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Isolation and Characterization of Effective Plant Growth Promoting Rhizobacteria from Rice Rhizosphere of Indian Soil

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ABSTRACT

The Plant Growth Promoting Rhizobacteria (PGPR) can substantially reduce the chemical inputs in agriculture. Furthermore, the use of indigenous PGPR can be an added advantage since it can easily acclimatize to the natural conditions and enhanced the plant–microbe interactions. The objective of my research is screening, characterization and selection of effective PGPR for rice production. Eight efficient PGPR isolates were selected and identified as *Pseudomonas aeruginosa* strain BHUJY12 (HQ-236532), *Pseudomonas putida* strain BHUJY14 (HQ-236533), *P. aeruginosa* strain BHUJY16 (HQ-236535), *Pseudomonas* sp. strain BHUJY19 (HQ-236519), *P. aeruginosa* strain BHUJY22 (HQ-236541), *P. putida* strain BHUJY23 (HQ-236542), *P. aeruginosa* strain BHUJY24 (HQ-236543) and *P. aeruginosa* strain BHUJY25 (HQ-236544). The bacterial strains were recorded range from 115.94 to 228.71 μg mL^{-1} phosphate solubilization in Pikovskaya broth medium. Also pH of the medium was ranged from 6.77 to 4.65 and SI index of different isolates ranged from 2.28 to 4.23 on solid agar plate at 6 days of incubation. Indole-3-acetic acid (IAA) production was varied range from 12.19 to 22.91 μg mL^{-1} at 6 days incubation. PGPR strains were also showed the growth inhibition of *Rhizoctonia solani* at 3 and 6 days incubation. Among all the strains, BHUJY23 strain (*Pseudomonas putida*) was found maximum significant phosphorus solubilization and IAA production followed by strain BHUJY16. Therefore, the *P. putida* strain BHUJY23 was effective PGPR for rice production under Indo-Gangetic plain of Eastern Uttar Pradesh.

Key words: Rhizosphere, PGPR, IAA, *Pseudomonas*, rice

INTRODUCTION

The rhizosphere microflora has been greatly affected and resulting decrease the soil productivity and nutrient use efficiency due to excessive use of the chemical fertilizers. Plant Growth Promoting Rhizobacteria (PGPR) is alternative of chemical fertilizer because this has ability of phosphorus solubilization, production of plant growth hormones and biocontrol activity. PGPR are free-living soil bacteria, isolated from the rhizosphere, when it applied as seed or crop inoculation, to promote plant growth and yield by several mechanism e.g., phytohormones production, provide available nitrogen by biological nitrogen fixation, available phosphorus by phosphate solubilization and suppression of phytopathogen (Vessey, 2003; Akhtar *et al.*, 2012). The direct mechanisms of plant growth by PGPR include the provision of phosphorus solubilization and its uptake by plants, biological nitrogen–fixation, sequestration of iron for plant by siderophores, production of plant hormones like auxins, cytokinins and gibberellins and lowering the plant ethylene level (Verma *et al*., 2010). PGPR strains of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to increase plant growth attributes, yield and nutrient content in various crop plants (Joseph *et al*., 2007; Mia *et al*., 2010). Bacterial inoculation of Phosphate Solubilizing Bacteria (PSB) with different crops has enhanced their plant growth and yield and also enhanced phosphorus content in soils (Domey and Lippmann, 1989; De Freitas *et al*., 1997). PSB inoculation has increase uptake of phosphorus as well as grain yields in several crops (Khalid *et al*., 2004). PSB has ability to promote plant growth by synthesis of phytohormones (indole-3-acetic acid (IAA), gibberellins and cytokinins) and various other plant growth promoting substances (Vikram *et al*., 2007; Verma *et al*., 2012). Phosphorus, the second most important nutrient after nitrogen, plays important role in development of root, stalk and stem, flower and seed formation, crop maturity, plant disease resistant and biological nitrogen fixation. Part of the phosphorus added through fertilizers in soil is utilized by plants and remaining portion is converted into insoluble forms like iron and aluminium phosphate in the acidic and calcium phosphate in alkaline or normal soil (Gyaneshwar *et al*., 2002).

