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Full Length Research Paper

Isolation and Characterization of *Pseudomonas fluorescens* in the rice rhizospheric soils of Rangareddy district in Telangana state

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Rice is an economically important food crop, which is subjected to infection by fungal, viral and bacterial pathogens. Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The potential negative effect of chemical fertilizers on the global environment and the cost associated with production had lead to research with the objective of replacing chemical fertilizers with bacterial inoculants. *Pseudomonas fluorescens* is an important among PGPR because it is having an ability to induce plant growth as well as control the growth of pathogens. Native population of these rhizobacteria play an important role in the sustainable agriculture as they majorly dominate the rhizosphere. In the present study thirty *P. fluorescens* isolates were isolated from the rhizosphere of rice from the Rangareddy district, Telangana and characterized by morphological and biochemical tests.

Key words: *Pseudomonas*, native population, plant growth promoting rhizobacteria (PGPR).

INTRODUCTION

The use of chemical fertilizers and pesticides has caused an incredible harm to the environment. These agents are both hazardous to animals and humans and may persist and accumulate in natural ecosystems and an answer to this problem is replacing chemicals with biological approaches, which are considered more environment friendly in the long term (Musa et al., 1976). One of the emerging research area for the control of different phytopathogenic agents is the use of plant growth promoting rhizobacteria (PGPR), which are capable of suppressing or preventing the phytopathogen damage (Nihorembere et al., 2011).

The soil bacteria that aggressively colonize the root zone and promote plant growth are generally termed as plant growth promoting rhizobacteria (PGPR). In this regard, the use of plant growth promoting rhizobacteria (PGPR) has depicted potential in developing sustainable agricultural systems for crop production and protection (Govindasamy et al., 2011). Plant growth promoting rhizobacteria consisting of primarily *Pseudomonas fluorescens* and *P. putida*. They were identified as important organisms with ability for plant growth promotion and effective disease management properties (Belkar and Gade, 2012). In the present study, *P. fluorescens* isolated from the rhizosphere of rice crop from Parigi and Doma mandals of Ranga Reddy district in Telangana were characterized for different morphological and biochemical tests.

MATERIALS AND METHODS

Soil sampling

Twenty two soil samples were collected from different villages of two mandals in Rangareddy district for the

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isolation of *Pseudomonas fluorescens* strains. The soil samples were mainly collected from rice rhizosphere. Crop plants were selected randomly in the field and the intact root system was dug out, carefully taken in plastic bags, labelled well and stored at 4^{o}C

Isolation:

King's B selective medium was used for the isolation of *P. fluorescens* (King's et al., 1954).

Cultural characterization:

All the bacterial isolates were studied for their colony morphology, cell morphology (Gram reaction), pigmentation and spore production as per Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Biochemical characterization of *Pseudomonas fluorescens* isolates:

Indole Production

Sterilized Hydrogen Sulfide-Indole-Motility agar (SIM agar) slants or Tryptophan broth tubes were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ± 2^{o}C. Following incubation, 10 drops of Kovac's indole reagent was added to each tube. The isolates showing production of red colour was recorded as positive for indole production (Aneja, 2001).

Methyl Red Test

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

Voges Prausker's Test

To the presterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37^{o}C 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 color in the broth was taken as positive for the test.

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^{o}C. Then the tubes were kept in the refrigerator for 30 minutes at 4^{o}C. The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative (Pickett et al., 1991).

Starch hydrolysis:

Sterile starch agar plates were spotted with 10µl overnight broth cultures of the isolates and incubated at 28 ± 2^{o}C for 24 - 48 h. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony indicated positive (Cappucino, 1983).

Citrate Utilization

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

Denitrification

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^{o}C for 10 -15 days. After incubation, the isolates which showed accumulation of gas in the Durham's tubes were scored as positive for denitrification (Aneja, 2001).

Catalase activity

Catalase test was performed by taking a drop of 3% hydrogen peroxide and added to 48 h old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicated catalase activity.

Oxidase test:

To the 24 h old bacterial culture oxidase discs are placed on them. The isolates showing blue colouration of discs were taken as positive.

Hydrogen sulphide production:

SIM agar medium tubes were stab inoculated by test isolates and incubated for 24 - 48 h at 37 ± 2^{o}C (Clarke, 1953). Tubes were observed for presence or absence of black coloration along the line of inoculation indicating hydrogen sulphide production.

