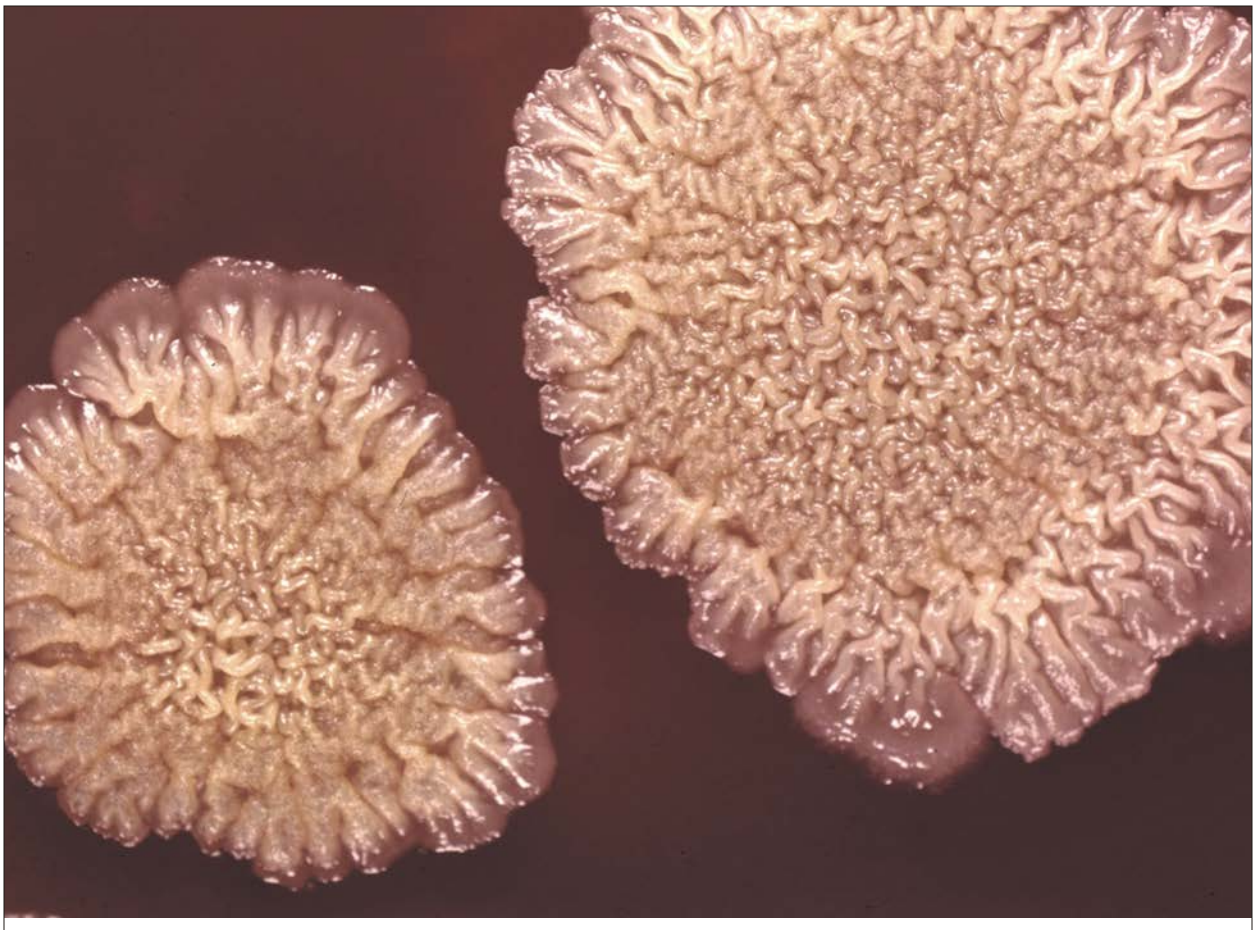

Bacteriology

Journal

ISSN 2153-0211

Academic
Journals Inc.

www.academicjournals.com

Bacteriology Journal 5 (1): 13-24, 2015
ISSN 2153-0211 / DOI: 10.3923/bj.2015.13.24

Isolation of Fluorescent *Pseudomonas* spp. from Diverse Agro-Ecosystems of India and Characterization of their PGPR Traits

[1]G. Praveen Kumar, [1]Suseelendra Desai, [1]E. Leo Daniel Amalraj and [2]Gopal Reddy
[1]Laboratory of Plant Pathology, Division of Crop Sciences, Central Research Institute for Dryland Agriculture (ICAR), Santoshnagar, Saidabad Post, Hyderabad, 500 059, India
[2]Department of Microbiology, Osmania University, Hyderabad, 500 007, India

Corresponding Author: G. Praveen Kumar, Laboratory of Plant Pathology, Division of Crop Sciences, Central Research Institute for Dryland Agriculture (ICAR), Santoshnagar, Saidabad Post, Hyderabad, 500 059, India

