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Screening And Characterization Of *Pseudomonas Fluorescence* Isolates From Different Rhizosphere Soils Of Telangana

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ABSTRACT
PGPR are beneficial bacteria that colonize the plant roots and enhance the plant growth by a wide variety of mechanisms. The use of PGPR steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers and pesticides. In this study, an attempt was made to collect rhizospheric soil samples isolation and enumeration of native Pseudomonas population from the different rhizospheric samples and to identify the native Pseudomonas fluorescens strains, a potent biocontrol agents as well as PGPR in the rhizosphere under UV light and further characterize them morphologically and culturally. Most P. fluorescence strains showed positive PGPR activity. The study showed that Pseudomonas as an effective plant growth promoting bacterium.
Keywords: *Pseudomonas fluorescens, plant growth promoting activity (PGPR), characterization.*

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INTRODUCTION

Microorganisms have capability to improve plant growth promoting activity. Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant root and increase plant growth by production of various plant growth hormones, P-solubilizing activity, N2 fixation and biological activity (Deshwal et al., 2003, 2010, 2011a). Few strains from genera such as Pseudomonas, *Azospirillium, Azotobacter, Bacillus, Burkholderia, Enterobacter, Rhizobium, Erwinia* and *Flavobacterium* are well known PGPRs (Rodriguez and Fraga, 1999; Misko and Germida, 2002).

Pseudomonas sp. is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. The most effective strains of Pseudomonas have been fluorescent *Pseudomonas* spp. Considerable research is underway globally to exploit the potential of one group of bacteria that belong to fluorescent pseudomonas. Recently Pandey *et al.* (2013) reported that *Pseudomonas* strains were plant growth promoting Endorhizospheric bacteria inhabiting sunflower (*Helianthus annus*). Deshwal et al. (2011a, b) mentioned that *Pseudomonas* strains isolated from Mucuna produced HCN. Gupta et al. (2002) isolated the IAA producing fluorescent pseudomonads in the potato rhizosphere. Glick et al. (1999) reported that IAA producing rhizobacteria enhanced the root length which is one of the plant growth promoting activity rhizobacteria.Bhatia *et al.* (2005) isolated fluorescent *Pseudomonas* strains from rhizosphere of sunflower, potato, groundnut and maize grown in farmer's fields of Haridwar district of Uttar Pradesh.

MATERIALS AND METHODS

Soil Sample Collection

Samples were collected from the rhizosphere soils of college farm, student farm, Rajendranagar, Hyderabad, Telangana.

Eight soil samples were collected upto the depth of 10 to 15cm from the rhizosphere of crop plants *i.e.* Groundnut, Sunflower, Maize, Black gram, Green gram, Rice, Soy bean and Redgram. The soil intimately adhering to the roots was collected and mixed to provide a composite soil sample

Isolation of *Pseudomonas fluorescence* isolates from different rhizosphere soils.

For isolation of Rhizobacteria, the method proposed by Vlassak *et al.* (1992) was followed. In this procedure 10g of soil from each soil sample was taken in a conical flask of 90ml saline. The sample was agitated for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. Dilutions prepared for bacteria were given below.

For *Pseudomonas* spp. – 10^{-3} to 10^{-5}.

0.1ml of respective dilutions were spread on sterilized petri plates containing specific media *i.e. Pseudomonas* agar for fluoresce in (*Pseudomonas*) the petri plates were incubated at room temperatures (28^0C ± 2^0C) for 24-72 h. Two replicates were maintained for each dilution. The plates were examined daily up to 3 days for bacterial colonies.

Enumeration

The plates incubated for a day at 30 ± 1°C were observed for the growth of *Pseudomonas* colonies on KB plates and the colonies are enumerated manually and recorded. Results are presented in the Table1.