Manivannan *et al*. (2012) has been reported that the PGPR isolates (PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2) were successfully isolated and characterized from rice rhizosphere soils and evaluate their PGPR activities such as production of IAA, siderophore, ammonium and solubilization of phosphate, and antagonistic activity against phytopathogenic fungi such as *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*.

Bacteria are able to solubilize organic and inorganic phosphorus from soil and made available to plant for growth and development (Narsian and Patel, 2000). Phosphate Solubilizing Bacteria (PSB) is a group of organisms that solubilize fixed form of organic and inorganic phosphorus and make it available to plants (Appanna, 2007). The most important phosphorus solubilizing bacterial genera includes *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* (Subbarao, 1998). The PSB are well known soil bacteria in rhizosphere and non rhizosphere soil which is various types of bacteria like *Pseudomonas* spp. and *Bacillus* spp. PSB population are decrease or increase on the basis of physic-chemical and biological properties of soil (Kim *et al*., 1998; Vazquez *et al*., 2000).

Bacteria solubilize insoluble inorganic phosphorus by secretion of organic acids, which through their hydroxyl and carboxyl groups chelate the cations (Al, Fe and Ca) bound to phosphate and decrease the pH of soil (Kpomblekou and Tabatabai, 1994; Stevenson, 2005). The major organic acids produced by PSB are gluconic and keto gluconic acids (Goldstein, 1995; Deubel *et al*., 2000), which solubilize phosphorus by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil (Nahas, 1996). Isolates of different phosphate solubilizing bacteria from different rhizospheric soil showed varying phosphate solubilizing capacity under *in vitro* condition (Ponmurugan and Gopi, 2006). Rangarajan *et al*. (2002) have also shown the diversity of *Pseudomonas* spp. isolated as PGPR from rice rhizosphere. The population of PSB were found maximum in rhizospheric soil of groundnut and minimum in rhizospheric soil of ragi, sorghum and maize. A wide variation in the capacity to solubilize phosphorous by the PSB isolates (BP01, CP01, CP22 etc.) was observed and they were able to secrete phytohormones like gibberellic acid (GA_3) and indole acetic acid (IAA) and acid phosphatase under *in vitro* condition (Ponmurugan and Gopi, 2006). Therefore the aim of present study was undertaken to screen of effective PGPR for rice as well as other crop production in Eastern Uttar Pradesh.

MATERIALS AND METHODS

Collection of soil sample: Twenty eight rice rhizosphere soil samples were collected from different places of Varanasi district of eastern Uttar Pradesh in year 2009. Rhizosphere soil were collected in polythene bag and stored at 4°C for further studies.

Isolation of PGPR strains: Ten gram rhizosphere soil was taken in 250 mL Erlenmeyer flask containing 90 mL sterile distilled water and mixed by shaking for 15 min. Serial dilutions were done in sterile distilled water. The dilution was done up to 10^{-2} to 10^{-6}. Aliquots of 0.1 mL (10^{-5} and 10^{-6}) were spread on plates containing Pikovskaya agar media [tricalcium phosphate 5 g, glucose 13 g, $(NH_4)SO_4$ 0.5 g, NaCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g, KCl 0.2 g, Yeast Extract 0.5 g, $MnSO_4$ trace, $FeSO_4.7H_2O$ trace, Agar 15 g, dissolved in 1000 mL distilled water and pH adjusted to 7.0]. Inoculated plates were placed into BOD at 28±2°C. After 72 h the inoculated plates were observed for growth and appearance of solubilization zone around the bacterial colonies. The bacterial colony that showed solubilization zone around their growth were isolated and recultured in another plates by streak plate method to isolates pure colony of phosphate solubilizing bacteria. Pure culture of PSB was maintained in agar slant for further analysis.

