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Research Article

ISOLATION AND CHARACTERIZATION OF FLUORESECENT PSEUDOMONADS FROM RICE AND CARROT RIZOSPHERIC SOIL

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ABSTRACT
The genus *Pseudomonas* represents a group of microorganisms with high metabolic versatility. Fluorescent pseudomonads are well known rhizospheric inhabitants. The present study reports the isolation and characterization of six isolates fluorescent pseudomonads from rice and carrot-rhizospere soils. The isolates were characterized morphologically and biochemically and were tested for their ability to produce siderophore, catalase and oxidase. Biosynthesis of siderophores by the isolates found dependent on the environmental factors like incubation temperature and the pH of the medium. Maximum siderophore production was observed at temperature 37^0C and pH range 7.0 to 9.0. The quantitative siderophore production was evaluated using chromeazurol-sulfonate assay, where the isolates R1 and C2 appeared predominant.

Keywords: *Fluorescent Pseudomonads, Siderophore, Rice Rhizosphere, Carrot Rhizosphere*

INTRODUCTION
Pseudomonads are gram-negative, straight or slightly curved rods with polar flagella. The aggressive colonization of plant root and rhizosphere soil by these organisms is due to their ability to utilize a variety of substrates such as organic acids, sugars and amino acids secreted by plant roots in the form of root exudates **(**Lugtenburg and Dekkers, 1999**)**. This group of bacteria is considered to be the most promising group among the rhizobacteria, involved in biocontrol of plant diseases, maintenance of soil health and influence plant growth directly or indirectly **(**Kloepper *et al.,* 1980**)**. Direct promotion of plant growth is through the production of siderophores **(**Cattelan *et al.,* 1999; Mavrodi *et al.,* 2001**)** and phosphatase enzyme (Katznelson and Bose, 1959) that can solubilize iron and phosphorus respectively from the soil and make them accessible to plants. Bacteria may fix nitrogen in the form that can be taken up and used by plants. Fluorescent pseudomonads produce fluorescent pigments like pyoverdine. Generally fluorescent pseudomonads produce lots of pigments like pyochelin, pyocyanin, pyorubin, oxiphenazin, chlororaphin etc. but in small quantities. *Pseudomonas* species thrive under moist conditions in soil (particularly in association with plants), and in sewage sediments and the aquatic environments. Presence of Pseudomonads in rhizosphere of different crops has been previously reported (Misko and Germida, 2002; Khalis *et al.,* 2004; Malik *et al.,* 1997; Lemanceau *et al.,* 1995). They also can produce Indole Acetic Acidand promote growth of plant roots and shoot elongation (Rodriguez and Fraga, 1999). Several studies reported that the in number of fluorescent as well as non-fluorescent Pseudomonads in rice rhizosphere is dominant (Kloepper *et al.,* 1989; Rangarajan *et al.,* 2002). *P. aeruginosa* is capable of growing in conditions of extremely low nutrient content (Palleroni, 1984). *P. fluorescens* is commonly found inhabiting plant rhizosphere or phyllosphere environments. The plant rhizosphere provides an environment in which the species may show improved survival and growth. *P. fluorescens* distributed homogeneously in soil and can result in significantly higher numbers in the rhizosphere of young wheat plants than in non-rhizosphere soil (Trevors *et al.,* 1990). Foliar plant pathogens such as *P. syringae* are generally not adapted to survival in the soil (Lindow and Panopoulos, 1988). However, *P. syringae* has been isolated from plant debris in the soil and can overwinter in temperate climates (Hirano and Upper, 1983**)**.

Interest in the *Pseudomonads* has increased recently because of the possible use of siderophores as biopesticides and the possible use of *pseudomonads* in detoxifying chemical wastes through a wide range of enzymatic metabolic activities (Angel *et al.,* 2013). Siderophores are known to play significant role in

bioremediation of heavy metals, where many microorganisms with heavy metal tolerance ability have been shown to produce siderophores (Ahmed and Holmstrom, 2014; Bitla and Sawant, 2014). The present study describes six *Pseudomonas* strains isolated from rhizosphere of carrot and rice respectively. The evaluation of siderophore production ability of these isolates yielded significant data regarding the iron uptake in the sampled rhizosphere.

MATERIALS AND METHODS

a) Collection of Samples and Isolation of Fluorescent Pseudomonads

The rice and carrot rhizospheric soil samples were collected in sterile petri dishes from Pethvadgaon, Kolhapur and Vihapur, Sangli, (MS) India. About 1gm of soil samples were serially diluted using sterile distilled water. Then 0.1ml of $10^{-4}$was spread inoculated on King's B agar (King *et al.*, 1954) medium and incubated at 37^0C for 48 h. After incubation all plates of all the plates were observed under UV light for detection of fluorescence from which, 6 colonies showing fluorescence were isolated, purified and maintained on King's B agar slopes.

b) Characterization of the Isolates

All the isolates were inoculated on Kings B agar medium incubated at 37^0C for 24h and examined for colony morphology, fluoresecens on King B medium and Gram reaction as per standard procedure.

Biochemical Tests

For the identification of isolates, certain biochemical tests were conducted according to Bergey's Manual for Determinative Bacteriology (Breed *et al.*, 1989).

Detection of Siderophore Production

Loopfull suspension of all the isolates of was spot inoculated on CAS agar (Schywn and Neilands, 1987) plates and incubated at room temperature for 4 days. During incubation all plates were observed for the presence of orange halo surrounding the growth.