IDENTIFICATION OF BACTERIAL ISOLATES

1. Morphological Characterization

All the 15 isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

2. Cultural Characterization

Morphological characteristics of the colony of each isolate were examined on Nutrientagar and specialized medium and incubated for according to isolate. Cultural characterization of isolates observed by different characteristics of colonies such as shape, size, elevation, surface, margin, colour, odour, pigmentation etc were recorded as per Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994).

3. Biochemical and Physiological Characterization

Different biochemical tests performed and the protocols followed are briefly outlined below.

3.1 Starch Hydrolysis

Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ±2° C for 24-48 h. After incubation, the plates were flooded with iodine solution . The formation of a transparent zone around the colony was taken as positive reaction for the test.

3.2 Hydrogen Sulfide Test

Sterilized Hydrogen sulfide- Indole-Motility agar stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 h at 28 ±2° C. Visualization of black colour along the line of inoculation indicated a positive reaction for the test.

3. 3 Indole Production

Sterilized SIM agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ±2° C. Following incubation, 10 drops of Kovac'sindole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

3.4 Catalase Test

This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of Pure isolates were taken on glass slides and one drop of H_2O_2 (30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

3.5 Oxidase Test

The overnight cultures of the test isolates were spotted on plates poured with sterile trypicase soy agar and the plates were incubated for 24 h at 28 ±2° C. After incubation, 2-3 drops of N, N, N', N'- tetramethyl-p-phenylenediaminedihydrochloride (Wurster's reagent) were added onto the surface of growth of each test organism. The isolates showing change of colour to maroon were noted as oxidase positive.

3.6 Gelatin liquefaction

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 h at 28 ±2° C. Then the tubes were kept in the refrigerator for 30 min at 4°C. The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative for the test.

3.7 Methyl Red Test

Sterilized glucose- phosphate broth tubes were inoculated with the test culture and incubated at 28±2^0C for 48h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test.

3.8 Voges Prausker's Test

To the presterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37^0C for 48h. After incubation ten drops of Baritt's reagent A was added and gently shaken followed by addition of 10 drops of Baritt's reagent B. Development of pink colour in the broth was taken as positive for the test.

3.9 Citrate Utilization

Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± 2^0C for 24h. Change in colour from green to blue indicates the positive reaction for citrate utilization.

3.10 Denitrification test

Sterilized nitrate broth tubes inserted with Durham's tube in inverted position were inoculated with overnight grown cultures of the test organisms and incubated at 25^oC for 10-15 days. After incubation, the isolates which showed accumulation of gas in the Durham's tubes were scored as positive for denitrification.

3.11 Carbohydrate Utilization

All pure bacterial isolates were screened for the carbohydrate fermentation abilities using 4 different carbohydrates (lactose, sucrose, dextrose and mannitol) in Peptone broth medium. Bacterial isolates were inoculated in broth containing specific carbohydrate. The change in colour of Peptone broth was observed for utilization of particular carbohydrate present in broth.

RESULTS AND DISCUSSION

Isolation of *Rhizobial* and *Pseudomonas fluorescence*

The microbial population in the rhizospheric soils of Groundnut, Black gram, Green gram, Red gram, Soy bean, Sunflower, Maize and Rice soils of different places were collected. Maximum population of *Pseudomonas fluorescence* was found in the Rice rhizospheric soils of College farm. The *Pseudomonas* population was at minimum in Maize rhizospheric soils of College farm. However the soils collected from different places the *Pseudomonas flourescence* population ranged between 1- 6.0 x 10^6cfu /g soil results are presented in the Table1.

Cultural and Morphological Characterization

Pseudomonas fluorescence (15 isolates), based on their colony morphology on different media, cell morphology and Gram reaction. The bacterial isolates were named according to the crop and cultural characters presented in Table.2

Pseudomonas fluorescence (15 isolates), based on their colony morphology on different media, cell morphology and Gram reaction presented in the Table 3.