Triple Sugar Iron agar (TSI) test

Isolates were streaked on TSI agar slants and incubated at 28 ± 2^{o}C for 24 - 48 h. Change in colour from yellow to reddish brown indicates the positive reaction for the test.

Carbohydrate Utilization test

All the pure bacterial isolates were screened for the carbohydrate fermentation abilities using four different carbohydrates (glucose, galactose and lactose) 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 (Aneja, 2001).

RESULTS AND DISCUSSION

Twenty two samples were collected from rice rhizospheric soils of parigi and doma mandals of Rangareddy district in Telangana for the isolation of fluorescent pseudomonads. The samples were serially

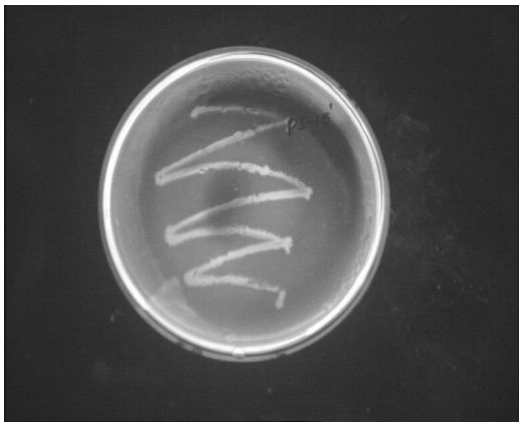
Table 1. Cultural and morphological characteristics of *Pseudomonas fluorescens* isolates on King's B medium.

S.No.	Isolate	Size	Margin	Color	Elevation	Surface	Pigmentation	Gram reaction	Shape	Sporulation
1	PRP1	Small	Round	Yellowish green	Convex	Smooth shiny	Yellowish green	Negative	Rods	Negative
2	DKP	Medium	Round	Dull white	Convex	Smooth shiny	Bluish green	Negative	Rods	Negative
3	PGP1	Small	Round	Bluish white	Convex	Smooth shiny	Bluish green	Negative	Rods	Negative
4	PVP1	Small	Round	Yellow	Convex	Smooth shiny	Yellowish green	Negative	Rods	Negative
5	PGoP	Small	Round	Yellowish green	Convex	Smooth shiny	Dark green	Negative	Rods	Negative
6	PLP	Small	Irregular	Yellowish green	Convex	Smooth shiny	Light green	Negative	Rods	Negative
7	PSP1	Small	Irregular	Green	Convex	Smooth shiny	Dark green	Negative	Rods	Negative
8	DOP	Medium	Round	White	Convex	Smooth shiny	Light green	Negative	Rods	Negative
9	DTP1	Small	Irregular	White	Convex	Smooth shiny	Light green	Negative	Rods	Negative
10	DDP	Small	Round	Bluish white	Convex	Smooth shiny	Light green	Negative	Rods	Negative
11	DBP	Small	Round	Yellow	Convex	Smooth shiny	Yellowish green	Negative	Rods	Negative
12	DGP1	Small	Irregular	Yellowish green	Convex	Smooth shiny	Light green	Negative	Rods	Negative
13	DPP	Medium	Irregular	Dull white	Convex	Smooth shiny	Light green	Negative	Rods	Negative
14	DMoP	Small	Round	Yellow	Convex	Smooth shiny	Yellowish green	Negative	Rods	Negative
15	DPiP	Small	Round	Green	Convex	Smooth shiny	Dark green	Negative	Rods	Negative
16	DRP	Small	Round	Dull white	Convex	Smooth shiny	Light green	Negative	Rods	Negative
17	DMuP	Small	Irregular	Dull white	Convex	Smooth shiny	Light green	Negative	Rods	Negative
18	DMP1	Small	Round	Dull white	Convex	Smooth shiny	Bluish green	Negative	Rods	Negative
19	DBoP	Medium	Round	Yellow	Convex	Smooth shiny	Yellowish green	Negative	Rods	Negative
20	PSmP	Small	Irregular	Dull white	Convex	Smooth shiny	Light green	Negative	Rods	Negative
21	PGuP1	Small	Irregular	Green	Convex	Smooth shiny	Light green	Negative	Rods	Negative
22	PKP	Small	Round	Yellowish white	Convex	Smooth shiny	Yellowish green	Negative	Rods	Negative
23	PRP2	Small	Irregular	Whitish green	Convex	Smooth shiny	Dark green	Negative	Rods	Negative
24	PGP2	Medium	Round	Yellowish green	Convex	Smooth shiny	Light green	Negative	Rods	Negative
25	PVP2	Small	Round	Green	Convex	Smooth shiny	Light green	Negative	Rods	Negative
26	PSP2	Small	Irregular	Yellow	Convex	Smooth shiny	Yellowish green	Negative	Rods	Negative
27	DTP2	Small	Irregular	Green	Convex	Smooth shiny	Dark green	Negative	Rods	Negative
28	DGP2	Medium	Round	Yellow	Convex	Smooth shiny	Yellowish green	Negative	Rods	Negative
29	DMP2	Small	Irregular	Dull white	Convex	Smooth shiny	Bluish green	Negative	Rods	Negative
30	PGuP2	Small	Round	White	Convex	Smooth shiny	Bluish green	Negative	Rods	Negative

