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Screening and characterization of native *Pseudomonas* sp. as plant growth promoting rhizobacteria in chickpea (*Cicer arietinum* L.) rhizosphere

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The search for diverse plant growth promoting rhizobacteria (PGPR) is gaining momentum as efforts are directed to exploit them as low-input biotechnology for sustainable agriculture. In search of efficient PGPR (*Pseudomonas* sp.) with multiple plant growth promoting (PGP) activities, a total of 35 isolates of rhizobacteria were isolated from 25 soil samples collected from healthy chickpea rhizospheric locations of Punjab (India). Ten isolates of rhizobacteria were characterized as *Pseudomonas* sp. on the basis of morphological, biochemical and growth promotion activities. PGPRs (*Pseudomonas* sp.) were screened for growth promotion activities [indole acetic acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore, phosphate (P) solubilization, catalase, antibiotic resistance spectra] and seed germination on water agar medium along with reference strain PGPR LK884 (*Pseudomonas diminuta*). Maximum amount of IAA was produced by PGPR-3 (70.05 µg/ml) followed by PGPR-2 (66.79 µg/ml) as compared to PGPR LK 884 (61.58 µg/ml) in the presence of L-Tryptophan as precursor of IAA. 70% of isolates showed capacity for P solubilization in the range of 5.08 to 13.45 mg/100 ml. Maximum P-solubilization was noticed with PGPR-3 (13.45 mg /100 ml) followed by PGPR-2 (13.15 mg/100 ml). Two isolates of *Pseudomonas* sp. PGPR-2 and PGPR-3 also produced siderophores, HCN, NH_3 and improved seed germination in *kabuli* and *desi* chickpea. Intrinsic antibiotic spectra (IAR) showed 70% of PGPRs (*Pseudomonas* sp.) resistance to ampicillin (10 µg/ml). Two native isolates of PGPRs (*Pseudomonas* sp.) PGPR-2 and PGPR- 3 with multiple PGP traits can be exploited for plant growth promotion due to their well adaptation in chickpea rhizosphere. Further evaluation of potential isolates for exhibiting their multiple PGP traits on soil plant system is needed to uncover their efficacy.

Key words: Plant growth promoting rhizobacteria (PGPR), *Pseudomonas* sp., Chickpea, Stress tolerance, Siderophores.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are associated with most plant species and commonly found in many environments. The early root colonizing microorganisms, in and around the growing roots of legumes, may interact with each other and with the plant resulting in symbiotic, associative, neturalistic or detrimental effects (Gulati et al., 2001). Rhizobacteria are rhizosphere competent bacteria that aggressively colonize plant roots. Traits associated with rhizosphere competence survival in soil include an ability to tolerate a reasonable range of abiotic factors including temperature, pH and moisture. The PGPRs are defined by three intrinsic characters:

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must be able to colonize root, survive and multiply in the micro habits associated with the root surface in competition with other micro biota at least for the time needed to express their plant promotion and pro-tection activities. A wide group of free living soil bacteria is considered to be PGPR including sps. of *Pseudo-monas,Azospirillum, Azotobacter,Klebsiella, Enterobacter, Alcaligens, Arthrobacter, Burkholderia, Bacillus,* etc. (Kloepper et al., 1989; Glick, 1995). *Pseudomonas* and *Bacillus* sps. are the most popular exploited PGPR (Roopa et al., 2012; Shau and Sindhu, 2011). Soil borne fluorescent pseudomonas have received particular attention throughout the global science because of their catabolic versatility, excellent root colonizing ability and their capacity to produce wide range of enzymes and metabolites that favour the plant to withstand various biotic and abiotic stress conditions. However, it is neither a single genus or species of bacteria nor a single trait that augments plant growth promotion, and rather it is a consortium of bacteria that possess several PGP properties. There are several mechanisms by which different PGPR may promote growth of crop plants. They may synthesize various phytohormones such as indole-3-acetic acid (IAA), produce siderophores that can provide iron (Fe) to plants, solubilize minerals such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase that can modulate plant growth and development (Rashedul et al., 2009). A particular PGPR strain may enhance plant growth and development using anyone or more of these mechanisms. Variability in the performance of PGPR may be due to various environmental factors which influence their growth and effects on plant. Exploitation of PGPR under variable environmental factors in improving crop productivity has an important role in sustainable agriculture. Therefore, it is necessary to explore soil microbial diversity of PGPR having combination of PGP activities and well adapted to particular soil environment. So keeping in view the above constraints, the present study was designed to screen potential native *Pseudomonas* sp. for their multiple plant growth promoting (IAA, P-solubilization) activities from chickpea rhizosphere.

MATERIALS AND METHODS

Isolation of rhizobacteria

Reference strain of plant growth promoting rhizobacteria *Pseudomons diminuta* (LK884) was procured from GB Pant University of Agricultural and Technology Uttarkhand, India and was subcultured on King's B medium (King et al., 1954). Rhizospheric soil samples (1 kg) were collected from seven districts of chickpea growing area of Punjab, India after rice. A total of 35 isolates of rhizobacteria were isolated from 25 soil samples collected from different chickpea rhizospheric locations of Punjab on King's B medium (*Pseudomonas* sp.) and nutrient agar (*Bacillus* sp.).

