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Biochemical and physiological characterizations of *Pseudomonas fluorescens*

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Abstract
Seven *Pseudomonas* isolates obtained from rhizosphere of different crops were designed as PF-1 to PF-7 which showed the characteristics of *Pseudomonas*. All the isolates were gram negative, rod shaped and produced yellow and greenish yellow pigment. Biochemical characterizations were studied *viz*., KOH test, catalase test, starch hydrolysis, gelatin liquefaction, H_2S production, acid and gas production, some of the isolates show both positive and negative to starch hydrolysis, H2S production, IAA production and phosphate solubilization. The bacterium failed to produce hydrogen cyanide (HCN). Physiological studies revealed that all isolates show maximum growth at 25 and 30°C temperature with OD value ≥ 60. All the isolates show maximum growth at pH 7 and 8 with OD vale ≥ 60. Antagonistic activity of *Pseudomonas fluorescens* isolates were studied against *Xanthomonas axonopodis* pv. *citri*. There were significant differences on inhibition zone. Isolate PF-7 recorded maximum zone of inhibition (29.1 mm) followed by PF-4 (28.7 mm), whereas no inhibition showed by isolate PF-6.

Keywords: *Pseudomonads fluorescens*, Isolation, Characterizations, *Xanthomonas axonopodis* pv. *citri*

Introduction
The Triclosan genus *Pseudomonas* is the most heterogeneous and ecologically significant group of known bacteria, and includes Gram-negative motile aerobic rods that are widespread throughout nature and characterized by elevated metabolic versatility. The nutritional requirements of *Pseudomonas* spp. are very simple, and the genus is foundin natural habitats like soil, fresh water, marine environments etc., but it has also been isolated from clinical instruments, aseptic solutions, cosmetics and medical products. The use of chemical fertilizer and pesticides has caused an incredible harm to environment. These agents are both hazardous to animal and humans and may persist and problem is replacing chemical with biological approaches, which are consider more environment friendly in the long term. One of the emerging research areas for the control of different phytopathogenic agents is the use of plant growth promoting rhizobacteria (PGPR), which are capable of suppressing or preventing the Phyto pathogen damage. In present study *P. fluorescens*iso late from different rhizosphere were characterized for different biochemical and physiological test.

Materials and Methods
Isolation of *Pseudomonas fluorescens:* The bio agent involve in the study i.e. *Pseudomonas fluorescens* were isolated from rhizosphere of different crops on King's B media by serial dilution and pour plate technique (King *et al.*, 1954) [9]. possible to perform on a small sample aliquot. The limit of quantification value in fish tissue was 0.083 mg g^{-1} and the limit of detection was 0.016 mg g^{-1}.

Morphological character of *Pseudomonas fluorescens*
Morphological character of *Pseudomonas fluorescens* such as shape, pigmentation and Gram reaction were studied as per Migula (1894) [12].

Biochemical characterization of *Pseudomonas fluorescens* isolates
Biochemical tests *viz*., KOH test, starch hydrolysis, gelatin liquefaction, H_2S production, acid and gas production, catalase test, was carried out for biochemical confirmation of *Pseudomonas fluorescens* according to Aneja, 2003.

Also all the isolates of *Pseudomonas fluorescens* were evaluated for plant growth promoting properties *viz*. indole production, phosphate solubilization and HCN production.

Physiological studies

Effect of temperature

The study was initiated to find the optimum temperature requirement for growth of *Pseudomonas fluorescens* using King's B medium. A loop full of 48 hrs old bacterial culture was inoculated in 100 ml conical flask containing 30 ml of King's B broth. The inoculated flasks were incubated at different temperature level *viz*., 5, 10, 15, 20, 25, 30, 35 and 40°C respectively for 72 hours. Observations were recorded for the optical density of the broth culture turbidiometrically by using spectrophotometer at 600 nm after 72 hr.

pH requirement

Effect of pH on the growth of *Pseudomonas fluorescens* was studied by adjusting pH of the King's B medium to various levels *viz*., 4, 5, 6, 7, 8, 9 and 10 using appropriate phosphate buffer. A loop full of 48 hour old bacterial culture was mixed in 100 ml conical flask containing 30 ml King's B broth. Inoculated flasks were incubated at room temperature for 72 hours. After the incubation period observations were recorded for the growth of bacterium turbidiometrically by using spectrophotometer at 600 nm.

