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Isolation and Biochemical Characterization of *Pseudomonas fluorescens* Isolated from Rhizosphere of Different Host Plant

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Abstract Soil is the reserve house for microorganisms, which provides nutrient and space for the survival and growth of microorganisms. Those microorganisms may be beneficial or harmful to the host plants. Therefore, isolation and characterization of antagonistic *Pseudomonas fluorescence* stains carried out at dept. of plant pathology, GBPUA and T. There are six *Pseudomonas fluorescence* strains were isolated from rhizosphere of different crop plants viz., Rice (namely PfR1 and PfR2), Sorghum (namely PfS1 and PfS2) and Sponge gourd (namely PfSGI and PtSG2). In visual observation of all isolates under UV light at 365nm showed fluorescence. Therefore, biochemical characterization of these isolates done by different biochemical test viz., Gram staining, Catalase test, starch hydrolysis, Milk proteolysis, Arginine dihydrolase test, Gelatin liquefaction, Tween 80 hydrolysis and Salt tolerance. All the isolates showed negative response in Gram staining and positive response in catalase test. Therefore, it shows that all the isolates were *Pseudomonas fluorescence.*

Keywords *Pseudomonas fluoresceence,* Rice, Sorghum, Sponge gourd, Biochemical.

Introduction

The rhizosphere, represents the thin layer of soil adhering around the roots of the plant. This rhizosphere provides congenial condition for the microbial activities, it contains lots of root exudates, metabolites and antibiotic which attracts the microorganisms. Plant growth promoting rhizobacteria rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface [1]. These organisms can also be beneficial to the plant by stimulating growth [2]. *Pseudomonas* species are the prominent plant associated bacteria which are widely used to induce systemic resistance against many plant pathogens by secretion of antimicrobial metabolites [3]. Among these organisms, genus *Pseudomonas* are gram negative, motile and oxidase positive belong to ÿ-proteobacteria. The Pseudomonads are capable to grow on very simple nutritional requirements as well as grow well under normal conditions. They produce secondary metabolites such as antibiotics, siderophores, volatile compound hydrogen cyanide (HCN) and phytohormones *P. fluorescens* was reported to provide 79-82% control of rice blast and sheath blight and thus increase grain yield in rice [4].

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P. fluorescens having ability to suppress soil borne fungal pathogens by the production of antibiotic metabolites such as pyoluteorin, phenazine, pyrrolnitrin, 2, 4-diacetylphloroglucinol (DAPG) and 1- carboxylic acid. The metabolites DAPG is a major factor in the biological control of a range of plant pathogens. In consideration of their immense potential in the management of plant diseases, it is utilizing as good a biocontrol agents. During recent years, the use of *P. fluorescens* as a biocontrol agent has been popularized so much. Therefore, the present studies have been done to isolate and characterize *P. fluorescens* isolates, which has been collected from different crop rhizosphere viz., Rice, Sorghum and Sponge gourd.

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Materials and Methods

Soil sample collection

The rhizospheric soil samples were collected from three different crops and three different localities of the G.B Pant University of Agricultur and Technology. The samples were collected from Rice field of Crop Research Center (CRC), Sorghum field of Livestock Research Center (LRC) and Sponge gourd field of Vegetable Research Center (VRC) of the university. Each samples were kept in polythene bag and brought to biocontrol lab of the department of plant pathology, GBPUA and T.

Isolation of *Pseudomonas fluorescens*

Isolation of *P. fluorescens* isolates were carried out on King's B medium. 1g of rhizospheric soil sample was suspended in 99 ml of sterile distilled water. Samples were serially diluted and 0.1 ml of sample was spreaded on King's B medium poured plates. After incubation at 27 ± 1^{0}C for 48 h the plates were exposed to UV light at 365 nm for few seconds and the colonies exhibiting the fluorescence were picked up and purified on King's B medium poured plates, for short term maintenance of the culture streaked on to the slants. The isolated samples were designated as PfR1 and PfR2 for rice samples, PfS1 and PfS2 for Sorghum and PfSG1 and PfSG2 for sponge gourd samples and kept for further studies.

Biochemical characterization of isolated *P. fluorescens*

In order to identify the species, the different biochemical tests were carried out. The Gram-reaction of each isolate was determined following the staining procedure. For catalase test, few drops of 3% hydrogen peroxide was added on the surface of 48 h old culture of each isolate on YPSA medium and bubble formation was recorded as positive for catalase activity [5]. For Gelatin liquefaction, Gelatin medium containing beef extract, 3g; Peptone, 5g and Gelatin, 120g in 1 liter distilled water was prepared, the medium was stab inoculated with each isolate grown for 48 h on YPSA medium and incubated at 28^{0}C. After 3, 7 and 21 days of incubation, each isolate was evaluated for gelatin liquefaction. The isolates in test tubes were kept at 4^{0}C for 30 minutes and gently tipped immediately. A medium that flows readily as the tube is gently tipped was took as positive for gelatin liquefaction [5]. For Starch hydrolysis, the isolates were streaked on starch agar medium (starch soluble, 20g; Peptone, 5g; Beef extract, 3g; agar, 15g dissolved in 1 liter distilled water, maintain pH 7 and autoclaved at 121^{0}C for 15 minutes) to evaluate their ability to hydrolyze starch (amylase production). The appearance of clear zone around the line of growth of each isolate indicated starch hydrolysis [6]. For Milk proteolysis, the ability of the isolates to degrade the protein case in by producing proteolytic exo-enzymes was tested by growing the isolates on milk agar plates (Skim milk powder, 100g; Peptone, 5g; Agar, 15g in 1 liter distilled water with PH 7.2 autoclaved at 121^{0}C for 15 minutes). Clear zone around the growth of the isolates was recorded as positive for casein hydrolysis [6]. For Arginine dihydrolase test, the test medium used contains (in g/l); Bacteriological peptone 1, NaCl 5, K_2HPO_4 0.3, phenol red 0.01, L+arginineHCl 10, pH 7.2. 4-5 ml of the medium was dispensed in test tubes and autoclaved. After autoclaving bacterial isolates were stab inoculated to base of the medium and incubated for 7 days at 27^{0}C. A positive alkaline reaction was indicated by a deep red color change compared with orange-pink by controls [7]. For, Tween 80 hydrolysis, Tween 80 hydrolysis medium used contain (g/l) Pep-