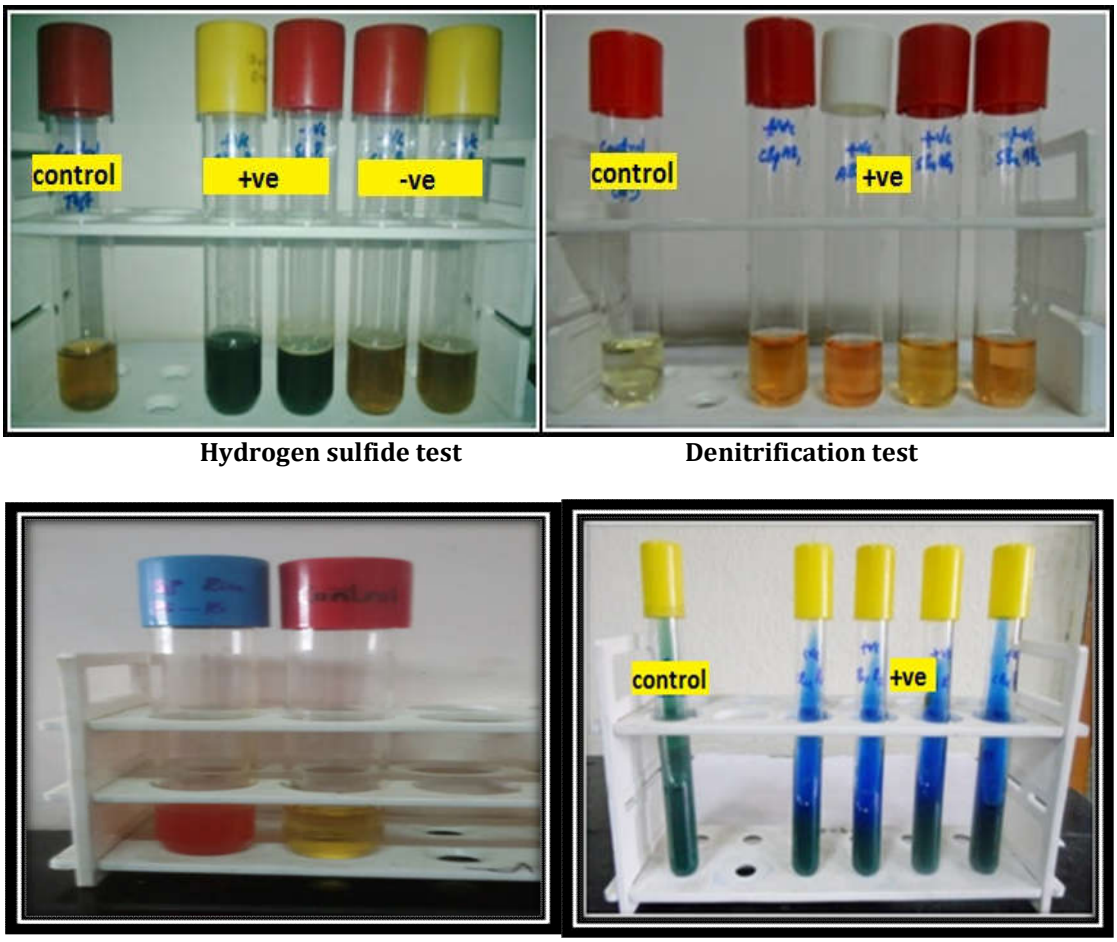
Fifteen of the total isolates took about 24 h to establish their growth on Kings B agar medium [Plate 1(A)]. All the isolates developed small to medium, smooth, glistening colonies, out of the 15 isolates 6 isolates showed yellowish green colour with light green pigmentation and the remaining isolates showed dull white colonies with no pigmentation. These isolates were Gram negative, small, single isolated rods without sporulation when observed under microscope and presented in the Table.4 [Plate 1 (B)]

(A) *Pseudomonas* colonies on Kings B agar medium (B)Microscopic Observation of *Pseudomonas*
Plate 1. Plant growth promoting rhizobacterial growth on medium &Microscopic observation

Biochemical and Physiological Characterization

After the study of cultural and cell morphology, the isolates of the *Pseudomonas fluorescence* (15 isolates) were tested for different biochemical test viz., IMVIC test, oxidase test, catalase test, carbohydrate fermentation, denitrification, H_2S production, starch hydrolysis, gelatin liquefaction etc. (Plate 2).