ABSTRACT

Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface. Among the Gram-negative soil bacteria, *Pseudomonas* is the most abundant genus in the rhizosphere and the PGPR activity of some of these strains has been known for many years. From diverse agro-ecosystems of India, 75 strain of fluorescent *Pseudomonas* spp. were isolated and all of them were tested for various PGPR traits like phytohormones production, HCN, EPS, siderophores and extracellular enzymes like cellulases, proteases, ureases, chitosanases and/or chitinases. Results showed that 50% of the isolates produced phytohormones, 31% isolates produced exopolysaccharides, 29% isolates produced ammonia, 16% were positive for hydrogen cyanide, 20% produced siderophores, 5% exhibited cellulases activity, 31% produce protease, 15% produced urease, 76% showed chitosanase and 59% isolates showed chitinase activity. P17 strain isolated from Bari Brahmana soil of Jammu and Kashmir state, among the tested isolates is marked as a candidate strain, having a potential with plethora of PGPR features along with CCME activity would prove and open-up options to use this strain as a biological agent for eco-friendly organic agriculture.

Key words: *Pseudomonas* sp. P17, PGPR traits, plant growth promotion, rhizosphere

INTRODUCTION

The rhizosphere, is represented as a thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria (Villacieros *et al*., 2003) known as Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper *et al*., 1980). Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface (Rangarajan *et al*., 2003). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg, 2001; Moeinzadeh *et al*., 2010).

PGPR can affect plant growth by different direct and indirect mechanisms (Glick, 1995; Gupta *et al*., 2000) which, increased mineral nutrient solubilization, nitrogen fixation, making nutrients available for the plant, repression of soil borne pathogens (by the production of hydrogen cyanide, siderophores, antibiotics, enzymes like chitinase and/or competition for nutrients), improving plant tolerance to drought, salinity and metal toxicity and production of phytohormones such as indole-3-acetic acid (IAA) (Gupta *et al*., 2000). Agriculturally Important Microorganisms (AIMs) are used in a variety of agro-ecosystems both under natural conditions and artificial inoculation for diverse applications such as augmenting nutrient supply, bio-control,

bio-remediation and rehabilitation of degraded lands (Vessey, 2003). A wide range of microorganisms are utilized in crop production and crop protection. They comprise nitrogen fixers like *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azotobacter*, *Azospirillum*, phosphate solubilisers like *Bacillus*, *Pseudomonas*, *Aspergillus* and Arbuscular Mycorrhizae (AM) like fungi, bacteria, viruses and nematodes used for pest and disease management in agriculture, horticulture and forestry.

Fluorescent pseudomonads have been predominantly recovered from the rhizoplane and rhizosphere of not only crop species but also from wood tree seedlings and fruit trees. Among the Gram-negative soil bacteria, *Pseudomonas* is the most abundant genus in the rhizosphere and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved (Patten and Glick, 2002; Garcia *et al*., 2004). *Pseudomonas* strains show high versatility in their metabolic capacity. Antibiotics, siderophores or hydrogen cyanide are among the metabolites generally released by these strains (Charest *et al*., 2005). These metabolites strongly affect the environment, because they inhibit growth of other deleterious microorganisms and they increase nutrient availability for the plant.

The use of multiple PGPR traits possessing *Pseudomonas* strains as bio-products in agro-ecosystems would benefit end-users in a significant way due to the synergistic effect of all the PGPR traits. Therefore, in view of this a study was carried out by isolating diverse fluorescent *Pseudomonas* isolates from various agro-ecosystems of India and preliminary work done to assess the presence of different PGPR traits in them and to draw a conclusion on the predominance of different traits in these strains.

MATERIALS AND METHODS

Bacterial cultures: Seventy five soil samples of different crops representing 31 locations from 13 states of India were used for isolation of fluorescent *Pseudomonas* spp. (Kumar *et al*., 2012b) using King's B medium (King *et al*., 1954).

Evaluation of PGPR traits: All the 75 *Pseudomonas* isolates were qualitatively and quantitatively characterized by standard protocols for the presence of PGPR traits which are known to play an essential role in growth promotion of plants.

Detection and estimation of indole acetic acid: IAA production in the culture medium of *Pseudomonas* isolates was detected following the method described by Bric *et al*. (1991). The quantitative analysis of IAA was carried out as per the modified method of Loper and Schroth (1986). Bacterial cultures were grown for 48 h at 28±2°C in mineral salts medium amended with 1% L-tryptophan. After their growth, cultures were centrifuged at 5000 rpm for 15 min. The clear supernatant (2 mL) was mixed with few drops of orthophosphoric acid and 2 mL of Salkowski reagent. Generation of pink colour indicates presence of IAA. The optical density of the colour produced was recorded at 530 nm using a UV-Visible spectrophotometer (Elico, India) concentration of the IAA produced in culture broth was estimated.