Biochemical analysis: The bacterial strains BHUJY12, BHUJY14, BHUJY16, BHUJY19, BHUJY22, BHUJY23, BHUJY24 and BHUJY25 were characterizes by their biochemical characteristic (Gram reaction, catalase reactions, Methyl red, Voges-Proskauer test, hydrolysis of starch and utilization of glucose, sucrose, mannitol) using standard methods (Cappuccino and Sherman, 1992; Aneja, 2003). Bacterial cultures were also molecularly characterized by 16S rDNA partial gene sequencing. Bacterial cultures were maintained on the respective slants media and store at 4°C further use. All media and chemical ingredient were purchased from Hi-media Pvt. Ltd. Mumbai.

Qualitative and quantitative estimation of phosphate solubilization on Pikovskaya medium: For estimation of phosphate solubilization on plates Pikovskaya agar medium were used (Pikovskaya, 1948). The plates were inoculated with bacteria and incubated at 28±2°C for 3 and 6 days. Colony, forming a clear halozone around them, indicating phosphate solubilisation (Fig. 1). The halo zone formation was measured after 3 and 6 days by scale and used for calculation of phosphate solubilization index. Solubilization Index (SI) was calculation according to Premono *et al.* (1996).

pH change: Bacterial culture were inoculated into 100 mL Pikovskaya's broth media in 250 mL conical flask and incubated at 28±2°C for 3 and 6 days on a rotatory incubating shaker (120 rpm). Sterile uninoculated medium served as control. Initial pH and change in pH after 3 and 6 days of inoculation were recorded by digital pH meter.

Quantitative estimation of phosphorus solubilization in broth medium: For the quantitative estimation of phosphorus in broth culture was estimated by Ammonium bicarbonate diethylene triamine pentaacetic acid (AB-DTPA) methods (Soltanpour and Workman, 1979). Available phosphorus was determined by ascorbic acid method (Watanabe and Olsen, 1965).

Indole acetic acid (IAA) production: Quantitative analysis of IAA was performed using the method of Loper and Schroth (1986).

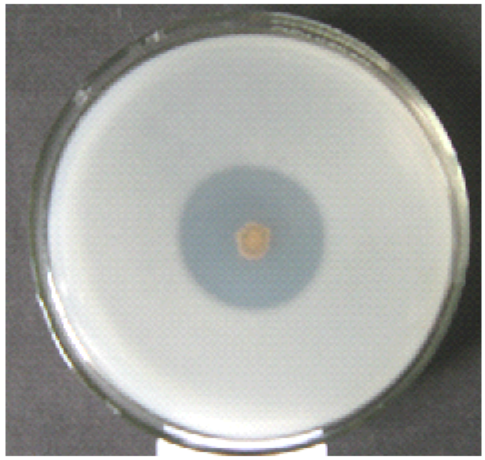

Fig. 1: Phosphate solubilization as clear halozone of *Pseudomonas putida* BHUJY23 on Pikovskaya agar

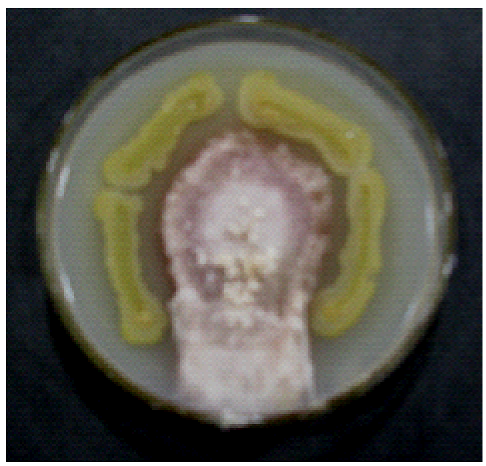

Fig. 2: Inhibition zone of *Pseudomonas aeruginosa* BHUJY16 against *Rhizoctonia solani* on PDA agar plate

Antifungal assay: Spores of fungal culture *Rhizoctonia solani* (fungal cultures obtained from Department of Plant Pathology and Mycology, Institute of Agricultural Sciences, BHU, Varanasi, India) grown on Potato Dextrose Agar (PDA) medium. A small block of agar with fungal growth was cut using sterile blade and placed in the centre of a fresh PDA plate. Test bacteria were streaked at three ends of the plate and allowed to incubate at 28±2°C for 48-96 h and zone of inhibition recorded (Fig. 2) positive for antifungal activity (Rajendran *et al*., 2008).