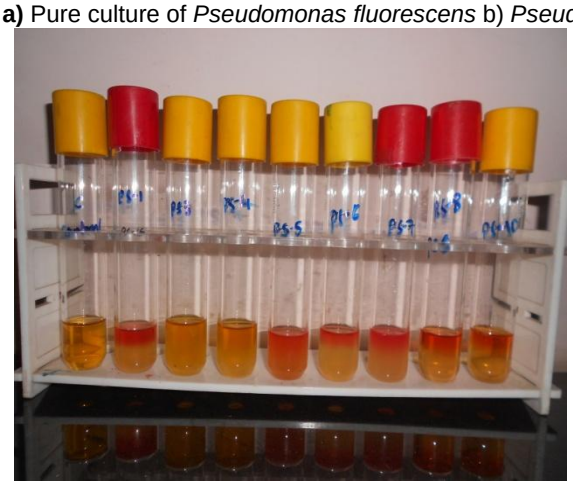
Table 2. Biochemical characterization of *Pseudomonas fluorescens* isolates from rice rhizosphere of Rangareddy district.

S.No.	Isolate	Indole test	MR test	VP test	Citrate utilization	Catalase	Oxidase	Starch hydrolysis	Gelatin liquefaction	H_2S	TSI test	Carbohydrate utilization			Denitrification
												Glucose	Galactose	Lactose	
1	PRP1	-	+	+	+	+	+	-	+	+	-	-	+	+	+
2	DKP	-	-	+	+	+	+	-	+	-	+	+	+	-	+
3	PGP1	-	-	-	+	+	+	-	+	+	-	+	+	+	+
4	PVP1	-	-	+	+	+	+	+	+	+	+	-	+	+	+
5	PGoP	-	+	+	+	+	+	-	+	+	+	+	-	+	+
6	PLP	-	+	-	+	+	+	+	+	+	-	+	-	+	+
7	PSP1	-	+	-	+	+	+	-	+	-	+	+	+	+	+
8	DOP	-	+	-	+	+	+	+	+	+	+	+	-	-	+
9	DTP1	-	-	-	+	+	+	+	+	+	+	+	-	+	+
10	DDP	-	+	+	+	+	+	-	+	+	-	-	+	+	+
11	DBP	-	+	+	+	+	+	-	+	-	+	+	+	-	+
12	DGP1	-	-	+	+	+	+	-	+	+	+	-	-	+	+
13	DPP	-	+	+	+	+	+	-	+	+	+	+	+	+	+
14	DMoP	-	+	+	+	+	+	+	+	+	+	+	+	+	+
15	DPiP	-	+	+	+	+	+	-	+	+	+	+	+	-	+
16	DRP	-	+	+	+	+	+	-	+	+	-	-	+	+	+
17	DMuP	-	+	+	+	+	+	+	+	+	+	-	+	-	+
18	DMP1	-	+	-	+	+	+	+	+	+	-	+	+	-	+
19	DBoP	-	+	-	+	+	+	+	+	+	-	+	-	-	+
20	PSmP	-	-	-	+	+	+	-	+	+	+	-	+	-	+
21	PGuP1	-	+	+	+	+	+	-	+	+	+	+	+	+	+
22	PKP	-	-	+	+	+	+	-	+	+	+	-	+	+	+
23	PRP2	-	-	+	+	+	+	+	+	+	-	+	+	-	+
24	PGP2	-	-	+	+	+	+	+	+	+	+	+	-	+	+
25	PVP2	-	+	+	+	+	+	-	+	+	-	+	+	+	+
26	PSP2	-	+	+	+	+	+	+	+	+	+	-	+	-	+
27	DTP2	-	+	+	+	+	+	+	+	+	-	-	-	+	+
28	DGP2	-	+	+	+	+	+	-	+	+	+	+	+	+	+
29	DMP2	-	+	+	+	+	+	-	+	+	+	+	-	-	+
30	PGuP2	-	+	+	+	+	+	-	+	-	-	-	+	-	+