Estimation of gibberellic acid: Gibberellic acid was quantified by using the method of Holbrook *et al*. (1961) with minor modifications. Bacterial cultures were in King's B broth medium and centrifuged after growth as described above to separate the cells. The pellet was used for protein estimation. The supernatants pH was adjusted to 2.5 using 0.1 M HCl and using equal volume of ethyl acetate. The extraction of gibberellic acid was done into organic phase twice. To 1.5 mL of extract, 0.2 mL of potassium ferrocyanide was added and centrifuged at 1000 rpm for

10 min. To the supernatant an equal volume of 30% HCl was added and mixture was incubated at 20°C for 75 min. 5% HCl was used as blank and absorbance was measured in a UV-Visible spectrophotometer (Elico, India) at 254 nm. The concentration of gibberellic acid was deduced using a standard graph and the quantity was expressed as milligram gibberellic acid per milligram protein.

Estimation of exo-polysaccharides: *Pseudomonas* isolates were grown in 50 mL of tryptone soy broth in 150 mL conical flask, incubated at 28±2°C, 120 rpm in an incubator shaker (Orbitek, India) for 48 h. Cells were separated by centrifugation at 10,000 rpm for 10 min. Pellet was dissolved in 0.4% KCl and re-centrifuged at 5,000 rpm for 10 min. To 1 mL of supernatant 3 mL absolute ethanol was added and incubated overnight at 4°C in a refrigerator. The solution was centrifuged at 15,000 rpm for 20 min. Pellet obtained was washed with sterile distilled water followed by centrifugation at 10,000 rpm for 15 min. Pellet was dissolved in 0.5 mL sterile distilled water. One hundred milliliter of this sample was used for the total EPS estimation by Anthrone method using glucose as a standard (Robert and William, 1960).

Hydrogen cyanide detection: King's B medium amended with 0.44% of L-Glycine was used for detection and quantification of hydrogen cyanide production following the method of Bakker and Schippers (1987). Whatman No. 1 filter paper strips soaked in a 0.5% picric acid+2% Na_2CO_3 solution. Test tubes with the liquid media were inoculated with 100 µL of bacterial inoculum. The tubes were sealed with cotton plugs and parafilm and incubated at 28±2°C for 48 h. HCN production was indicated by change in colour of the filter paper from yellow to light brown or reddish brown. The total cyanides (in ppm) content in the filter paper were estimated by using the following equation.

$$\text{Total cyanides contents (ppm)} = 396 \times A_{510}\ \text{nm}$$

Siderophores detection and quantification: Siderophore production by *Pseudomonas* isolates was detected by observing orange halos production around the bacterial colony on CAS agar plates (Schwyn and Neilands, 1987) after 72 h of growth. For quantification of siderophores, 0.5 mL of cell free culture supernatant grown in liquid CAS medium 0.5 mL of CAS reagent was added and absorbance was measured at 630 nm against a blank. Siderophores contents were expressed as percentage siderophore units using the formula:

$$\text{Siderophore units}\,(\%) = \frac{Ar - As}{Ar} \times 100$$

Where:
Ar = Absorbance of reference at 630 nm (CAS reagent)
As = Absorbance of sample at 630 nm

Detection of cellulase, protease and chitosanase and/or chitinases: Test for the detection of cellulase and protease was carried out by amending mineral salts medium with 1% carboxy methyl cellulose as sole carbon source (Booth, 1971) and nutrient agar amended with 1% skimmed milk powder for protease, respectively. Bacterial cultures grown for 24 h were spot inoculated on to media in plates and incubated for 48 h for detection. Isolates that produced a clearing zone around the colony were considered positive for protease. The media plates were spot-inoculated

with 5 µL actively growing culture and incubated at 28±2°C for 48 h. The detection of cellulases production was analyzed by staining with 0.1% congo red solution for 10 min, excess dye solution was removed by 1 M NaCl. To stabilize the colour, 1 M acetic acid was flooded over the plates for 10 min. Presence of clear zone around the colony was considered as a positive result for cellulase production.

For detecting chitinases and/or chitosanases in test strains the following method was adopted. Substrate (chitosan of DA 1.6, 11, 35 and 56%) was prepared by dissolving 1 mg in 1 mL of 100 mM glacial acetic acid and incubated overnight by shaking. Freeze dried crude protein samples were dissolved in 50 mM sodium acetate buffer (pH 5.2) at a final concentration of 10 mg mL^{-1} and were inoculated as 3 µL spots onto the prepared gels. The gels were incubated at 37°C overnight in a moist chamber. After incubation, the gels were stained with calcofluor white for 5 min and washed with water for 20 min twice and observed in a gel documentation system under ultra violet light for chitinase activity.

Detection of ammonia production: Isolates under test were checked for ammonia production, after their growth in test tubes containing peptone water medium (10.0 g peptone; 5.0 g NaCl; 1000 mL distilled water; 7.0 pH (Dye, 1962). The tubes were inoculated with 100 µL of 24 h grown cultures and incubated at 28±2°C for 72 h. The accumulation of ammonia was detected by addition of 0.5 mL of Nessler's reagent to each tube. A faint yellow colour indicated a small amount of ammonia (+) and deep yellow to brownish colour indicated high production of ammonia (++).