All the 15 isolates of *Pseudomonas fluorescence* showed positive results for catalase test and oxidase test whereas they were negative for Voges Prausker's test. For methyl red test RGP-1, RP-1, RP-4, MP-1, MP-2, SFP-1and SFP-2 isolates showed positive results. Out of 15 isolates 12 isolates showed positive results for starch hydrolysis, only 3 isolates showed positive results for gelatin liquefaction, 12 isolates showed positive results for citrate utilization, only 5 isolates showed positive results for H_2S test, denitrification, RGP-2, RP-3, MR-2, MR-3 and SFP-1 isolates respectively showed positive results. Results are presented in the Table5.



Hydrogen sulfide test **Denitrification test**

Methyl red test **Citrate utilization test**

Plate2. Biochemical and physiological characterization of bacterial isolates

CONCLUSION

Future study with these isolates using them in pot cultures and followed by field experiments will help in establishing their potential to be used as biofertilizers. The data obtained in the present study suggest that Pseudomonas flourescence isolates GGP-1, MP-1 would be ideal organisms for further study in pot culture and field experiments to exploit their PGPR potential for a good biofertilizers production.

Table 1. Microbial population in the Rhizosphere soils of different crops

S.No	Crop	*Pseudomonas fluorescence* (x 10 ^{6}cfu / gm soil)
1	Ground nut	2.65
2	Red gram	3
3	Green gram	3
4	Black gram	4
5	Soya bean	3
6	Sunflower	2.35
7	Maize	2
8	Rice	6

Table.2 Labelling of isolates according to crop and cultural characters

	Pseudomonas fluorescence
Isolate name	**Soil sample /crop**
RP-1	Rice
RP-2	Rice
RP-3	Rice
RP-4	Rice
MP-1	Maize
MP-2	Maize
MP-3	Maize
SFP-1	Sun flower
SFP-2	Sun flower
GNP-1	Ground nut
GGP-1	Green gram
BGP-1	Black gram
SYP-1	Soy bean

Table.3 Cultural characteristics of *Pseudomonas fluorescence* isolates on different media

Isolates	**Colony morphology on Nutirent Agar**	**Colony morphology on king's B media**
RGP-1	Yellow , round, non spreading	Round, yellowish green, convex
RGP-2	Yellow , round, non spreading	Round, white, convex
RP-1	Off white, irregular, non-spreading, smooth, flat, opaque	Irregular, dull white, convex
RP-2	Yellow , round, non spreading	Round, yellowish green, convex
RP-3	Yellow , round, non spreading	Round, white, raised
RP-4	Yellow , round, non spreading	Irregular, yellowish green, convex
MP-1	Pale white and raised	Round, off white, flat
MP-2	Yellow , round, non spreading	Round, yellowish green, convex
MP-3	Irregular, dull white, convex	Irregular, white, raised
SFP-1	Off white, irregular, non-spreading, smooth, flat, opaque	Round, off white, flat
SFP-2	Yellow , round, non spreading	Round, yellowish green, convex
GNP-1	Irregular, dull white, convex	Irregular, dull white, convex
GGP-1	Off white, irregular, non-spreading, smooth, flat, opaque	Round, off white, flat
BGP-1	Round, white, raised	Round, white, raised
SYP-1	Pale white and convex	Medium, dull white, convex