Biochemical characterization of rhizobacteria

Selected isolates were biochemically characterized by Gram's reaction, oxidase, catalase, citrate utilization, blood agar, McConkey's lactose bile salt agar (MLA), Methyl red (MR), Voges-Proskauer (VP) and nitrate reduction test according to the standard procedure (Cappuccino and Sherman, 1992).

Characterization of rhizobacteria for PGP traits

Growth promotional activities

Production of indole acetic acid (IAA): IAA production in different *Pseudomonas* sp. isolated as PGPRs strains was detected according to Gordon and Weber (1951) method by inoculating the *Pseudomonas* sp. in 5 ml Luria Bertanni broth supplemented with 0.01% tryptophan separately and incubated for 3 days at 28±2°C. Appearance of pink colour after the addition of 4 ml of Salkowski's reagent to 2 ml of culture supernatant confirmed the production of IAA. Quantitative measurement of IAA was determined by recording absorbance at 535nm.

Seed germination assay: Four healthy seeds of chickpea were surface sterilized with 0.1% $HgCl_2$ for 3 min followed by treatment with 95% ethanol for 5 minand then successive washing with sterilized distilled water. The surface sterilized seeds were inoculated with broth cultures of different strains of PGPR grown in their respective medium for 24 h containing at least 10^6 cells/ml for 10 min (Saxena and Matta, 2005). Un-inoculated and inoculated seeds were germinated in 0.02% water agar at 28±2°C under controlled conditions with three replications. Germination of seeds was observed after 3 days.

Siderophore production: Siderophore production was detected according to Teintze et al. (1981) study. The bacterial cultures were streaked on the King's B medium with and without (50 mg/l) $FeCl_3$ and incubated at 28 ± 2°C for 48 h. Fluorescent pigment formed was considered as an indication of siderophore production. Further qualitative production of siderophore was tested using Chrome Azurol S (CAS) agar (Schwyn and Neilands, 1987). Presence of siderophore production was indicated by orange halos around the colony due to chelation of iron which bound to CAS dye.

Phosphate solubilisation

Qualitative measurement of phosphate solubilisation

Petri plates containing Pikovaskaya (Pikovaskaya, 1948) and NBRIP (National Botanical Research Institute's phosphate growth medium) (Nautiyal, 1999) media were inoculated with different *Pseudomonas* sp. Formation of halo and yellow zone around the bacterial zone on Pikovaskaya's and NBRIP media respectively indicated the qualitative phosphate solubilization activity of the micro-organism.

Quantitative measurement of phosphate solubilization

100 ml of Pikovaskaya's broth was dispensed in 250 ml conical flasks 100 mg P_2O_5 as tri-calcium phosphate (TCP) was added separately to each flask and the contents were sterilized at 121°C for 15 min. The flasks were inoculated with 1 ml suspension of overnight grown culture and incubated at 28±2°C for 15 days. Presence of yellow colour after addition of ammonium molybdate and ammonium vandate in equal ratio to culture supernatant confirmed phosphate solubilizing activity. The yellow colour intensity of the solution was measured at 420 nm after 25 minutes incubation for quantitative estimation of phosphate solubilisation (Jackson, 1973).

Stress tolerant activities

Production of ammonia

The different isolates of *Pseudomonas* sp. (PGPR) were grown in peptone water in tubes and were incubated at 30°C for 4 days. 1 ml Nessler's reagent was added in each tube. Tubes were observed for presence of a yellow to brownish colour for maximum production of ammonia (Cappuccino and Sherman, 1992).

Table 1. Location of rhizospheric soil samples.

District	Location of Rhizospheric soil
Bathinda	Bugar
	Phul
	Bhundari
Gurdaspur	Gurdaspur I
	Gurdaspur II
	Gurdaspur III
Jalandhar	Nava Pind
	Ughi
	Sukhpura
	Kudi kalan
Hoshiarpur	Patar kalan
	Sarowal
	Dhami kalan
	Barial
Ludhiana	Nangal kalan
	Talwandi Mallian
	Sheikh Daulat
	Handiayen
	P.A.U I
	P.A.U II
	Kotli
	Galib Khurad
Kapurthala	Kapurthala I
Barnala	Parvahi I
	Parvahi II

HCN production

Exponentially grown different *Pseudomonas* sp. (PGPR) strains were separately streaked on King's B medium supplemented with 4.4 g of glycine per litre with simultaneous supplementation of a filter paper soaked in 0.5% picric acid in 5% Na_2CO_3 in the upper lid of Petri dishes. The plates were incubated at 28±1ºC for 2to 3 days. Change in colour from yellow to light brown for moderate (brown) or strong (reddish-brown) indicated HCN production (Bakker and Schippers, 1987).

Catalase production

Pseudomonas cultures were grown in a nutrient agar medium for 18 to 24 h at 28 ± 1°C. The cultures were mixed with appropriate amount of 3% of hydrogen peroxide (H_2O_2) on a glass slide to observe the evolution of oxygen (Cappuccino and Sherman, 1992).

Intrinsic antibiotic spectra (IAR)

IAR test was carried out to identify the bacterial sensitivity or resistance to antibiotics. A plate of King's B was used by spreading an aliquot of bacterial culture evenly across the agar surface. Filter paper discs containing different concentration of antibiotics were placed on it. Plates were incubated at 28±2ºC for two days and zone of inhibition around the disc was recorded (Bauer et al., 1966). Each test was performed three times.