Statistical analysis: The experiment was done in three replication under laboratory condition. Data of phosphate solubilization were analysed through Duncan multiple range test (DMRT) at $p \leq 0.05$ by using SPSS software version 17.0.

RESULTS

Characterization of PGPR strains: Eight bacterial strains isolated from rice rhizosphere and screened on the basis of their plant growth promoting activities of PGPR strains on Pikovskaya media. Morphologically and biochemically characterized as per methods described in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). All the isolates showed rod shape cell under microscope and positive for catalase, oxidase and citrate while, negative for Gram reaction, MR and VP test (Table 1). The bacterial strains BHUJY12, BHUJY14, BHUJY16, BHUJY19, BHUJY22, BHUJY23, BHUJY24 and BHUJY25 were molecularly characterized through 16S rDNA partial gene sequencing and submitted in NCBI-GenBank with different accession number. Accession number had allotted for each bacterial strain *Pseudomonas aeruginosa* strain BHUJY12 (HQ-236532), *Pseudomonas putida* strain BHUJY14 (HQ-236533), *P. aeruginosa* strain BHUJY16 (HQ-236535), *Pseudomonas* species strain BHUJY19 (HQ-236519), *P. aeruginosa* strain BHUJY22 (HQ-236541), *P. putida* strain BHUJY23 (HQ-236542), *P. aeruginosa* strain BHUJY24 (HQ-236543) and *P. aeruginosa* strain BHUJY25 (HQ-236544).

Phosphate solubilization index on solid agar plates: Solubilization Index (SI) of phosphate solubilizing bacterial strains were recorded at 3 and 6 days of incubation. Solubilization index ranged between 2.28 to 30.03 and 3.23 to 4.23 after 3 and 6 days of incubation, respectively (Table 2). All bacterial strain showed significantly higher solubilization than control. Maximum SI

Table 1: Different parameter of biochemical properties of different PGPR strains

	Biochemical test							
Strains	Shape	Gram reaction	Catalase	Oxidase	Citrate	MR	VP	HCN
BHUJY12	Rod	-	+	+	+	-	-	+
BHUJY14	Rod	-	+	+	+	-	-	-
BHUJY16	Rod	-	+	+	+	-	-	+
BHUJY19	Rod	-	+	+	+	-	-	+
BHUJY21	Rod	-	+	+	+	-	-	+
BHUJY22	Rod	-	+	+	+	-	-	+
BHUJY23	Rod	-	+	+	+	-	-	-
BHUJY27	Rod	-	+	+	+	-	-	+

MR: Methyl red, VP: Voges Praskaur, HCN: Hydrogen cyanide, +: Positive, -: Negative

Table 2: Phosphate solubilization activities of different PGPR strains in Pikovskaya medium

		Solubilization index (SI)		pH change		Phosphate solubilization (μg mL^{-1})	
Strain	Bacteria name	3 days	6 days	3 days	6 days	3 days	6 days
Control	Un-inoculated	0.00^{a}	0.00^{a}	7.00^{c}	7.00^{d}	0.00^{a}	0.00^{a}
BHUJY12	*P. aeruginosa*	2.39^{bc}	3.47^{b}	6.60^{b}	5.79^{c}	132.46^{cd}	151.62^{b}
BHUJY14	*P. putida*	2.51^{bc}	3.68^{b}	6.77^{b}	5.98^{c}	160.48^{e}	183.83^{c}
BHUJY16	*P. aeruginosa*	2.28^{b}	3.47^{b}	5.93^{a}	5.20^{b}	167.05^{e}	191.29^{c}
BHUJY19	*Pseudomonas*	2.70^{c}	3.47^{b}	6.27^{ab}	5.96^{c}	115.94^{b}	141.55^{b}
BHUJY22	*P. aeruginosa*	2.72^{c}	3.23^{b}	6.66^{b}	5.72^{c}	122.97^{bc}	149.49^{b}
BHUJY23	*P. putida*	3.03^{d}	4.23^{c}	5.83^{a}	4.65^{a}	186.32^{f}	228.71^{d}
BHUJY24	*P. aeruginosa*	2.67^{c}	3.18^{b}	6.63^{b}	5.82^{c}	138.47^{d}	156.40^{b}
BHUJY25	*P. aeruginosa*	2.41^{bc}	3.57^{b}	6.66^{b}	5.54^{ab}	135.30^{cd}	155.36^{b}