RESULTS AND DISCUSSION

Isolation of fluorescent *Pseudomonas* spp.: *Pseudomonas* spp. isolated from different soil samples showing fluorescent production on King's B medium were selected and purified. The seventy-five fluorescent *Pseudomonas* spp. were isolated from soil samples obtained from 31 different locations representing 13 states of India (Fig. 1). Isolates were designated as P1-P75 and added to the culture collection of Central Research Institute for Dryland Agriculture, Hyderabad. All the isolates were stored as 30% glycerol stocks at -20°C and revived periodically for further studies.

Phytohormones detection and quantification: Indole Acetic Acid (IAA) and Gibberellic Acid (GA_3) are important phytohormones and many rhizobacteria are known to produce them extracellularly. Among seventy-five isolates screened for the production of IAA and GA_3, about 50% of isolates produced both of these phytohormones. Whereas, 15% isolates synthesized IAA and 13% isolates synthesized only GA_3, remaining 21% isolates (i.e., 16) neither produced IAA nor GA_3 in the culture medium (Table 1). The quantitative estimation of the phytohormones was carried out by spectrophotometric method. Among all the strains, 17 isolates found to produce higher quantity of phytohormones. Among these, P17 produced highest quantities of both IAA (35.4 µg mL^{-1}) and GA_3 (47.3 µg mL^{-1}). The next best isolate that produced highest IAA was P73 (28.5 µg mL^{-1}) and P59 produced 29.3 µg mL^{-1} GA_3. The isolates P1, P2, P20, P29, P62 and P75 produced either IAA or GA_3 only (Table 2).

Among PGPR, fluorescent pseudomonads occur commonly in the rhizosphere of plants and they have immense potential in agriculture for use as biofertilizer, biocontrol agent and in bioremediation due to their plant growth-promoting ability, antagonistic activity and degradation of pollutants.

Fig. 1: Map showing soils samples from different agro-ecosystems. Figures in parentheses in legends represent number of samples collected

Table 1: Qualitative screening of *Pseudomonas* isolates for production of IAA and GA_3

IAA	GA_3	IAA and GA_3
P2, P10, P25-P27, P29, P30, P32, P52, P64, P65(11 strains)	P1, P20, P41, P44, P54, P58, P60, P62, P72, P75 (10 strains)	P3-P7, P9, P11, P13-P19, P28, P33, P34, P36, P42, P43, P47-51, P53, P56, P57, P59, P63, P66-P71, P73, P74 (38 strains)

IAA: Indole acetic acid, GA_3: Gibberellic acid, P: *Pseudomonas*

Out of 75 isolates of *Pseudomonas,* when screened for plant growth promoting traits, 38 isolates produced Indole Acetic Acid (IAA) and Gibberellic Acid (GA_3) and isolate P17 produced highest amounts of both IAA and GA_3 (Table 1, 2). Glick (1995) and Arshad and Frankenberger (1998) have also attributed growth promotion by rhizomicroorganisms to mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities. The beneficial

Table 2: Quantitative screening of *Pseudomonas* isolates for production of IAA and GA_3 selected for pot culture studies

Pseudomonas Isolates	Quantity of IAA (mg mL^{-1}±SD)	Quantity of GA_3 (mg mL^{-1}±SD)
P1	-	24.6±2.26
P2	22.6±2.08	-
P4	24.8±2.28	18.9±1.74
P5	21.6±1.99	19.6±1.80
P13	11.4±1.05	17.5±1.61
P14	16.7±1.53	21.1±1.94
P17	35.4±3.26	47.3±4.35
P20	-	24.3±2.23
P28	12.1±1.11	18.4±1.69
P29	19.4±1.78	-
P59	27.5±2.53	29.3±2.70
P62	-	18.4±1.69
P67	8.90±0.82	14.6±1.34
P68	11.8±1.08	16.4±1.51
P71	17.8±1.64	15.9±1.46
P73	28.5±2.62	18.6±1.71
P75	-	27.8±2.56

IAA: Indole acetic acid, GA_3: Gibberellic acid, SD: Standard deviation

effects exerted by fluorescent pseudomonads are known for production of gibberellic acid (Karakoc and Aksoz, 2004), IAA (Hameeda *et al*., 2006) and other plant growth regulators.

Exo-polysaccharides estimation: All the isolates were characterized for the quantity of EPS they produced. Isolates differed significantly for their ability to produce EPS. It was observed that the highest EPS production of 237 μg mL^{-1} was seen in P12 strain. P17 followed the series with 85.5 μg mL^{-1} of EPS. Lowest production of EPS of 1.61 μg mL^{-1} was observed in P73 (Table 3).

All the strains differed in synthesizing the EPS (Table 3) which could be due to their metabolic diversity. Plant growth promoting effect of EPS producing bacteria has been well established (Alami *et al*., 2000). Role of EPS producing *Rhizobium* and its impact on wheat growth and soil structure was demonstrated by Kaci *et al*. (2005). In the present study, highest EPS production was observed in P12. Isolate P17 produced only 85.5 μg mL^{-1} EPS (Table 3). The plant-growth-promoting pseudomonads have been reported to discontinuously colonize the root surface, developing as small biofilms along epidermal fissures (Bloemberg *et al*., 2000). Plant growth promoting effect of IAA and EPS producing *Pseudomonas* spp. in combination with AM fungi in sorghum has been proved by Kumar *et al*. (2012a) recently.