RESULTS AND DISCUSSION

A total of 35 isolates of rhizobacteria were isolated from 25 soil samples collected from different rhizospheric locations of Punjab (Table 1). On nutrient agar, 10 out of 35 isolates produced round shaped and raised colonies having smooth, shiny surface with smooth margin. The colonies of these 10 isolates were light yellow to off white, but all were odourless. Six of them produced a fluorescent green pigment when streaked on King's B medium. On the basis of cultural and morphological appearance, these were tentatively assigned belonging to genera *Pseudomonas.* These isolates were designated as PGPR 1, PGPR 2, PGPR 3, PGPR 4, PGPR 5, PGPR 6, PGPR 7, PGPR 8, PGPR 9 and PGPR 10 (Table 2).

Microscopic observations were performed to investigate some characteristics of PGPR isolates such as shape, gram's reaction and motility. All the isolates were rod shaped, motile and gram negative in reaction and evaluated in detail for their cultural (Figure 1), morphological and biochemical characteristics (Tables 2 and 3) (Cappuccino and Sherman, 1992).

These rhizobacterial isolates were characterised biochemically and were found to be positive for oxidase test (within 10 s), catalase, citrate utilization, McConkey's lactose bile salt agar and nitrate reduction but negative for Methyl Red and Voges-Proskauer tests. Out of 35 isolates, 28.6% belonged to *Pseudomonas* sp.

Similarly, Joseph et al. (2007) reported that the population of *Pseudomonas* spp. (1.1-2.1 X 10^6cfu/g of soil) dominated in chickpea rhizosphere. These results are also in close agreement where out of 121 isolates from mungbean rhizosphere, 65% were gram negative represented by *Pseudomonas, Bacillus, Enterobacter, Proteus* and *Klebsiella* (Gupta, 1995). Similarly, Cattlen et al. (1998) reported *Pseudomonas, Burkholderia, Bacillus* and *Alcaligens* as predominant genera in rhizosphere of soybean.

Plant growth promoting rhizobacteria were assayed for their ability to produce IAA in pure culture in the presence and absence of precursor L-tryptophan. In the absence of L-tryptophan, all the PGPR isolates produced very low amount of IAA which ranged between 1.00 to 4.91 µg/ml (Figure 2). In the presence of L-tryptophan, the concentration of IAA produced by the rhizobacterial isolates

Table 2. Morphological characteristics of *Pseudomonas* sp. (PGPR) on nutrient agar and King's B media.

Isolate	Shape	Elevation	Surface	Margin	Color	Odour	Pigmentation on King's B
PGPR 1	Round	Raised	Smooth Shiny	Smooth	light yellow	Odourless	Fluorescent green
PGPR 2	Round	Raised	Smooth Shiny	Smooth	light yellow	Odourless	Fluorescent green
PGPR 3	Round	Raised	Smooth Shiny	Smooth	light yellow	Odourless	Fluorescent green
PGPR 4	Round	Raised	Smooth Shiny	Smooth	light yellow	Odourless	Fluorescent green
PGPR 5	Round	Raised	Smooth Shiny	Smooth	Off white	Odourless	-
PGPR 6	Round	Raised	Smooth Shiny	Smooth	light yellow	Odourless	Fluorescent green
PGPR 7	Round	Raised	Smooth Shiny	Smooth	Off white	Odourless	-
PGPR 8	Round	Raised	Smooth Shiny	Smooth	Off white	Odourless	-
PGPR 9	Round	Raised	Smooth Shiny	Smooth	Off white	Odourless	-
PGPR 10	Round	Raised	Smooth Shiny	Smooth	Off white	Odourless	-

Figure 1. Cultural and biochemical characteristics of *Pseudomonas* sp. on Nutrient agar.

ranged between 30.58 to 70.05 µg/ml. Out of the 10 rhizobacterial isolates, PGPR 2 and PGPR 3 were able to produce high amount of IAA that is 66.79 and 70.05 µg/ml respectively as compared to reference *Pseudomonas* sp. LK884 (61.58 µg/ml of IAA) in the presence of L-tryptophan. Similar variation in IAA production by *Bacillus* and *Pseudomonas* sps. was also reported by Minaxi et al. (2011), Kumar et al. (2012) and Jangu and Sindhu (2012). In the present studies, production of IAA was in agreement with the study of Yasmin et al. (2009) which reported higher IAA production in the presence of precursor L-tryptophan and t significant difference in the concentration of IAA produced among the isolates (4.97 to 46.66 mg/l).

Joseph et al. (2007) showed the highest IAA production in all isolates of *Bacillus, Pseudomonas* and *Azotobacter* (100%) followed by *Rhizobium* (85.7%). Ashrafuzzaman et al. (2009) reported that the IAA production was also influenced by cultural conditions, growth stage and substrate availability.

Chickpea seeds bacterized with *Pseudomonas* sp. PGPR 2 showed a significant increase in percentage seed germination (89.7%) followed by PGPR 3 (88.9%) and LK 884 (86.4%) in *desi* variety PBG1 (Table 4) whereas, seed germination percentage was maximum in *kabuli* variety, BG 1053 when inoculated with reference culture LK884 (89 %) followed by PGPR 3 (85%) and PGPR 2 (84%). In the control treatment, seed germination was 81.6% in *desi*

Table 3. Cultural and biochemical characteristics of *Pseudomonas* sp. (PGPR) isolates.