Table.4: Cultural and morphological characteristics of *Pseudomonas fluorescens* isolates on King's B medium

aracteristics of isolates	**size**	**Colony shape**	**colour**	**elevation**	**surface**	**margin**	**pigmentation**	**Gram reaction**	**shape**	**Sporulation**
RGP-1	Small	Round	Yellowish green	convex	Smooth	irregular	Yellowish green	Negative	Rod	Negative
RGP-2	Small	Round	white	Convex	Smooth, shiny	Regular	Bluish grren	Negative	Rod	Negative
RP-1	Small l	irregular	Dull white	Convex	Smooth	Irregular	Light green	Negative	Rod	Negative
RP-2	Small	Round	Yellowish green	Convex	Smooth, Shiny	Regular	yellowish green	Negative	Rod	Negative
RP-3	Small	Round	White	Raised	Smooth,	Regular	Light green	Negative	Rod	Negative

					mucoid					
RP-4	Medium	irregular	Yellowish green	Convex	Smooth, shiny	Irregular	yellowish green	Negative	Rod	Negative
MP-1	Small	Round	Off white	Flat	Smooth	Irregular	Light green	Negative	Rod	Negative
MP-2	Small	Round	Yellowish green	Convex	Smooth, shiny	Regular	yellowish green	Negative	Rod	Negative
MP-3	Medium	irregular	White	Raised	Smooth, mucoid	Regular	Yellowish green	Negative	Rod	Negative
SFP-1	Small	Round	Off white	Flat	Smooth	Irregular	Bluish grren	Negative	Rod	Negative
SFP-2	Small	Round	Yellowish green	Convex	Smooth, shiny	Regular	yellowish green	Negative	Rod	Negative
GNP-1	Medium	irregular	Dull white	Convex	Smooth, shiny	Regular	Light green	Negative	Rod	Negative
GGP-1	Small	Round	Off white	Flat	Smooth	Irregular	Bluish green	Negative	Rod	Negative
BGP-1	Small	Round	White	Raised	Smooth, mucoid	Regular	light green	Negative	Rod	Negative
SYP-1	medium	irregular	Dull white	Convex	Smooth, shiny	Regular	Light green	Negative	Rod	Negative

Table. 5 Biochemical and physiological characteristics of *Pseudomonas fluorescence* isolates

S.No	Isolates	Indole test	MR	Vp test	Citrate utilization test	Catalase test	Oxidase test	Starch hydrolysis test	Gelatine liquefaction test	Denitrification	H_2S test	Carbohydrate Utilization			
												Lactose	Sucrose	Dextrose	Mannitol
1	RGP-1	-	-	-	+	+	+	+	+	-	+	+	-	+	+
2	RGP-2	-	+	-	-	+	+	+	-	+	+	+	-	+	+
3	RP-1	-	+	-	+	+	+	+	-	-		+	+	+	+
4	RP-2	-	-	-	+	+	+	+	-	-	+	+	-	-	+
5	RP-3	-	-	-	+	+	+	+	-	+	-	-	-	-	-
6	RP-4	-	+	-	+	+	+	+	-	-	+	-	-	+	-
7	MP-1	-	+	-	+	-	+	+	-	-	-	+	+	+	+
8	MP-2	-	+	-	+	+	+	-	+	+	+	-	+	-	-
9	MP-3	-	-	-	+	+	+	+	-	+	-	+	+	+	+
10	SFP-1	-	+	-	+	+	+	+	-	+	-	+	_	+	-
11	SFP-2	+	+	-	+	+	+	-	-	-	-	+	+	+	+
12	GNP-1	-	-	-	-	-	+	+	-	-	-	-	-	+	+
13	GGP-1	-	-	-	-	+	+	+	+	-	-	-	-	+	+
14	BGP-1	-	-	-	+	-	+	_	-	-	-	+	-	+	-
15	SYP-1	-	-	-	+	+	+	+	-	-	-	+	+	-	-

MR - Methyl red , + positive result, VP - VogesPraskaur's test, - Negative result

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