Antagonism of *Pseudomonas fluorescens* against *Xanthomonas axonopodis* pv. *citri*

Xanthomonas axonopodis pv. *citri* isolatewas collected from Plant Pathology Section, COA, Nagpur and antagonistic effect of *Pseudomonas fluorescens* was tested. Different isolates of *Pseudomonas fluorescence* were evaluated for their efficacy against the growth of *Xanthomonas axonopodis* pv. *citri* by inhibition zone assay method. A suspension (3 day old) of *Xanthomonas axonopodis* pv. *citri* multiplied in nutrient broth was mixed with lukewarm nutrient agar medium. Fifteen to twenty ml of seeded medium poured into the sterilized petriplates and allowed to solidify. A loop full culture of each *Pseudomonas fluorescens* isolate placed in the centre of petriplates containing the seeded medium. The inoculated plates then incubated at 28°C for 72 hours. Antagonistic activity of different *Pseudomonas fluorescens* isolates were determined by measuring inhibition zone of pathogenic bacteria in petriplates.

Result and Discussion

Six isolates of Pseudomonas fluorescens were obtained from rhizosphere of different crops and one from Plant Pathology Section, College of Agriculture, Nagpur were designed as PF-1 to PF-7. Isolate PF-1 was procured from Pathology Section, College of Agriculture, Nagpur, PF-2 from groundnut rhizosphere and PF-3 from chickpea rhizosphere. Two isolates namely PF-4 and PF-5 were isolated from soybean and maize rhizosphere.

Isolates namely PF-6 and PF-7 isolated from jowar and citrus rhizosphere, respectively. The association of bacterial bioagent i.e. *Pseudomonas fluorescens* has also been reported by (Dave and Dube, 2000, Yeole and Dube, 2001, Gholve *et al.*, 2006 and Kaur *et al*., 2007) [4, 17, 5, 7] from rhizosphere of various crop.

Morphological and biochemical characterizations

Result presented in Table 1 showed that all isolates were rod and Gram negative in reaction. All the isolates were able to liquefy the gelatin and were capable of H_2S production. Among these all the isolates shows positive for KOH test, catalase test, acid and gas production. Out of seven isolates of *Pseudomonas fluorescens* only the four isolates were able to hydrolyse the starch.

Different biochemical tests were carried out for confirmation of *Pseudomonas fluorescens*. Among them test gelatin liquefaction, H_2S production, starch hydrolysis, catalase test, KOH test, acid and gas production test gave positive reaction and confirmed the isolate as Gram negative, similar studies have also reported by several workers.

Meera and Balabaskar (2012) [10] studied seven isolates of *P. fluorescens* in detail for colony, colour, growth type, fluorescence and cell shape. *P. fluorescens* showed that all the isolates produced similar results with regard to Gram staining (negative), starch hydrolysis (negative), gelatin liquefaction (positive), catalase test (positive), oxidase test (positive) and fluorescent pigmentation (positive).

Saravanan *et al*. (2013) [14] reported biochemical characteristics of fluorescent pseudomonas showed that all ten isolates were positive to catalase, amylase, gelatinase and Siderophores production.

Table 1: Biochemical characterizations of *Pseudomonas fluorescens* isolated from rhizospheric soil of field crop.

Sr. No.	Characters	Reaction of isolates						
		PF-1	PF-2	PF-3	PF-4	PF-5	PF-6	PF-7
Morphological characterization								
1	Gram staining	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
3	Colony colour	WY	WY	WY	WY	GY	WY	WY
Biochemical characterization								
4	KOH test	+	+	+	+	+	+	+
5	Catalase test	+	+	+	+	+	+	+
6	Gelatin liquefaction	+	+	+	+	+	+	+
7	H_2S production	+	+	+	+	+	+	+
8	Starch hydrolysis	+	-	+	-	-	+	+
9	Acid and gas production	+	+	+	+	+	+	+

+: positive reaction -: negative reaction WY: whitish yellow GY: greenish yellow

Plant growth promoting studies of *Pseudomonas fluorescens*

Table 2 indicate plant growth promoting characteristics of *Pseudomonas fluorescens.* Development of cherry red colour upon addition of Kovac's reagent to culture supernatant of *Pseudomonas fluorescens* strain confirmed IAA production. All the isolates were positive for production of IAA except (PF-3). Shinde (2003) studied the plant growth promoting activities of rhizobacteria and noted IAA production of nine out of ten *Pseudomonas fluorescens*. Phosphate solubilization

by bacterial strain was found positive as they formed clear zone on Pikovskaya's agar medium. All the isolates were shows positive to solubilize the phosphate. Similarly 19 isolates of phosphate solubilizing bacteria showed the highest halo colony ratios, the halo colony ratio indicate that these isolates were the putative bacteria with phosphate solubilizing activities reported by Ruangsanka (2014) [13]. Change in colour from yellow to reddish brown of filter paper confirmed production of strong HCN. All the isolates of *Pseudomonas fluorescens* showed negative reaction for HCN production. Mahesh (2007) [11] also studied ten isolates of *Pseudomonas fluorescens* and found that all tested isolates showed negative for HCN production.