Detection of HCN production: Among the isolates characterized for HCN production ability, only 16% of isolates (i.e., 12) were found to produce HCN in culture medium. Further, it was observed that P17 produced 31.2 ppm of HCN which was the highest, where as lowest production was recorded in P38 which was 6.6 ppm (Fig. 2 and Table 3).

Siderophores production: All the isolates were screened for production of siderophores. Among them, only 20% (i.e., 15) were found to produce siderophores. Quantitative assay for siderophore production showed that P17 produced the highest quantity of Siderophore Units (114 SU) followed by P19 which was 74 SU. The lowest siderophore production was observed in P28 which was 12 SU (Fig. 2 and Table 3).

Interest in pseudomonads has increased because of the possible use of their siderophores as biopesticides (Wilson, 1997). Siderophores of fluorescent pseudomonads can also induce systemic resistance (Leeman *et al*., 1996). Therefore, siderophore production by these isolates may also

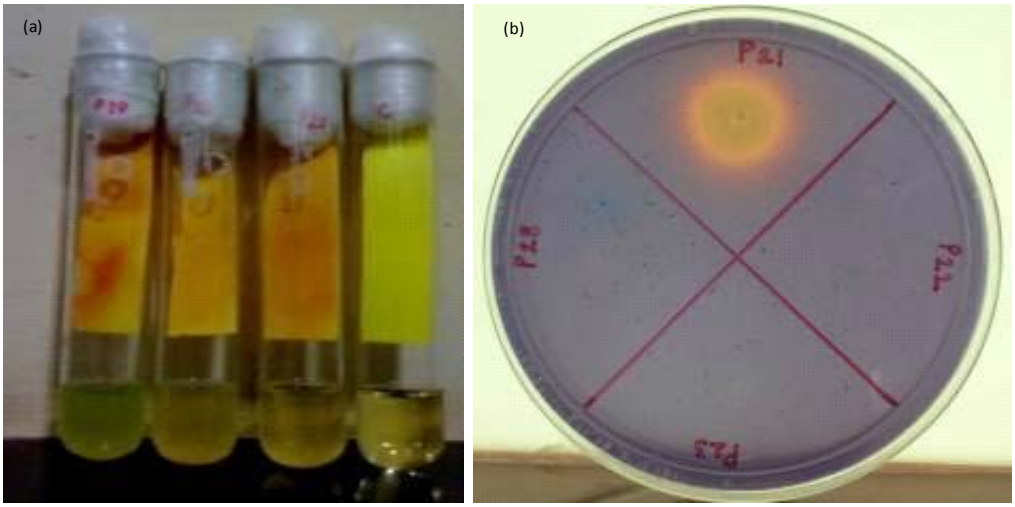

Fig. 2(a-b): (a) Detection of HCN production in culture medium and (b) Siderophores production by *Pseudomonas* isolates *in vitro*

Table 3: Quantitative estimation of exopolysaccharide production by *Pseudomonas* isolates

Pseudomonas isolates	Quantity of EPS produced (μg mL^{-1}±SD)	HCN (ppm)	No. of siderophore units
P1	48.30±4.45	-	-
P4	54.00±4.97	-	22
P5	-	-	31
P8	25.80±2.37	-	-
P10	6.45±0.59	-	20
P11	24.20±2.23	-	-
P12	237.00±21.84	-	-
P13	27.40±2.52	-	42
P14	38.00±3.50	-	-
P17	85.50±7.88	31.20	114
P18	25.80±2.37	-	60
P19	19.30±1.77	-	74
P20	8.00±0.73	10.80	69
P21	24.10±2.22	11.88	58
P23	3.20±0.29	-	-
P28	22.50±2.07	29.04	12
P29	19.30±1.77	-	-
P30	19.30±1.77	-	-
P32	43.00±3.96	-	-
P33	21.00±1.93	-	-
P35	37.00±3.41	-	-
P36	75.80±6.98	-	-
P37	-		24
P38	-	6.60	-
P42	63.00±5.80	14.60	34
P43	58.00±5.34	18.70	22
P48	51.60±4.75	-	-
P54	27.00±2.48	-	-
P58	19.35±1.78	-	-
P59	36.00±3.31	29.40	71
P62	19.35±1.78	-	-
P63	-	19.60	-
P67	-	21.20	44
P73	1.61±0.14	-	-
P74	37.00±3.41	14.60	-
P75	53.00±4.88	13.30	-

EPS: Exopoloysaccharide, HCN: Hydrogen cyanide, ppm: Parts per million, SD: Standard deviation

induce systemic resistance in test crops. Highest siderophore production was observed in the strain P17 with 114 SU (Table 3). Beneficial siderophore producing rhizobacteria suppressed some soil-borne fungal pathogens (Gupta *et al*., 2002) and there is convincing evidence to support a direct role of siderophore-mediated iron competition in biocontrol ability.

