields (Rossolini *et al*. 1998) [35]. The present study indicated that many isolates of pseudomonad found to be phosphorus solubilizer and thereby expected to enhance uptake by the plants, resulting in increased plant biomass. Apart from phosphorus solubilization, phytohormone production like IAA is another of mechanism that directly influences plant growth (Glick *et al*. 1995) [21].

Results from the present investigation reveal interesting observation that the PUR46 has also other growth promoting attributes like phosphorus solubilization and ammonification, which may contribute to enhancement of growth, yield and nutrient uptake of plant.

References

1. Anuratha CS, Gnanamanickam SS. Biological control of bacterial wilt caused by *Pseudomonas solanacearum* in India with antagonistic bacteria. Plant Soil. 1990; 124:109-116.
2. Araujo MAV, Mendoncea-Hagler LC, Hagler AN, Van Elsas JD. Survival of genetically modified *Pseudomonas fluorescence* introduced into subtropical soils microcosms. FEMS Microbiology Ecology. 1994; 13:205-216.
3. Bakker AW, Schippers B. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biology and Biochemistry. 1987; 19:451-457.

4. Baligh M, Conway KE, Delgado MA. Development of a bioassay system to compare quantities of ammonia produced by strains of *Pseudomonas* spp., potential biocontrol agents against soilborne fungi. Phytopathology. 1991; 81(10):1228.
5. Baligh M, Conway KE, Delgado MA. Production of ammonia by *Pseudomonas cepacia* and *Pseudomonas aeruginosa:* Quantification and effect on host and pathogen. In: Pandalai S.G, editor. Recent research developments in plant pathology Trivandrum, India: Research Signpost. 1996; 1:7-19.
6. Baligh M, Conway KE, Delgado MA. Production of ammonia by *Pseudomonas cepacia* interferes with seed germination and root elongation. Phytopathology. 1990; 80(10):1048.
7. Barber SA. Soil nutrient bioavailability: a mechanistic approach. Oxford, UK: Wiley-Blackwell, 1984.
8. Beauchamp CJ, Dion P, Kloepper JW, Antoun H. Physiological characterization of opine-utilizing rhizobacteria for traits related to plant growth-promoting activity. Plant Soil. 1991; 132:273-279.
9. Borowitz JJ, Stankie-Dicz M, Lewicka T, Zukowska Z. Inhibition of fungal cellulase, pectinase and xylanase activity of plant growth-promoting fluorescent pseudomonads. Bulletin of. OILB/SROP. 1992; 15:103-106.
10. Chahboune R, Barrijal S, Moreno S, Bedmar EJ. Characterization of *Brady rhizobium* species isolated from root nodules of *Cytisus villosus* grown in Morocco. Systematic and Applied Microbiology. 2011; 34:440-445.
11. Conway KE, Foor CJ, Malvick D, Bender C. Inhibition by *Pseudomonas cepacia*, a potential biocontrol agent, of selected soilborne pathogens. Phytopathology. 1989; 79:1159.
12. De Freitas JR, Banerjee MR, Germida JJ. Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). Biology and Fertility of Soil. 1997; 24:358-364.
13. Deepa CK, Dastager SG, Pandey A. Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) Seedling growth. World Journal of Microbiology and Biotechnology. 2010; 26:1233-1240.
14. Defago G, Berling CH, Burger U, Hass D, Kahr G, Keel C. *et al*. Suppression of black root rot of tobacco and other root diseases by strains of *Pseudomonas* y*uorescens*: potential applications and mechanisms. In: Hornby, D. (Ed.), Biological Control of Soilborne Plant Pathogens. CAB International, Wellingford, Oxon, UK, 1990, 93-108.
15. Dowling DN, O'Gara F. Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends in Biotechnology. 1994; 12:133-141.
16. Duffy BK, Defago G. Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas* f*uorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. Phyotpathology. 1997; 87:1250-1257.
17. Dye DW. The inadequacy of the usual determinative tests for identification of *Xanthomonas spp.* New Zealand Journal of Science. 1962; 5:393-416.
18. Flaishman MA, Eyal Z, Zilberstein A, Voisard C, Hass D. Suppression of *Septoria tritici* blotch and leaf rust of wheat by recombinant cyanide-producing strains of *Pseudomonas putida*. Mol. Plant-Microbe Interact. 1996; 9:642-645.
19. Frindlender M, Inbar J, Chet I. Biological control of soilborne plant pathogens by a β-1, 3 glucanase producing *Pseudomonas cepacia*. Soil Biology and Biochemistry. 1993; 25:1211-1221.
20. Glick BR, Penrose DM, Li J. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. Journal of Theoretical Biology. 1998; 190:63-68.
21. Glick BR. The enhancement of plant growth by free living bacteria. Canadian Journal of Microbiology. 1995; 41:109-117.
22. Johnson LF, Curl EA. Methods for research on ecology of soil borne plant pathogens. Burgess Publishing Co., Monneapolis, 1972, 247.
23. Keating JD, Beck L, Materon A, Yurtsever N, Karuc K, Altuntas S. The role of D.P rhizobial diversity in legume crops productivity in the west Asian Highlands. Experimental Agriculture. 1995; 31:473-483.
24. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluoresecin. Journal of Laboratory Medicin. 1954; 44:301-307.
25. Kucey RMN, Janzen HH, Leggett ME. Microbially mediated increases in plant-available phosphorus. Advances in Agronomy. 1989; 42:198-228.
26. Kumar A, Maurya BR, Raghuwanshi R. Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). Biocatalysis and Agricultural Biotechnology. 2014; 3:121-128.
27. Lee JH, Lee J. Indole as an intercellular signal in microbial communities. FEMS Microbiology Reviews. 2010; 34:426-444.
28. Lim H, Kim Y, Kim S. *Pseudomonas stutzeri* YLP-1 genetic transformation and antifungal mechanism against *Fusarium solani*, an agent of plant root rot. Applied and Environmental Microbiology. 1991; 57:510-516.
29. Majeed A, Abbasi MK, Hameed S, Imran A Rahim N. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in Microbiology. 2015; 6:198.
30. O'Sullivan DJ, O'Gara F. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiological Review. 1992; 56:662-676.
31. Obukowicz MG, Perlak FJ, Kusano-Kretzmer K, Mayer EJ, Bolten SL, Watrud LS. Tn-5-mediated integration of delta-endotoxingene from *Bacillus thuringiensis* into chromossome of root colonizing pseudomonads. Journal of Bacteriology. 1986; 168:982-989.
32. Potgieter H, Alexander M. Susceptibility and resistance of several fungi to microbial lysis. Journal of Bacteriology. 1996; 91:1526-1532.
33. Rangeswaran R, Prasad RD. Screening and selection of rhizobacteria for biological control of *Sclerotium rolfsii* and *Rhizoctonia solani*. National Symposium on Development of Microbial Pesticides in Insect Pest Management. Nov. 12-13, HAL, Pune, 1998, 41.
34. Richardson AE, Baréa JM, McNeill AM, Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 2009; 321:305-339.