Characteristic	*Pseudomonas*
Cell shape	Rod
Motility	Motile
Gram reaction	Gram negative
Oxidase	+ (with in 10 sec)
Catalase	+
Citrate utilization	+
Methy red (MR)	-
Voges Proskauer (VP)	-
McConkey's Lactose Bile Salt Agar	+
Nitrate reduction (NR)	+

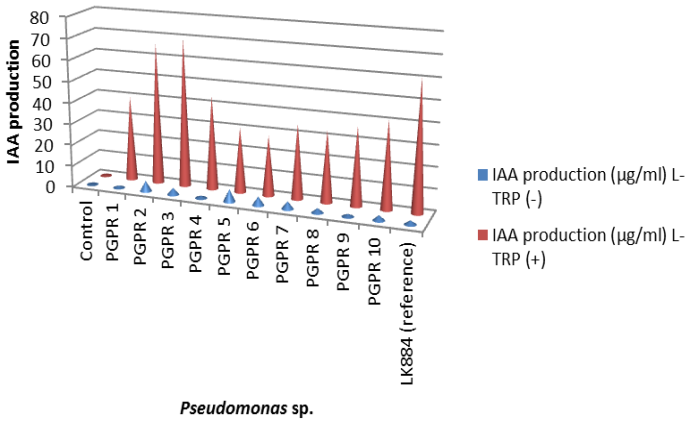

Figure 2. Quantitative measurement of IAA production by different *Pseudomonas* sp. (PGPR) in presence and absence of L-tryptophan.

Table 4. Effect of different *Pseudomonas* sp. (PGPR) on seed germination in chickpea.

Isolate	Seed germination (%)	
	Variety	
	***desi* PBG1**	***kabuli* BG1053**
Control	81.6	82.0
PGPR 1	80.4	81.0
PGPR 2	89.7	84.0
PGPR 3	88.9	85.0
PGPR 4	82.6	80.5
PGPR 5	80.9	83.1
PGPR 6	79.4	80.4
PGPR 7	83.2	82.6
PGPR 8	81.0	83.1
PGPR 9	82.4	80.4
PGPR 10	82.0	81.1
LK884 (reference)	86.4	89.0

PBG1 and 82% in *kabuli* BG1053. 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 over the control. 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 enhance growth and seed emergence in peanut. Two isolates, PGPR 2 and PGPR 3 were able to produce siderophores as shown by fluorescent pigment on King's B medium without $FeCl_3$ (Table 5) with orange zone formation after incubation for 48 h. The diameter of orange zone was 1.8 and 2.2 cm for PGPR 2 and PGPR 3 respectively (Figure 3).

These findings are in close corroboration with those of Yasmin et al. (2009) which showed that *Serratia* UPMSP3, *Pseudomonas* UPMSP13 and *Pseudomonas* UPMSP20 produced fluorescent pigment on King's B medium indicating the presence of siderophore. Buysens et al. (1996) also reported that *Pseudomonas* sp. are known to produce pyoverdines and pseudobactins, which can be detected by their yellow-green fluorescence under ultraviolet light when grown on iron deficient medium. Several workers also agreed with our results where siderophores production has been reported by *Pseudomonas* sp. in chickpea (Akhthar and Siddiqui, 2009; Verma et al., 2010) and wheat (Silini et al., 2012). Siderophores are known to chelates with iron and other metals and contribute to disease resistance by limiting the supply of essential trace minerals in natural habitats. Siderophores may directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to bacteria (Sayyed et al., 2005) and can play an important role in systematic host resistance. 70% of total isolates showed P-solubilization and resulted into formation of halo and yellow zone on Pikovaskaya's (Figure 4a) and NBRIP (Figure 4b) respectively (Table 5). Efficiency of the seven isolates in solubilizing tri-calcium phosphate (TCP) in liquid medium as a function of time was further investigated at different intervals (3,6,9,12 and 15 days). It was seen that increasing amount of P was released by different isolates with increasing period of incubation up to the 12^{th} day. The phosphate solubilizing activity was observed up to the 15^{th} day. These isolates showed maximum phosphate solubilization at the 12^{th} day which ranged between 5.08 to 13.45 mg/100 ml. Maximum phosphate was solubilized by PGPR 3 (13.45 mg/100 ml) followed by PGPR 2 (13.15 mg/100ml) whereas reference *Pseudomonas* sp. LK884 was able to solubilize 6.40 mg/100ml of phosphorus at 12^{th} day (Table 6). After 12 days, there was decline in phosphate solubilizing activity which might be due to deficiency of nutrients in the culture media.

This investigation was found coherent to the result of Yasmin et al. (2009) who reported that 6 out of 15 rhizobacteria belonging to genera *Pseudomonas, Bacillus,*

Table 5. Qualitative screening of phosphate solubilization and siderophore production by *Pseudomonas* sp. (PGPR).