Volatile and non-volatile compounds have been known to be one of the mechanisms of biocontrol (Sarangi *et al*., 2010). In the present investigation common isolates were observed with HCN, EPS and siderophores production. Isolates of P59 and P17 were pointed out with marked volatiles production (Table 3). These strains with both siderophores and HCN production could be employed effectively in the control of phytopathogenic fungi as viable alternatives to chemical pesticides. Fernando *et al*. (2005) reported the biocontrol activity of volatiles producing (cyclohexanol, n-decanal and dimethyl trisulfide) *Pseudomonas,* against *S. sclerotiorum*. Hydrogen cyanide produced by pseudomonads was demonstrated to control of root rot of tobacco (Voisard *et al*., 1989).

Production of hydrolytic enzymes: Characterization of the test isolates for different hydrolytic enzymes showed that only 5% of isolates (4) were positive for cellulase production and 31% (23) were positive for protease production. About 15% isolates (11) were positive for urease production. The details of hydrolytic enzymes produced by isolates is summarized in Table 4.

All the isolates were also characterized for their ability to produce chitin/chitosan modifying enzymes (chitosanases and/or chitinases-CCME). Out of these 75 isolates, about 20 isolates hydrolyzed chitosan of DA 1.6%. About 85% of isolates (37) hydrolyzed chitosan of DA 11% of which 10 isolates showing very strong activity on dot blot gels. Fourteen isolates (33%) hydrolyzed DA 35% chitosan of which 3 isolates showed high activity on gels, 13 isolates (45%) hydrolyzed DA 56% chitosan of which only 2 isolates showed high activity. About 43% (17) isolates hydrolyzed glycol chitin substrate (Table 5).

In the present work, *Pseudomonas* isolates differed in their ability to produce enzymes like cellulases, proteases and chitinases (Table 4-5). The production of various hydrolytic enzymes could play an important role in soil fertility as they hydrolyze complex polysaccharides, proteins and urea into simpler form and add to soil again improving soil fertility (Pidwirny, 2006). Dunne *et al.* (1997) have demonstrated that biocontrol of *Pythium ultimum* in the rhizosphere of sugar beet was due to the production of extra cellular protease.

Table 4: Qualitative assay for production of hydrolytic enzymes and classification of *Pseudomonas* isolates into weak, moderate and high groups based on *in vitro* ammonia production

Pseudomonas isolates		
Cellulases	Proteases	Ureases
P17, P59, P67, P68 (4 strains)	P10, P17-P21, P28-P31, P33, P38-P40, P42,P43, P47, P54, P59, P67, P71, P74, P75 (23 strains)	P3, P4, P23, P37, P46, P47, P52, P65, P68, P71, P73 (11 strains)
Weak (+)	Moderate (++)	High (+++)
P1, P2, P4, P22, P39, P40,P45,P47, P57-P67, P69-P75 (26 strains)	P3, P5, P18, P20, P23-P26, P30-P35, P41-P44, P46, P48-P52, P54, P55, P68 (27 strains)	P6-P17, P19, P21, P27-P29, P36-P38, P53, P56 (22 strains)

Table 5: Detection of production of chitinases/chitosanases by *Pseudomonas* isolates using various percentage degree of acetylated (DA) chitosans and glycol chitin

Substrates	*Pseudomonas* isolates
DA 1.6 (%)	P8-P17, P19, P22, P24, P29, P30, P32, P35, P43, P59, P60 (20 strains)
DA 11 (%)	P8-P17, P19-P24, P28-P32, P35, P36, P42, P43, P59, P60, P62, P64, P65, P67-P71, P74, P75 (37 strains)
DA 35 (%)	P17, P19-P23, P30, P35, P43, P60, P62, P67, P74, P75 (14 strains)
DA 56 (%)	P17, P29, P30, P35, P36, P43, P59, P60, P62, P67, P68, P74, P75 (13 strains)
Glycol chitin	P19-P23, P28-P30, P35, P36, P43, P59, P60, P62, P67, P74, P75 (17 strains)

DA: Degree of acetylation, P: *Pseudomonas*

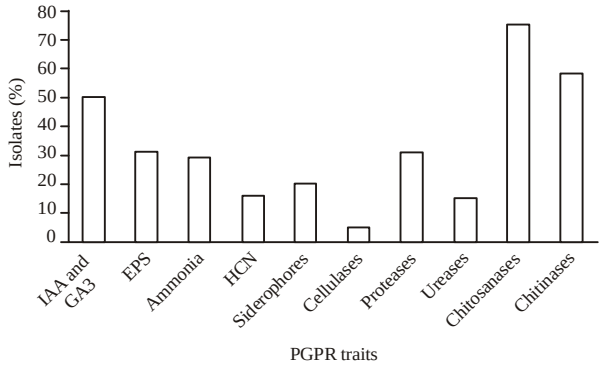

Fig. 3: Percent distribution pattern of various PGPR traits across test isolates

Only four isolates produced cellulases and all of them were not positive for protease production. When characterized for CCME using different chitin/chitosan substrates, 40 isolates responded differentially (Table 5). Low DA hydrolyzing enzymes are categorized as chitosanases having anti microbial action and high DA chitosan degrading enzymes usually are known to enhance plant growth and induced systemic resistance in plants. On the whole, a particular PGPR trait is not responsible for enhanced plant growth whereas, a synergistic action of all the traits is more beneficial in rhizosphere region for higher plant growth (Glick *et al*., 1999). The impact of abiotic stress tolerant *Pseudomonas* spp. has been demonstrated recently by Kumar *et al*. (2014).