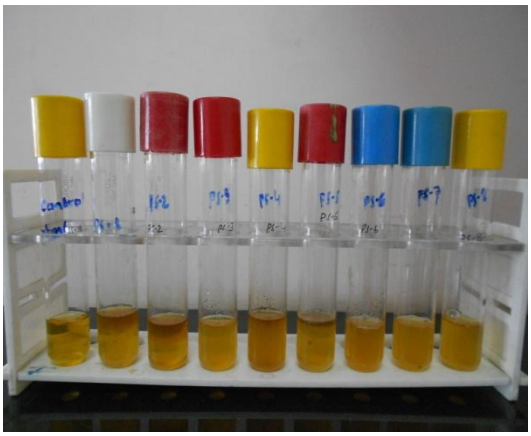
+ positive **–** negative **MR**-Methyl Red test **VP**-Voges Prausker's test **H_2S-** Hydrogen sulphide test **TSI**-Triple Sugar Iron test.



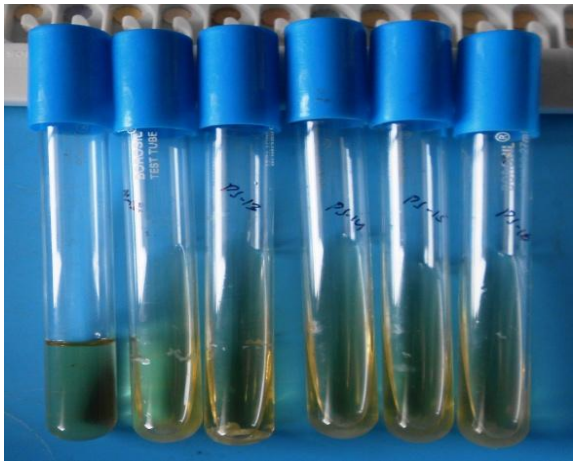
a) Pure culture of *Pseudomonas fluorescens* b) *Pseudomonas fluorescens* under UV light.