***Pseudomonas* sp.**	**Phosphate solubilisation**	**Siderophore production**
PGPR 1	+	-
PGPR 2	+	-
PGPR 3	+	+(1.8 cm)
PGPR 4	+	+ (2.2 cm)
PGPR 5	+	-
PGPR 6	+	-
PGPR 7	+	-
PGPR 8	-	-
PGPR 9	-	-
PGPR 10	-	-
LK884 (reference)	+	-

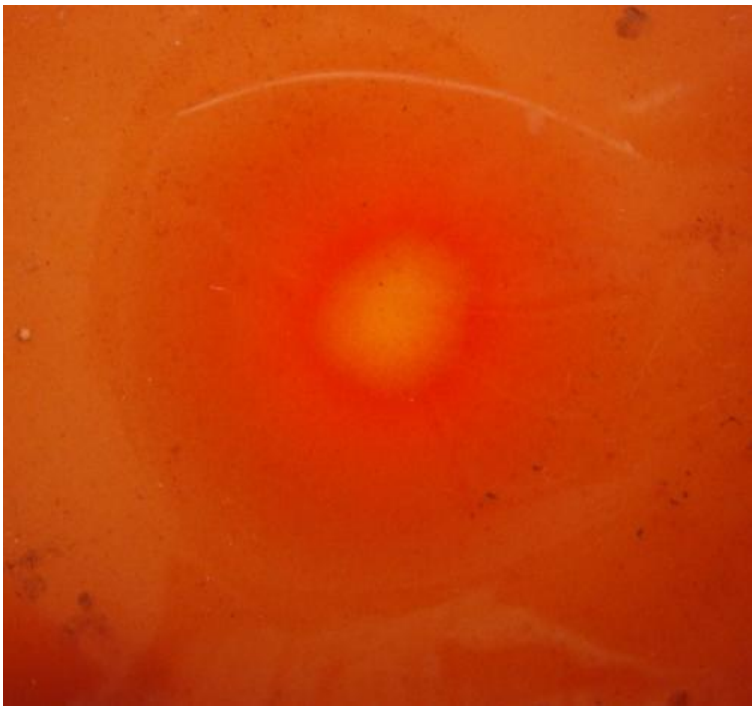

Figure 3. Siderophore production by PGPR (*Pseudomonas* sp.): orange zone on CAS agar.

Azospirillum and *Enterobacter* isolated from sweet potato rhizosphere solubilized calcium phosphate in liquid medium and also produced clear zones ranging from 0.86 cm (*Pseudomonas* UPMSP20) to 2.03 cm (*Erwinia* UPMSP10) on Potato dextrose yeast extract agar (PDYA) - calcium phosphate plates. Poonguzhali et al. (2008) observed that solubilization of TCP in liquid medium by *Pseudomonas* spp. varied in the range of 24.7 to 44.0 mg/100 ml. Phosphate solubilization by rhizobacterial isolates has been shown to be related to the production of organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids (Silni 2012). In tropical soil, the low pH influences solubilization of phosphate by rhizobacteria (Yasmin et al., 2009). Quantitatively, out of 35 isolates, 20% of strains exhibited P-solubilization. Relatively low number of P-solubilizers among the tested strains is not surprising as Rashedul et al. (2009) and Hameeda et al. (2008) also reported similar results; 23.5 and 2.41% of phosphate solubilizers in rice and maize plants respectively. Verma et al. (2010) reported phosphate solubilization by PGPR spp. from chickpea rhizosphere whereas largest halo zone produced by *Pseudo-*

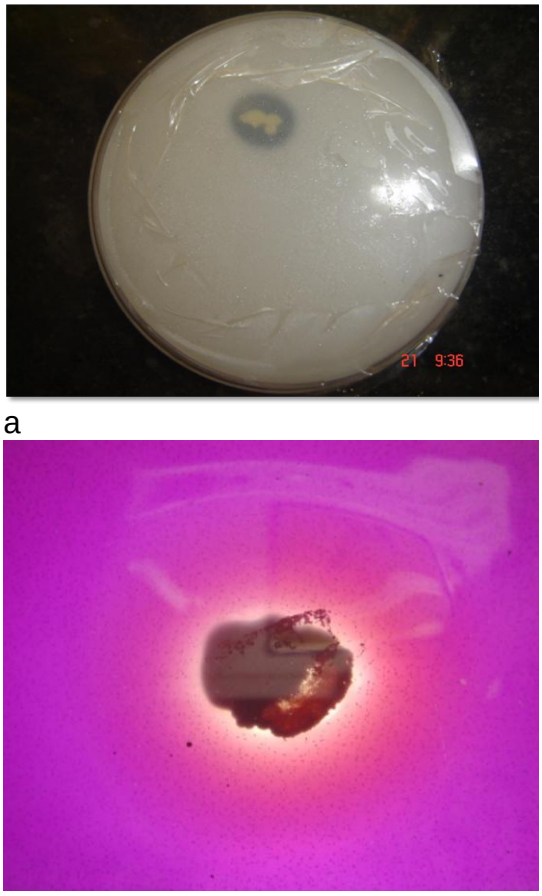

Figure 4. Phosphate solubilization by PGPR (*Pseudomonas* sp.). **(a)** Halo zone on Pikovaskaya's medium. **(b)** Yellow halo zone on NBRIP medium

Table 6. Quantitative measurement of phosphate solubilization by *Pseudomonas* sp. (PGPR) in Pikovaskaya's medium as a function of time.