[a]Values are mean of three replicates, Mean with different letters in the same column differ significantly at $p \leq 0.05$ according to DMRT analysis

was recorded in strain BHUJY23 followed by BHUJY22, BHUJY19, BHUJY24, BHUJY14, BHUJY25, BHUJY12 and BHUJY16 at 3 days of incubation. Bacterial strain BHUJY23 was recorded maximum SI followed by BHUJY14, BHUJY25, BHUJY19, BHUJY16, BHUJY12, BHUJY22 and BHUJY24 at 6 days of incubation. *Pseudomonas putida* strain BHUJY23 was showed significant 3.03 and 4.23 maximum SI at 3 and 6 days incubation, respectively (Table 2).

pH change: The pH change of the medium was recorded after 3 and 6 days of incubation. The pH of the culture broth was dropped significantly as compared to the control where it remained constant around pH 7.0. pH change was varied from 6.77 to 5.83 after 3 days of incubation and it varied from 5.96 to 4.65 after 6 days of incubation (Table 2). Maximum pH drop (5.83) was recorded with strain BHUJY23 followed by BHUJY16, BHUJY19, BHUJY12, BHUJY24, BHUJY22, BHUJY25 and BHUJY14 after 3 days of incubation. After 6 days of incubation maximum pH drop (4.65) was observed with strain BHUJY23 followed by BHUJY16, BHUJY25, BHUJY22, BHUJY12, BHUJY24, BHUJY19 and 14 (Table 2).

Phosphate solubilization in broth medium: Phosphate solubilization in broth medium was recorded after 3 and 6 days of incubation. It varied from 115.94 to 186.32 and 141.55 to 228.71 μg mL^{-1}, respectively. Significantly higher solubilization was recorded 186.32 and 228.71 μg mL^{-1} in strain BHUJY23 at 3 and 6 days of inoculation, respectively. *Pseudomonas putida* strain BHUJY23 showed more significant phosphate solubilizer followed by BHUJY16, BHUJY14, BHUJY24, BHUJY25, BHUJY12, BHUJY22 and BHUJY19 after 3 and 6 days incubation at 30°C temperature in BOD incubator (Table 2).

Indole-3-acetic acid (IAA) production: Plant growth promoting hormones IAA was determined by 100 and 150 μg mL^{-1} ryptophan supplemented as precursor of IAA synthesis under laboratory condition (Table 3). IAA production range was varied from 9.00 to 16.23 and 12.19 to 22.91 μg mL^{-1} at 100 and 150 μg mL^{-1} tryptophan concentrations, respectively at 24 h of incubation. More significant IAA production was recorded in PGPR strain BHUJY23 followed by BHUJY16 and BHUJY19 over others strains. *Pseudomonas putida* strains BHUJY23 was produced 16.23 and 22.91 μg mL^{-1} IAA at 100 and 150 μg mL^{-1} tryptophan concentrations, respectively (Table 3).