Table 1. Biochemical tests of *P. fluorescence* isolates.

Biochemical tests	PfR1	PfR2	PfS1	PfS2	PfSG1	PfSG2
Gram staining	-	-	-	-	-	-
Fluorescence	+	+	+	+	+	+
Milk proteolysis	-	+	+	-	-	+
Catalase activity	+	+	+	+	+	+
Tween 80 hydrolysis	+	+	+	+	+	+
Arginine dehydrolase test	+	+	+	+	+	+
Starch hydrolysis	-	+	+	-	-	+
Gelatin liquefaction after 3 days	+	+	+	+	+	+
7 days	+	+	+	+	+	+
21 days	+	+	+	+	+	+
Salt tolerance 1%	+	+	+	+	+	+
2%`	+	-	-	+	+	+
3%	-	+	+	-	-	+

tone 10, NaCl 5, $CaCl_2$. $2H_2O$ 0.1, Agar 20 and pH was adjusted to 7.4. Tween 80 was autoclaved separately in 10 ml quantities added to IL of the medium, mixed well and poured into the plates and bacterial isolates were streaked at the center of the plate and the plates were incubated for 7 days at 25-27^{0}C. Development of opaque zones on medium around the bacterial growth indicates positive reaction [7]. For Salt tolerance, the bacterial isolates were inoculated on 1%, 2% and 3% salt concentration containing autoclaved YS (Yeast Salt) broth medium (g/L) [$NH_4H_2PO_4$-0.5; K_2HPO_4-0.5; $MgSO_4.7H_2O$-0.2; Yeast extract-5 and required concentration of NaCl 1,2 or 3g/L] and incubated at 28 ± 1^{0}C for 14 days. Salt tolerance was observed by seeing the visible turbidity after incubation [7].

Results and Discussion

In this research six isolates of *P. fluorescens* (two from each crop) were isolated from different host rhizosphere namely Rice (PfR1 and PfR2), Sorghum (PfS1 and PfS2) and Sponge gourd (PfSP1 and PfSP2) on King 'B Agar medium and observed under UV light at 366nm for few second. All the isolates namely PfR1, PfR2, PfS1, PfS2, PfSP1 and PfSP2 were showed fluorescence under UV light. Therefore, the colonies were picked up from fluorescent region for pure culture maintenance and further studies. All the six isolates were identified as *P. fluorescence* strains using Bergey's manual of Determinative Bacteriology based on phenotypic and biochemical characteristics. All the six isolates were studies showed negative Gram staining reaction (Table 1).

All the six isolates showed positive catalase test, Tween 80 hydrolysis, Gelatine liquefaction and Arginine dehydrolase test (Table 1). Similar observation of *P. fluorescence* towards positive test to gelatin hydrolysis was found earlier [8]. Maleki et al. [9] and Mayz et al. [10] observed similar result of positive response of *P. fluorescence* strains in catalase, gelatin liquefaction and Arginine dehydrolase test. While, only three isolates of *P. fluorescence* namely PfR2, PfS2and PfSG2 showed positive reaction in the milk proteolysis and starch hydrolysis (Table 1). The ability of *P. fluorescens* P60 in hydrolyzing starch was a specific character of most negative gram bacteria.

In the salt tolerance test, at 1% concentration all the six *P. fluorescens* isolates showed positive reaction. While, at 2% salt concentration only four isolates namely PfR1, PfS2, PfSG1 and PfSG2 showed positive reaction. Similarly at 3% salt concentration only three isolates namely PfR2, PfS1 and PfSG2 showed positive reaction. The isolates PfSG2 found to be tolerant at all the concentration (1%, 2% and 3%) of the salt (Table 1). Johri et al. [11] isolated and characterized salinity tolerant phosphate solubilizing bacteria that could survive at 5% NaCl concentration. Recently. Tank and Saraf [12] reported the plant growth promoting effect of *P. fluorescens* and *P. aeruginosa* on tomato and these strains were able to survive at 6% NaCl. Paul et al. [13] explained that *P. fluorescens* strain MSP-393 synthesized novel proteins which nullified detrimental effects of high osmolarity. Salt stress tolerance is an important aspect of competitiveness and saprophytic ability among rhizobial isolates.

Conclusion

This study provides the biochemical and physical identification of the different isolates of the *P. fluorescens* which were isolated from different host rhizosphere. The identification of species and strains have necessary to understand the nature and biology of the microorganisms. Therefore, the identified strains could be used for the isolation and identification of effective antifungal metabolites and antibiot-

ics. It will also help to know about the effective biocontrol strains of the identified isolates.

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