***Pseudomonas* sp.**	**P-solubilization (mg/100ml)**				
	Incubation period (days)				
	3rd	**6th**	**9th**	**12th**	**15th**
PGPR 1	2.45	3.89	4.86	5.67	4.39
PGPR 2	7.45	10.70	10.95	13.15	5.25
PGPR 3	8.75	9.95	12.35	13.45	2.20
PGPR 4	3.21	4.59	5.89	5.95	2.50
PGPR 5	2.18	3.54	5.12	5.10	3.91
PGPR 6	3.95	4.17	4.98	5.08	2.85
PGPR 7	2.89	3.90	4.01	5.65	3.63
LK884 (reference)	3.35	5.25	5.85	6.40	4.15

monas sp. was documented by Minaxi et al. (2011) in cowpea rhizosphere.

Out of the 10 isolates, only four isolates of *Pseudomonas sp* were found positive for HCN production (Figure 5 a and b). PGPR 2 and PGPR 3 were potent HCN producers followed by PGPR 4 and PGPR 7 (Table 7) whereas reference *Pseudomonas sp* LK884 was not able to produce HCN. These results are in close agreement as reported by Siddiqui and Shakeel (2009) for HCN production by 21 *Pseudomonas* isolates from pigeonpea rhizosphere, out of which 11 were moderate producers but three of them were potent producers of HCN. Bakker and Schippers (1987) also observed that nearly 50% of the pseudomonads from potato and wheat rhizospheres produce HCN which has a primary mechanism in suppression of root fungal pathogens. Selvakumar et al. (2009) also gave evidence in support of our results where *Pseudomonas fragi* CS11RH1, a psychotolerant bacterium produced HCN and seed bacterization with the isolate significantly increased the percent germination and rate of germination, plant biomass and nutrient uptake of wheat seedlings. Another volatile compound produced by rhizobacteria is ammonia which also plays an important role in biocontrol activity of PGPRs. Production of ammonia was indicated by the development of deep yellow to brown colour after the addition of Nessler's reagent to inoculated peptone water. Out of the ten isolates tested, PGPR 2 and PGPR 3 were found to be ammonia producers (Table 7). These results are in close agreement with those of Joseph et al. (2007) who revealed the production of ammonia commonly detected in the isolates of *Bacillus* (95%) followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45%). Similarly, Chacko et al. (2009) isolated the *Pseudomonas putida* from the rhizosphere of *Pisum sativum*. The organism exhibited a battery of PGPR characteristics and was also found positive for the production of ammonia. Howell et al. (1988) described the role of ammonia in antagonism. Pavlica et al. (1978) concluded that ammonia is the only gas present in sufficient concentration in soil to inhibit soil fungus. Catalase activity was detected in strains of *Pseudomonas* which may be potentially very advantageous. *Pseudomonas* isolates with catalase activity must be highly resistant to environmental, mechanical and chemical stresses (Joseph et al., 2007).

Ten rhizobacterial isolates were tested for their reactivity to antibiotics along with reference strain LK884. The data in Table 8 shows that of 30% of the rhizobacterial isolates, MR and LK884 were resistant to tetracycline. All the isolates and MR showed resistance against ampicillin whereas LK884 was sensitive to this antibiotic. Isolates showed 30, 70, 50, 30, 40 and 50% resistance to kanamycin, erythromycin, chloramphenicol, gentamycin, amoxycillin and streptomycin, respectively.

The data was supported by Siddiqui et al. (2006) results where *Pseudomonas* sp. were resistant to ampicillin. Kundu et al. (2009) in their studies found that the isolates belong to genera *Pseudomonas* from chickpea; wheat and mustard rhizosphere were resistant to ampicillin. They also reported that these isolates were resistant to tetracycline and kanamycin. Yasmin et al. (2009) revealed that six rhizo-

Table 7. HCN, NH_3 and catalase production by *Pseudomonas* sp. (PGPR).

HCN production	NH_3 production	Catalase production
-	-	+
-	-	+
+	+	+
+	+	+
+	-	+
-	-	+
-	-	+
+	-	+
-	-	+
-	-	+
-	-	+
-	-	+

Table 8. Intrinsic antibiotic spectra of *Pseudomonas* sp. (PGPR).

Isolate	Tetracycline (30 µg/disc)	Amphicillin (10 µg/disc)	Kanamycin (30 µg/disc)	Erythromycin (15 µg/disc)	Chloramphenicol (25 µg/disc)	Gentamycin (10 µg/disc)	Amoxycillin (10 µg/disc)	Streptomycin (25 µg/disc)
PGPR 1	S	R	S	R	R	S	S	S
PGPR 2	R	R	S	R	S	S	S	R
PGPR 3	R	R	R	R	S	S	S	R
PGPR 4	S	R	S	S	R	R	S	S
PGPR 5	S	R	S	S	R	S	R	R
PGPR 6	S	R	R	R	S	R	S	S
PGPR 7	S	R	S	R	S	R	R	S
PGPR 8	S	R	S	R	S	S	R	S
PGPR 9	S	R	S	R	R	S	R	R
PGPR 10	R	R	R	S	R	S	S	R
LK844 (reference)	R	S	S	R	R	S	R	S

R, Resistant; S, sensitive.

bacterial isolates were intrinsically resistant to the antibiotics tested. Similar results were reported by Jangu and Sindhu, (2011) where *Pseudomonas* strain MPS90 showed resistance to chloramphenicol, ampicillin and streptomycin but were sensitive to kanamycin from the rhizosphere of green gram and urdbean. Resistance of PGPR to several antibiotics might have an ecological advantage of survival in the rhizosphere when they are introduced as inoculum.