Table 3: Indole-3-acetic acid (IAA) production and growth inhibition activities of PGPR strains

		R. solani inhibition (mm)		IAA production	
Bacterial strains	Bacterial name	After 3 day	After 6 day	100 (μg mL^{-1})	150 (μg mL^{-1})
Control	Un-inoculated	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
BHUJY-12	*P. aeruginosa*	7.33^{cd}	9.00^{cde}	11.32^{cd}	14.30^{c}
BHUJY-14	*P. putida*	5.00^{bc}	8.00^{bc}	11.48^{cd}	14.17^{c}
BHUJY-16	*P. aeruginosa*	11.67^{e}	15.00^{f}	14.35^{de}	21.22^{e}
BHUJY-19	*P. seudomonas*	6.00^{bc}	7.00^{b}	13.41^{de}	16.94^{d}
BHUJY-22	*P. aeruginosa*	7.00^{c}	8.33^{bc}	9.00^{b}	12.19^{b}
BHUJY-23	*P. putida*	6.00^{bc}	8.00^{bc}	16.29^{f}	22.91^{e}
BHUJY-24	*P. aeruginosa*	9.33^{d}	12.00^{e}	10.83^{bc}	16.08^{d}
BHUJY-25	*P. aeruginosa*	8.00^{cd}	10.00^{de}	10.28^{bc}	13.20^{bc}

[a]Values are mean of three replicates, Mean with different letters in the same column differ significantly at p≤0.05 according to DMRT analysis

Antifungal activity: All PGPR strains were showed antagonistic against phytopathogenic fungus such as *Rhizoctonia solani*. Fungal growth inhibition activity by the PGPR strains on the solid plate of PDA media was recorded in millimeters. Growth inhibition range was varied from 6.00 to 11.67 mm and 7.00 and 15.00 mm at 3 and 6 days incubation (Table 3). PGPR strain BHUJY16 was showed more significant growth inhibition of *R. solani* followed by BHUJY24 and BHUJY25 at 3 and 6 days of incubation. Overall results showed that *Pseudomonas putida* strains BHUJY23 has ability of phosphate solubilisation, IAA production and antifungal activity followed by strains BHUJY16 and BHUJY19 (Table 3).

DISCUSSION

In present investigation, *Pseudomonas* strains were mainly found in rhizosphere soil of rice on the basis of biochemical analysis. *Pseudomonas aeruginosa* and *P. putida* were screened as good phosphate solubilizing bacteria. Biochemical test of different *Pseudomonas* strains were also observed by other workers (Pandey *et al.*, 2006; Ayyadurai *et al.*, 2006). The *P. putida* strain BHUJY23 was showed more solubilization index 4.23 on Pikovskaya agar plates at 6 days incubation. According to De Freitas *et al.* (1997), good phosphate-solubilizers were able to produce halos of more than 15 mm diameters around their colonies. Several species of fluorescent pseudomonas such as *P. fluorescens*, *P. aeruginosa* and *Bacillus* sp. were reported as good phosphate solubilizers (Ahmad *et al.*, 2008; Verma *et al.*, 2010; Panhwar *et al.*, 2012). The *P. putida* strain BHUJY23 was showed 186.32 and 228.71 μg mL^{-1} phosphate solubilization at 3 and 6 days incubation, respectively. Similarly (Chakkaravarthy *et al.*, 2010) investigated that PB08 was most efficient phosphate solubilizer on phosphate agar plates with SI 4.80 at sixth day incubation and phosphate solubilization was noted ranged from 9.6 to 136 ppm in broth culture of Pikovaskaya media at 10 days incubation. Other worker, Pandey *et al.* (2006) reported that phosphate solubilization by *Pseudomonas putida* (247 μg mL^{-1}) after 15 days of inoculation. Lowest pH drop in the broth culture was recorded with strain BHUJY23 4.23 after 6 days of incubation. The phosphate solubilization activity by different microorganisms was due to the production of different organic acids, in which Hydroxyl and carboxyl groups of acids chelate cation (AL, Fe, Ca) and decrease the pH of the medium (Stevenson, 2005). The production of IAA production was highest in isolates of *P. putida* strains BHUJY23 followed by *P. aeruginosa* strains BHUJY16 and *Pseudomonas* sp. strains BHUJY19. Similarly, IAA production was recorded in *Pseudomonas* sp., *Bacillus, Azotobacter*, *Rhizobium* by other workers (Ahmad *et al.*, 2008; Verma *et al.*, 2010; Panhwar *et al.*, 2012). The antifungal activity of the test isolates might be due to the production of antifungal enzyme and antibiotics activities. All PGPR strains showed positive for growth inhibition of *R. solani*. Several studies have demonstrated that production of lytic enzymes by pseudomonas strains was most effective in controlling the plant root pathogens including *Fusarium oxysporum* f. sp. *ciceris* and *R. solani* (O'Sullivan and O'Gara, 1992; Ahmad *et al.*, 2008).