(c) MR test



(d) VP test

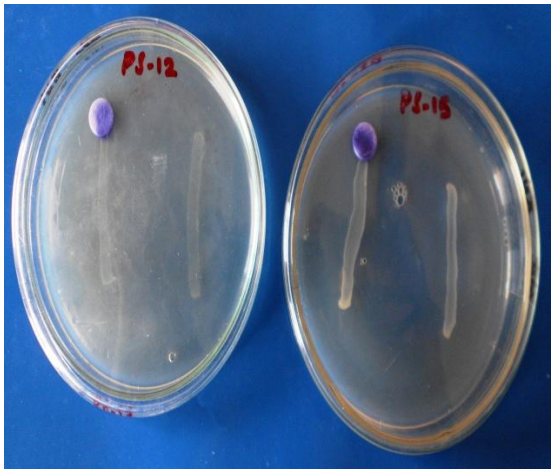


(e) Gelatin liquefaction

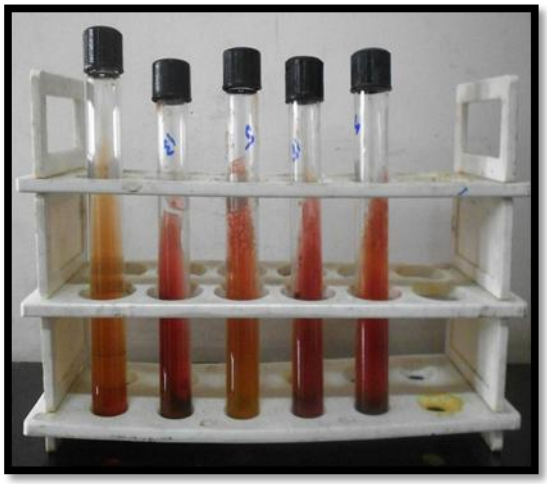
(f) Starch hydrolysis

diluted and plated onto KB medium. All the isolates developed small to medium, smooth, glistening colonies, convex elevation and these isolates were Gram negative, rods without sporulation when observed under microscope. Out of the total 30 isolates, 8 isolates showed yellowish green pigmentation, 12 showed light green pigmentation, 5 showed bluish green and 5 showed dark green pigmentation under UV light. Small size was shown by 24 isolates and medium size was shown by 6 isolates i.e., DKP, DOP, DPP, DBoP, PGP2 and DGP2. Margin was round in 18 isolates and irregular in 12 isolates. 11 isolates were green, 13 isolates were

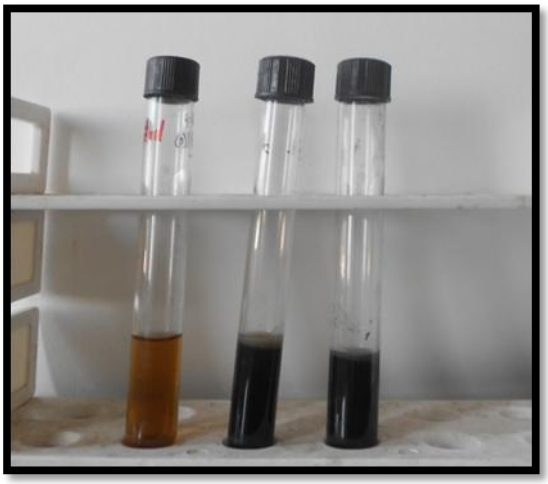
g) Citrate utilization



h) Oxidase test



i) TSI test



j) H_2S production

k) Catalase test

white and 6 were yellow coloured. All the isolates showed, smooth shiny surface. Based on the colony morphology and cultural characteristics of the isolates on the KB medium and observation of pigmentation under

UV light about thirty colonies from above plates were selected, purified and the pure cultures obtained was stored in refrigerator at 4^{o}C (Table 1). Similar results were obtained with Jayashree et al. 2000, who isolated fluorescent pseudomonads from the rhizospheres of blackgram, carrot, banana, pepper, rice and forest trees grown in several geographical areas of Tamil Nadu and later on confirmed the fluorescent colonies by viewing under UV-light.

Biochemical characterization

Results revealed that all the 30 isolates of *Pseudomonas fluorescens* were negative for indole production and 21 isolates for methyl red test, 22 isolates for Voges Prausker's test showed positive results. All the thirty *Pseudomonas fluorescens* isolates were positive for gelatin liquefaction i.e., the isolates of *P. fluorescens* produced gelatinase enzyme in nutrient broth agar media supplemented with gelatine substrate as 1% and gelatinase belongs to proteolytic enzyme resulting in gelatinous hydrolysis and starch was hydrolysed by only 12 isolates i.e., PVP1, PLP, DOP, DTP1, DMoP, DMuP, DMP1, DBoP, PRP2, PGP2, PSP2 and DTP2. All the isolates showed positive for citrate utilization, denitrification, catalase and oxidase tests. The *P. fluorescens* organisms produced the enzymes catalase, oxidase and hence showed positive for the tests. For Triple Sugar Iron test 19 isolates i.e., PVP1, DBP, DMoP, PKP, PSP2, DGP2, DOP, DTP1, DGP1, DPP, DMuP, PSmP, PGuP1, PGP2, DKP, DMP2, PGoP, PSP1 and DPiP showed positive results. For H_2S test 26 *Pseudomonas fluorescens* isolates were positive.

In carbohydrate utilization test glucose, galactose and lactose sugars were supplemented in media and noticed glucose was utilized by the 19 isolates, Galactose was utilized by the 22 isolates and Lactose was utilized by the 18 isolates i.e., *P. fluorescens* have the ability to utilise the carbohydrates and these tests confirmed the strains biochemically as *Pseudomonas fluorescens* (Table 2).

Our results agreement with Akter *et al.* 2014 who isolated 325 bacteria and 14 of them were identified as fluorescent pseudomonads by morphological and biochemical characterization. Fifty *Pseudomonas fluorescens* and 28 *Rhizobium* strains were isolated from rhizospheric soil and root nodules of pigeonpea, biochemically characterized and identified as *Pseudomonas fluorescens* and *Rhizobium* (Basha *et al.* 2014).

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