This study showed PGPR-2 and PGPR-3 strains of *Pseudomonas* sp. with multiple PGP activities (maximum IAA production, phosphate solubilisation and seed germination) and stress tolerant activities (HCN, siderophore, catalase production and antibiotic resistance). In future, consortium of PGPR with *Mesorhizobium* sp*. cicer* can be developed for improvement in symbiotic nitrogen fixation and yield in chickpea. Therefore, these native isolates can be explored as potent bio-fertilizers for sustainable agriculture with *Mesorhizobium* sp*. cicer* in chickpea. However, any practical application of these results should be preceded by further evaluation under field conditions. Besides exploring the potential PGPR with PGP functions,

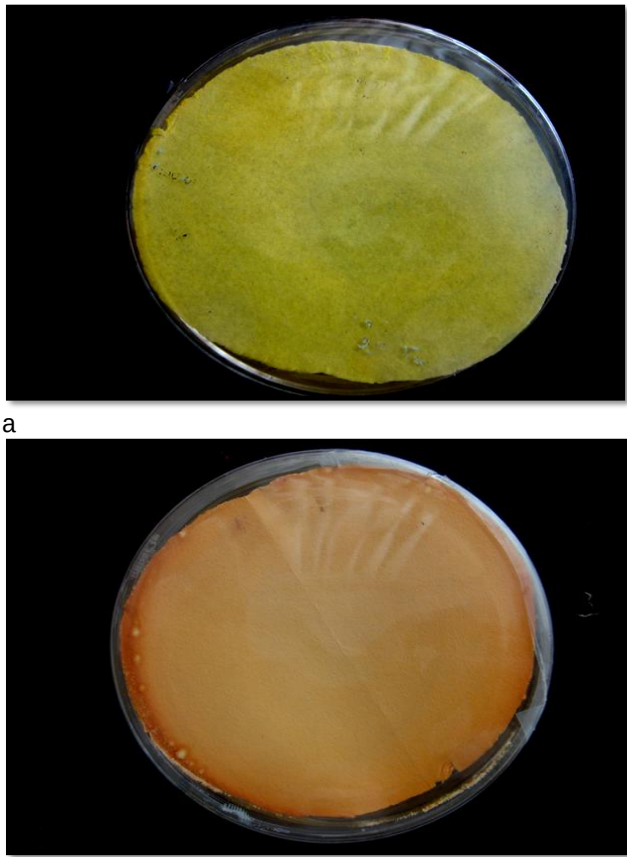

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Figure 5. HCN production by PGPR (*Pseudomonas* sp.). **(a)** Uninoculated. **(b)** Inoculated.

it is also important that bacteria are well adapted to environmental conditions before they are utilized as inoculant strains. Selection of useful PGP traits of PGPR will pave the way for minimizing the use of hazardous chemical fertilizers and pesticide for sustainable agriculture.

REFERENCES

Akhthar MS, Siddiqui ZA (2009). Use of plant growth promoting rhizobacteria for the biocontrol of root-rot disease complex of chickpea. Aust. Pl. Pathol. 38:44-50.

Ashrafuzzaman M, Hossen FA, Ismail MR, Hoque Md A, Islam MZ, Shahidullah SM, Mcon S (2009). Efficiency of plant growth promoting rhizobacteria (PGPR) for the enhancement of rice growth. Afr. J. Biotechnol. 8:1247-1252.

Bakker AW, Schippers B (1987). Microbial cyanide production in the rhizosphere in relation to potato yield and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biol. Biochem. 19:451-457.

Bauer AW, Kirby WM, Sherris, JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. American. J. Clin. Pathol. 45:493-496.

Buysen S, Heungens K, Poppe J, Hofte M (1996). Involvement of pyochelin and pyoverdin in suppression of Pythium-induced damping-off of tomato by *Pseudomonas aeruginosa* 7 NSK2, Appl. Environ. Microbiol, 62:865-871.

Cappuccino JC, Sherman N (1992). In: Microbiology: A Laboratory Manual, New York, pp. 125-179.

Cattlen AJ, Hartel PG, Fuhrmann JJ (1998). Bacterial composition in the rhizosphere of nodulating and non-nodulating soybean. Soil Sci. Soc. Am. J. 62:1549-1555.

Chacko S, Ramteke PW, John SA (2009). Amidase from plant growth promoting rhizobacterium. J. Bact. Res. 1: 46-50.

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004). Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol. Res. 159:371-394.

Glick BR (1995). The enhancement of plant growth by free living bacteria. Can. J. Microbiol. 41:109-114.

Gordon SA, Weber RP (1951). Calorimetric estimation of Indole acetic acid. Plant Physiol. 26:192-195.

Gulati SL, Mishra SK, Gulati N, Tyagi MC (2001). Effect of inoculation of plant growth promoting rhizobacteria on cowpea. Ind. J. Microbiol. 41:223-224.

Gupta A (1995). Associative effects of plant growth promoting rhizo-bacteria on mungbean-*Bradyrhizobium* symbiosis. Ph.D. dissertation. Indian Agricultural Research Institute, New Delhi, India.

Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G (2008). Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. Microbiol. Res. 163:234-242.