Detection of ammonia production: Isolates were checked for their ability to produce ammonia. Based on colour intensity, isolates were categorized into three groups viz., weak, moderate and high ammonia producers (Table 4). Among all the isolates screened, 35% (i.e., 26) isolates were weak NH_3 producers, 36% (i.e., 27) were moderate and 29% of isolates (i.e., 22) were high producers of ammonia (Table 4).

P17 was also a strong producer of ammonia (Table 4), which is an inorganic volatile, may be useful in biocontrol as demonstrated by Howell *et al*. (1988) where ammonia, produced by *Enterobacter cloacae*, appeared to be one of the many mechanisms that bacteria use in the biocontrol of pre-emergence damping-off caused by *Pythium* spp.

It was observed that among the characterized isolated for various PGPR traits 50% of the isolates produced phytohormones, 31% isolates produced exopolysaccharides, 29% isolates produced ammonia, 16% were positive for hydrogen cyanide, 20% produced siderophores, 5% exhibited cellulases activity, 31% produce protease, 15% produced urease, 76% showed chitosanase and 59% isolates showed chitinase activity (Fig. 3).

CONCLUSION

In recent years, considerable attention has been paid to plant growth promoting rhizobacteria, primarily fluorescent pseudomonads, as they are ubiquitous soil microorganisms and aggressive root colonizers. They are also considered as cost-effective and viable alternatives to chemical pesticides for biological control of plant diseases. However, present study focused on isolation and preliminary characterization of various *Pseudomonas* isolates from diverse crop production systems of India in order to identify the strains with multiple PGPR traits and P17 among the tested

isolates is marked as a candidate strain having a potential with plethora of PGPR features along with CCME activity would prove and open-up options to use this strain as a biological agent for sustainable agriculture.

ACKNOWLEDGMENTS

Authors thank Indian Council of Agricultural Research, New Delhi for generous financial support under a network project on Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)-Nutrient Management and PGPR.

REFERENCES

Alami, Y., W. Achouak, C. Marol and T. Heulin, 2000. Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobiumsp*. Strain isolated from sunflower roots. Applied Environ. Microbiol., 66: 3393-3398.

Arshad, M. and W.T. Frankenberger, 1998. Plant growth-regulating substances in the rhizosphere: Microbial production and function. Adv. Agron., 62: 45-151.

Bakker, A.W. and B. Schippers, 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* SPP-mediated plant growth-stimulation. Soil Biol. Biochem., 19: 451-457.

Bloemberg, G.V., A.H.M. Wijfjes, G.E.M. Lamers, N. Stuurman and B.J.J. Lugtenberg, 2000. Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different autofluorescent proteins in the rhizosphere: New perspectives for studying microbial communities. Mol. Plant Mic. Interact., 13: 1170-1176.

Bloemberg, G.V. and B.J.J. Lugtenberg, 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr. Opin. Plant Biol., 4: 343-350.

Booth, C., 1971. Fungal Culture Media. In: Methods in Microbiology, Booth, C. (Ed.). Vol. 4, Academic Press, London, pp: 49-67.

Bric, J.M., R.M. Bostock and S.E. Silverstone, 1991. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied Environ. Microbiol., 57: 535-538.

Charest, M.H., C.J. Beauchamp and H. Antoun, 2005. Effects of the humic substances of de-inking paper sludge on the antagonism between two compost bacteria and *Pythium ultimum*. FEMS Microbiol. Ecol., 52: 219-227.

Dunne, C., J.J. Crowley, Y. Moenne-Loccoz, D.N. Dowling and F. O'Gara, 1997. Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. Microbiology, 143: 3921-3931.

Dye, D.W., 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. N. Z. J. Sci., 5: 393-416.

Fernando, W.G.D., R. Ramarathnam, A.S. Krishnamoorthy and S.C. Savchuk, 2005. Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol. Biochem., 37: 955-964.

Garcia, J.A.L., J. Domenech, C. Santamaria, M. Camacho, A. Daza and F.J.G. Manero, 2004. Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: Relationship with microbial community structure and biological activity of its rhizosphere. Environ. Exp. Bot., 52: 239-251.

Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109-117.

Glick, B.R., C.L. Patten, G. Holguin and D.M. Penrose, 1999. Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria. Imperial College Press, London, UK., ISBN-13: 9781860941528, pp: 134-179.

Gupta, A., M. Gopal and K.V.B.R. Tilak, 2000. Mechanism of plant growth promotion by rhizobacteria. Indian J. Exp. Biol., 38: 856-862.