35. Rossolini GM, Shipa S, Riccio ML, Berlutti F, Macaskie LE, Thaller MC. Bacterial non-specific acid phosphatases: physiology, evolution, and use as tools in microbial biotechnology. Cellular and Molecular Life Sciences. 1998; 54:833-850.
36. Saharan BS, Nehra V. Plant growth promoting rhizobacteria: A critical review. Life Science and Medicine Research. 2011; 21:1-30.
37. Saxena A, Sharma A, Johri BN. Bacterial characterization of a growth promoting fluorescent pseudomonad from Rajniganda rhizosphere, 37th Ann. Conf. Assoc. Microbiol. India, Dec. 4-6., IIT, Chennai, 1996, 135.
38. Saxena AK, Lata AK. Charecterization of plant growth-promoting rhizobacteria. Training manual on biofertilizer technology. 2003, 31-37.
39. Selvakumar G, Mishra PK, Bhatt JC, Joshi P, Joshi GK, Gupta HS. *Pseudomonas lurida* M2RH3 (MTCC 9245), a psychrotolerant bacterium from the Uttarakhand Himalayas, solubilizes phosphate and promotes wheat seedling growth. World Journal of Microbiology and Biotechnology. 2011; 27:1129-1135.
40. Selvakumar G, Reetha R, Thamizhiniyan P. The PGPR as elicitors of plant defence mechanisms and growth stimulants on tomato (*Lycopersicum esculentum* Mill.) Botany Research International. 2013; 6:47-55.
41. Shakeela S, Padder SA, Bhat ZA. Isolation and characterization of plant growth promoting rhizobacteria associated with medicinal plant *Picrorhiza Kurroa*. Journal of Pharmacognosy and Phytochemistry. 2017; 6(3):157-168.
42. Sharma S, Kumar V, Tripathi RB. Isolation of phosphate solubilizing microorganism (PSMs) from soil. Journal of Microbiology and Biotechnology Research. 2011; 1:90-95.
43. Sivaprasad P. Microbial inoculant technology for plant disease management. Research Extension Interface, Farm information Bureau, government of Kerala, 2002, 23-30.
44. Tien TM, Gaskins MH, Hubbell DH. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Applied and Environmental Microbiology. 1979; 37:1016-1024.
45. Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y. Plant growth-promoting rhizobacteria and root system functioning. Frontier in Plant Science. 2013; 4:356.
46. Van Elsas JD, Van Overbeek LS, Feldmann AM, Dullemans AM, de Leeuw O. Survival of genetically engineered *Pseudomonas fluorescence* in soil in competition with the parent strain. FEMS Microbiology Ecology. 1991; 85:53-64.
47. Velazhahan R, Samiyappan R, Vidhyasekaran P. Relationship between antagonistic activities of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of lytic enzyme. Journal of Plant Diseases and Protection. 1999; 106:244-250.
48. Whitelaw MA, Harden TJ, Bender GL. Plant growth promotion of wheat inoculated with *Penicillium radicum* sp. Nov. Australian Journal of Soil Research. 1997; 35:291-300.
49. Xie H, Pasternak JJ, Glick BR. Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida G*R12-2 that overproduce indole acetic acid. Current Microbiology. 1996; 32:67-71.
50. Yeole RD, Dube HC. Siderophore mediated antibiotics of rhizobacterial fluorescent pseudomonads against soilborne fungal plant pathogens. Journal of Mycology and Plant Pathology. 2000; 30:335-338.