CONCLUSION

In present study, *Pseudomonas putida* strain BHUJY23 was found efficient plant growth promoting rhizobacteria for rice production. It could be an alternative to chemical fungicides and chemical fertilizer to promote plant growth in rice and other field crops.

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REFERENCES

Ahmad, F., I. Ahmad and M.S. Khan, 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res., 168: 173-181.

Akhtar, A., Hisamuddin, M.I. Robab, Abbasi and R. Sharf, 2012. Plant growth promoting Rhizobacteria: An overview. J. Nat. Prod. Plant. Resour., 2: 19-31.

Aneja, K.R., 2003. Experiments in Microbiology, Plant pathology and Biotechnology. 4th Edn., New Age International Publishers, Daryaganj, New Delhi, India.

Appanna, V., 2007. Efficacy of phosphate solubilizing bacteria isolated from vertisols on growth and yield parameters of sorghum. Res. J. Microbiol., 2: 550-559.

Ayyadurai, N., P.R. Naik, M.S. Rao, R.S. Kumar, S.K. Samrat, M. Manohar and N. Sakthivel, 2006. Isolation and characterization of novel banana rhizosphere bacterium as fungal antagonist and microbial adjuvant in micropropagation of banana. J. Applied Microbiol., 100: 926-937.

Cappuccino, J.C. and N. Sherman, 1992. Microbiology: A Laboratory Manual. 3rd Edn., Benjamin/Cummings Publishing Company, New York, USA., ISBN-13: 9780805310528, pp: 125-179.

Chakkaravarthy, V.M., R. Arunachalam, S. Vincent, K. Paulkumar and G. Annadurai, 2010. Biodegradation of tricalcium phosphate by phosphate solubilizing bacteria. J. Biol. Sci., 10: 531-535.

De Freitas, J.R., M.R. Banerjee and J.J. Germida, 1997. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). Biology Fertility Soils, 24: 358-364.

Deubel, A., Gransee and W. Merbach, 2000. Transformation of organic rhizodeposites by rhizoplane bacteria and its influence on the availability of tertiary calcium phosphates. J. Plant Nutr. Soil Sci., 163: 387-392.

Domey, S. and G. Lippmann, 1989. Stimulation of Plant Growth by Phosphate Solubilizing Bacteria. In: Interrelationships between Microorganisms and Plants in Soil, Kunc, F. and V. Vancura (Eds.). Czechoslovak Academy of Sciences, Prague, Czech Republic, ISBN-13: 9780444408822, pp: 457-461.

Goldstein, A.H., 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. Biol. Agric. Hortic., 12: 185-193.

Gyaneshwar, P., G.N. Kumar, L.J. Parekh and P.S. Poole, 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil, 245: 83-93.

Holt, J.G., N.R. Kneg, P.H.A. Sneath, J.T. Stanly and S.T. Williams, 1994. Bergeys Mannual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, Baltimore.

Joseph, B., R.R. Patra and R. Lawrence, 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). Int. J. Plant Prod., 2: 141-152.

Khalid, A., M. Arshad and Z.A. Zahir, 2004. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. J. Applied Microbiol., 96: 473-480.

Kim, K.Y., D. Jordan and G.A. McDonald, 1998. Effect of phosphate solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol. Fert. Soils, 26: 79-87.

Kpomblekou, K. and M.A. Tabatabai, 1994. Effect of organic acids on release of phosphorus from phosphate rocks1. Soil Sci., 158: 442-453.

Loper, J.E. and M.N. Schroth, 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. Phytopathology, 76: 386-389.