Table 2: Plant growth promoting properties of *Pseudomonas fluorescens*

SN.	Characters	Reaction of isolates						
		PF-1	PF-2	PF-3	PF-4	PF-5	PF-6	PF-7
1	IAA	+	+	-	+	+	+	+
2	Phosphate solubilization	+	+	+	+	+	+	+
3	HCN	-	-	-	-	-	-	-

'+' positive test '–' negative test

Physiological Characterization

Temperature

Table 3 indicates all the isolates shows the maximum growth at temperature ranges between 25 and 30°C with an OD value ≥60 followed by 20 and 35°C with an OD value ranging between 0.31 to 0.60 and minimum growth of bacteria observed at 5, 10, 15 and 40°C with an OD value ranging between 0.01 to 0.30 indicating the physiological properties of *Pseudomonas fluorescens*. The present result are in conformity with the result obtained by Waghunde *et al.* (2013) [16] they isolated seven *Pseudomonas* isolates from different locations of South Gujarat, all the isolates were able to grow at 10 to 42°C temperature and fail to grow at 4°C.

Table 3: Effect of temperature regimes on growth of *Pseudomonas fluorescens*

SN.	Temperature	PF-1	PF-2	PF-3	PF-4	PF-5	PF-6	PF-7
1	5°C	+	+	+	+	+	+	+
2	10°C	+	+	+	+	+	+	+
3	15°C	+	+	+	+	+	+	+
4	20°C	++	++	++	++	++	++	++
5	25°C	+++	+++	+++	+++	+++	+++	+++
6	30°C	+++	+++	+++	+++	+++	+++	+++
7	35°C	++	++	++	++	++	++	++
8	40°C	+	+	+	+	+	+	+

+: 0.01 to 0.30 ++: 0.31 to 0.60 +++: ≥60

pH

All the isolates showed maximum growth at 6 and 7 pH range with an OD value ≥60 followed by 8 pH with an OD value ranging between 0.31 to 0.60. The least growth was observed only at 4, 5, 9 and 10 pH with an OD value ranging between 0.01 to 0.30 (Table 4). The results corroborate with the finding of Stolp and Gadkari, (1970) [15] indicated that optimum temperature and pH for *Pseudomonas* spp., were 30°C and pH 7-8.5, respectively.

Table 4: pH requirement for growth of *Pseudomonas fluorescens*

SN.	pH requirement	PF-1	PF-2	PF-3	PF-4	PF-5	PF-6	PF-7
1	4	+	+	+	+	+	+	+
2	5	+	+	+	+	+	+	+
3	6	+++	+++	+++	+++	+++	+++	+++
4	7	+++	+++	+++	+++	+++	+++	+++
5	8	++	++	++	++	++	++	++
6	9	+	+	+	+	+	+	+
7	10	+	+	+	+	+	+	+

+: 0.01 to 0.30 ++: 0.31 to 0.60 +++: ≥60

Table 5: Antagonistic activity of Pseudomonas fluorescens against Xanthomonas axonopodis pv. Citri

SN.	Bacterial strain	Inhibition zone (mm)
1	*Pseudomonas fluorescens* (PF-1)	13.2
2	*Pseudomonas fluorescens* (PF-2)	20.1
3	*Pseudomonas fluorescens* (PF-3)	15.3
4	*Pseudomonas fluorescens* (PF-4)	28.7
5	*Pseudomonas fluorescens* (PF-5)	18.2
6	*Pseudomonas fluorescens* (PF-6)	–
7	*Pseudomonas fluorescens* (PF-7)	29.1
8	Control	00

Antagonism of *Pseudomonas fluorescens* against *Xanthomonas axonopodis* pv. *citri*

The result of antagonistic activity of *Pseudomonas fluorescens* isolates against *Xanthomonas axonopodis* pv. *Citri in vitro* condition are shown in table 5. It shows inhibition zone induced by antagonistic bacteria *Pseudomonas fluorescens* isolates. The highest inhibition zone observed in strain PF-7 (29.1 mm) followed by strain PF-4 (28.7 mm). No inhibition zone was induced by strain PF-6. The results corroborate with the finding of several workers (Kalita *et al*., 1996; Khodakaramian *et al.*, 2008; Abhang *et al.*, 2015; Apet *et al.*, 2018) [6, 8, 2, 3].

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