Howell RC, Beier RC, Stipanociv RD (1988). Production of ammonia by *Enterobacter cloacae* and possible role in biologicalcontrol of *Pythium* pre-emergence damping-off by bacteria. Phytopatholog*y.* 78:1075-1078.

Jackson ML (1973). Phosphorous determination for soils. In: *Soil Chemical Analysis.* Prentica Hall of India Pvt. Ltd, New Delhi India. pp 134-182.

Jangu OP, Sindhu SS (2011). Differential response of inoculation with indole acetic acid producing *Pseudomonas* sp. in green gram (*Vigna radiata* L.)and black gram(*Vigna mungo* L.). Microbiol J. 1:159-173.

Joseph B, Patra RR, Lawrence R (2007). Characterization of plant growth promoting rhizobaacteria associated with chickpea (*Cicer arientinum L.). Int. J. Plant Production. 2:141-152.*

King EO, Ward MK, Raney DE (1954). Two simple media for the demonstration of pyocyanin and fluorecein. J. Lab. Clin. Med. 44:301-307.

Kloepper JW, Lifshitz R, Zablotowicz RM (1989). Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. 7:39-43.

Kumar A, Devi S, Payal C , Negi S (2012). Isolation ,screening and characterization of bacteria from rhizospheric soils for different plant growth promotion (PGP) activites :*in vitro* study. Recent Res Sci Technol. 4(1): 1-5.

Kundu BS, Nehra K, Yadav R, Tomar M (2009). Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. Ind. J. Microbiol. 49:120-127.

Minaxi NL, Yadav RC, Saxena J (2011). Characterization of multi-faceted *Bacillus* sp. RM-2 for its use as plant growth promoting bi-oinoculant for crops grown in semi arid deserts. J. Appl. Soil Ecol. 59: 124-135.

Nautiyal CS (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett. 170:265–270.

Pavlica DA, Hora TI, Bradshaw JJ, Skogerboe RK, Baker R (1978). Volatiles from soil influencing activities of soil fungi. Phytopathology. 68:758-765.

Pikovaskaya RI (1948). Mobilization of phosphate dissolving bacteria in rhizosphere of some cultivated legumes. Pl Soil. 35:127-132.

Poonguzhali S, Madhaiyan M, Sa TM (2008). Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and P-utilization of plants. J. Microbiol. Biotechnol. 18:773-777.

Rashedul Md I, Madhaiyan M, Boruah HPD, Yim W, Lee G, Saravanam VS, Fu Q, Hu H, Sa T (2009). Characterization of plant growth-promoting traits of free-living diazotrophic bacteria sand their inoculation effects on growth and nitrogen uptake of crop plants. J. Microbiol. Biotechnol. 19:1213-1222.

Roopa B Maya C, Makar HK (2012). Effect of different PGPR strains along with *Rhizobium* on nodulation and chickpea productivity. Asian J. Exp. Biol. Sci.3: 424-426.

Sahu GK, Sindhu SS (2011). Disease control and plant growth promo-

tion of greengram by siderophores producing *Pseudomona*s sp. Res. J. Microbiol. 6:735-749.
Saxena MAK and Matta NK (2005). Selection and cultivable PGPR from diverse pool of bacteria inhabiting pigeonpea rhizossphere. Indian J. Microbiol. 45(1):21-26.
Sayyed R, Badgujar MD, Sonawane HM, Mhaske MM, Chincholkar SB (2005). Production of microbial iron chelators (siderophores) by fluorescent pseudomonads. Ind. J. Biotechnol. 4:484-490.
Schwyn B, Neilands JB (1987). Universal chemical assay for detection and determination of siderophores. Anal. Biochem. 160:47-56.
Silini C, Silini A, Ghoul M, Yadav S (2012). Isolation and characterization of plant growth promoting traits of rhizobacteria: *Pantoea agglomerans* Ima2. Pak. J. Boil. Sci. 15 :267-276.
Siddiqui IA, Shaukat SS, Sheikh IH, Khan A (2006). Role of cyanide by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. World J. Microbiol. Biotechnol. 22:641-650.
Siddiqui ZA, Shakeel U (2009). Biocontrol of wilt disease complex of pigeon pea (*Cajanus cajan* L.) by isolates of *Pseudomonas* spp. Afr. J. Pl. Sci. 3:1-12.
Selvakumar G, Josh P, Nazim S, Mishra PK, Bisht JK, Gupta HS (2009). Phosphate solubilization and growth promotion by *Pseudomonas fragi* CS11RH1(MTCC8984), a psychotolerant bacterium isolated from a high altitude Himalayan rhizosphere.J. Biol.64:239-245.
Teintze M, Hossain MB, Baines CL, Leong J, Van der Helm D (1981). Structure of ferric pseudobactin, a siderophore from a plant growth promoting *Pseudomonas*. Biochem. 20: 6446-6457.
Verma JP,Yadav J, Tiwari KN (2010). Application of Rhizobium sp. BHURC01 and plant growth promoting rhizobacteria on nodulation, plant biomass and yield of chickpea(Cicer arietinum L.). Inter. J. Agri. Res. 5:148-156.
Yasmin F, Othman R, Sijam K, Saad MS (2009). Characterization of beneficial properties of plant growth promoting rhizobacteria isolated from sweet potato rhizosphere. Afr. J. Microbiol. Res. 3:815-821.