Manivannan, M., P. Ganesh, R.S. Kumar, K. Tharmaraj and B.S. Ramya, 2012. Isolation, screening, characterization and antagonism assay of PGPR isolates from rhizosphere of rice plants in Cuddalore District. Internat. J. Pharmaceut. Biol. Archi., 3: 179-185.

Mia, M.A.B., Z.H. Shamsuddin and M. Mahmood, 2010. Use of plant growth promoting bacteria in banana: A new insight for sustainable banana production. Int. J. Agric. Biol., 12: 459-467.

Nahas, E., 1996. Factors determining rock phosphate solubilization by microorganism isolated from soil. World J. Microbiol. Biotechnol., 12: 18-23.

Narsian, V. and H.H. Patel, 2000. *Aspergillus aculeatus* as a rock phosphate solubilizer. Soil Biol. Biochem., 32: 559-565.

O'Sullivan, D.J. and F. O'Gara, 1992. Traits of fluorescent *Pseudomonas* spp. involved in the suppression of plant roots pathogens. Microbiol. Mol. Biol. Rev., 56: 662-676.

Pandey, A., P. Trivedi, B. Kumar and L.M.S. Palni, 2006. Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-alpine location in the Indian central Himalaya. Curr. Microbiol., 53: 102-107.

Panhwar, Q.A., R. Othman, Z.A. Rahman, S. Meon and M.R. Ismail, 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. Afr. J. Biotechnol., 11: 2711-2719.

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with vital activities by some microbial species. Microbiologia, 17: 362-370.

Ponmurugan, P. and C. Gopi, 2006. Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J. Agron., 5: 600-604.

Premono, M.E., A.M. Moawad and P.L.G. Vlek, 1996. Effect of phosphate solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere. Indonasian J. Crop Sci., 11: 13-23.

Rajendran, G., F. Sing, A.J. Desai and G. Archana, 2008. Enhanced growth and nodulation of pigeon pea by co-inoculation of *Bacillus* strains with *Rhizobium* spp. Bioresour. Technol., 99: 4544-4550.

Rangarajan, S., L.M. Saleena and S. Nair, 2002. Diversity of *Pseudomonas* spp. isolated from rice rhizosphere populations grown along salinity gradient. Micob. Ecol., 43: 280-290.

Soltanpour, P.N. and S.M. Workman, 1979. Oil testing methods used at Colorado State University soil testing laboratory for the evaluation of fertility, salinity, sodicity and trace element toxicity. Colorado State University Experiment Station, Fort Collins, Colorado, USA., pp: 14.

Stevenson, F.J., 2005. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients. John Wiley and Sons, New York, USA.

Subbarao, N.S., 1998. Phosphate Solubilizing Microorganisms. In: Biofertilizer in Agriculture and Forestry, Subharao, N.S. (Ed.). Regional biofertilizer Development Centre, Hissar, India, pp: 133-142.

Vazquez, P., G. Holguin, M.E. Puente, A. Lopez-Cortes and Y. Bashan, 2000. Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi arid coastal lagoon. Biol. Fertil. Soils, 30: 460-468.

Verma, J.P., J. Yadav and K.N. Tiwari, 2012. Enhancement of nodulation and yield of chickpea by co-inoculation of indigenous *Mesorhizobium* spp. and plant growth-promoting rhizobacteria in Eastern Uttar Pradesh. Communic. Soil Sci. Plant Anal., 43: 605-621.

Verma, J.P., J. Yadav, K.N. Tiwari, Lavakush and V. Singh, 2010. Impact of plant growth promoting rhizobacteria on crop production. Int. J. Agric. Res., 5: 954-983.

Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571-586.

Vikram, A., H. Hamzehzarghani, A.R. Alagawadi, P.U. Krishnaraj and B.S. Chandrashekar, 2007. Production of plant growth promoting substances by phosphate solubilizing bacteria isolated from vertisols. J. Plant Sci., 2: 326-333.

Watanabe, F.S. and S.R. Olsen, 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proc., 29: 677-678.