Gupta, C.P., R.C. Dubey and D.K. Maheshwari, 2002. Plant growth enhancement and suppression of *Macrophomina phaseolina* causing charcoal rot of peanut by fluorescent *Pseudomonas*. Biol. Fert. Soil, 35: 399-405.

Hameeda, B., O.P. Rupela, G. Reddy and K. Satyavani, 2006. Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of Pearl millet (*Pennisetum glaucum* L.). Biol. Fertil. Soils, 43: 221-227.

Holbrook, A.A., W.J.W. Edge and F. Bailey, 1961. Spectrophotometric method for determination of gibberellic acid. Adv. Chem. Ser., 28: 159-167.

Howell, C.R., R.C. Beier and R.D. Stipanovic, 1988. Production of ammonia by *Enterobacter cloacae* and its possible role in the biological control of Pythium pre-emergence damping-off by the bacterium. Phytopathology, 78: 1075-1078.

Kaci, Y., A. Heyraud, M. Barakat and T. Heulin, 2005. Isolation and identification of an EPS-producing *Rhizobium* strain from arid soil (Algeria): Characterization of its EPS and the effect of inoculation on wheat rhizosphere soil structure. Res. Microbiol., 156: 522-531.

Karakoc, B.S. and N. Aksoz, 2004. Optimization of carbon-nitrogen ratio for production of gibberellic acid by *Pseudomonas* sp. Polish J. Microbiol., 53: 117-120.

King, E.O., K.M. Ward and D.E. Raney, 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med., 44: 301-307.

Kloepper, J.W., J. Leong, M. Teintze and M.N. Schroth, 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature, 286: 885-886.

Kumar, G.P., N. Kishore, E.L.D. Amalraj, S.M.H. Ahmed, A. Rasul and S. Desai, 2012a. Evaluation of fluorescent *Pseudomonas* spp. with single and multiple PGPR traits for plant growth promotion of sorghum in combination with AM fungi. Plant Growth Regulat., 67: 133-140.

Kumar, G.P., S. Desai, L.D.A. Emmanuel, M.H.A. Shaik and G. Reddy, 2012b. Plant growth promoting *Pseudomonas* spp. from diverse agro-ecosystems of India for *Sorghum bicolor*. J. Biofert. Biopestic.

Kumar, G.P., S.K.M.H. Ahmed, S. Desai, E.L.D. Amalraj and A. Rasul, 2014. *In vitro* screening for Abiotic stress tolerance in potent biocontrol and plant growth promoting strains of *Pseudomonas* and *Bacillus* spp. Int. J. Bacteriol. 10.1155/2014/195946

Leeman, M., F.M. den Ouden, J.A. van Pelt, F.P.M. Dirkx, H. Steiji, P.A.H.M. Bakker and B. Schippers, 1996. Iron availability affects induction of systemic resistance to Fusarium wilt of radish by *Pseudomonas fluorescens*. Phytopathology, 86: 149-155.

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.

Moeinzadeh, A., F. Sharif-Zadeh, M. Ahmadzadeh and F.H. Tajabadi, 2010. Biopriming of sunflower (*Helianthus annuus* L.) seed with *Pseudomonas fluorescens* for improvement of seed invigoration and seedling growth. Aust. J. Crop Sci., 4: 564-570.

Patten, C.L. and B.R. Glick, 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Applied Environ. Microbiol., 68: 3795-3801.

Pidwirny, M., 2006. Biogeochemical cycling: Inputs and outputs of nutrients to ecosystems. Fundamentals of Physical Geography, 2nd Edition. http://www.physicalgeography.net /fundamentals/9p.html.

Rangarajan, S., L.M. Saleena, P. Vasudevan and S. Nair, 2003. Biological suppression of rice diseases by *Pseudomonas* spp. under saline soil conditions. Plant Soil., 251: 73-82.

Robert, S. and B. William, 1960. Determination of protein-bound carbohydrate in serum by modified anthrone method. Anal. Chem., 32: 885-886.

Sarangi, N.P.A., W.G.D. Fernando, K.Y. Rashid and T. de Kievit, 2010. The role of volatile and non-volatile antibiotics produced by *Pseudomonas chlororaphis* strain PA23 in its root colonization and control of *Sclerotinia sclerotiorum*. Biocon. Sci. Technol., 20: 875-890.

Schwyn, R. and J.B. Neilands, 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem., 160: 47-56.

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

Villacieros, M., B. Power, M. Sanchez-Contreras, J. Loret and R.I. Oruezabal *et al*., 2003. Colonization behaviour of *Pseudomonas fluorescens* and *Sinorhizobium meliloti* in the alfalfa (*Medicago sativa*) rhizosphere. Plant Soil., 251: 47-54.

Voisard, C., C. Keel, D. Haas and G. Defago, 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. Eur. Mol. Biol. Organiz. J., 8: 351-358.

Wilson, M., 1997. Biocontrol of aerial plant diseases in agriculture and horticulture: Current approaches and future prospects. J. Ind. Microbiol. Biotechnol., 19: 188-191.