Effect of Temperature on Siderophore Production

The 0.1ml suspension of all isolates were inoculated in 3ml of sterile Kings B broth and incubated at different temperatures (4^0C, 10^0C, 28^0C, 37^0C and 50^0C) for 48 hours. After incubation, the tubes were centrifuged at 5000 rpm for 10 minutes and optical density at wavelength 480 nm was recorded.

Effect of pH on Siderophore Production

The 0.1ml suspension of all isolates were inoculated in 3ml of sterile Kings B broth and incubated at different pH values like 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 for 48 hours at 37^0C. After incubation, the tubes were centrifuged at 5000 rpm for 10 minutes and optical density at wavelength 480 nm was observed and recorded.

RESULTS AND DISCUSSION

Six isolates-three from each, rice and carrot field each of fluorescent Pseudomonads were obtained by serial dilution on Kings B agar medium and observed under UV light.

Table 1: Carbohydrate Utilization

Sugar	R1	R2	R3	C1	C2	C3
Glucose	+	+	+	+	+	+
Sucrose	-	+	-	-	-	-
Mannitol	+/-	-	-	-	-	-
Lactose	-	-	-	-	-	-
Galactose	-	-	-	+	+	-
Maltose	-	-	-	+	-	-
Xylose	+	-	+	+	+	+
Arabinose	+	-	-	+	-	+
Ribose	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-

The isolates showed distinct morphological characters (Table 1). All isolates were actively motile and were Gram negative short rods.
All isolates showed fluorescence under UV light and were Oxidase and Catalase positive. The isolates differed in sugar fermentation abilities.

Table 2: Enzymatic Properties

Enzyme	R1	R2	R3	C1	C2	C3
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Gelatinase	+	-	-	-	-	-
Pectinase	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-
Lecithinase	+	+	+	+	+	+
Lipase	-	-	-	-	-	-

Table 3: Hugh and Leifson's Test

Incubation condition	R1	R2	R3	C1	C2	C3
Aerobic	-	+	+	+	+	+
Anaerobic	+	+	+	+	+	+

Table 4: Biochemical characters

Test	R1	R2	R3	C1	C2	C3
Arginine hydrolysis	+	+	+	+	-	+
Phenyl alanine	-	-	-	-	+	-
Growth at 4^0C	-	-	-	-	-	-
Growth at 41^0 C	+	+	+	+	+	+
Fluorescens at 41^0C	+	+	+	-	-	-

Detection of Siderophore Production
The presence of orange halo on CAS agar, surrounding the colonies of all the isolates showed active siderophore production ability of the isolates-for sequestration of iron from surrounding environment in iron starvation conditions.

Effect of Temperature on Siderophore Production
The impact of temperature on siderophore production was evaluated by cultivating all the isolates at various temperatures. Depending on OD at 480 nm wavelength, we found that, siderophore production started at 10^0C; however, at the temperature 37^0C all the isolates exhibited maximum siderophore production. None isolate shows siderophore production at 50^0C; which may be because at these temperatures fluorescent pseudomonas was not able to grow or produce the siderophore. The temperature range of 28^0C to 37^0C found suitable for optimum siderophore production for all isolates.
Isolate R3 and C1 showed maximum siderophore production. R1 and R2, C2 and C3 showed similar siderophore production (Figure 1). The results are presented graphically.

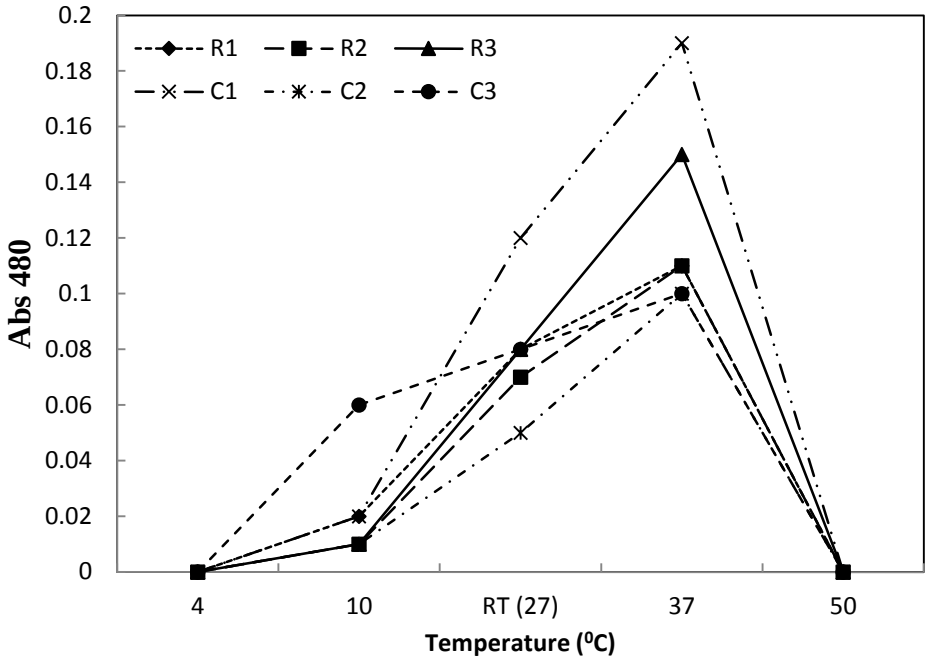

Figure 1: Effect of temperature on siderophore production

Effect of pH on Siderophore Production

The influence of pH on siderophore production was determined using King's B medium with various pH.

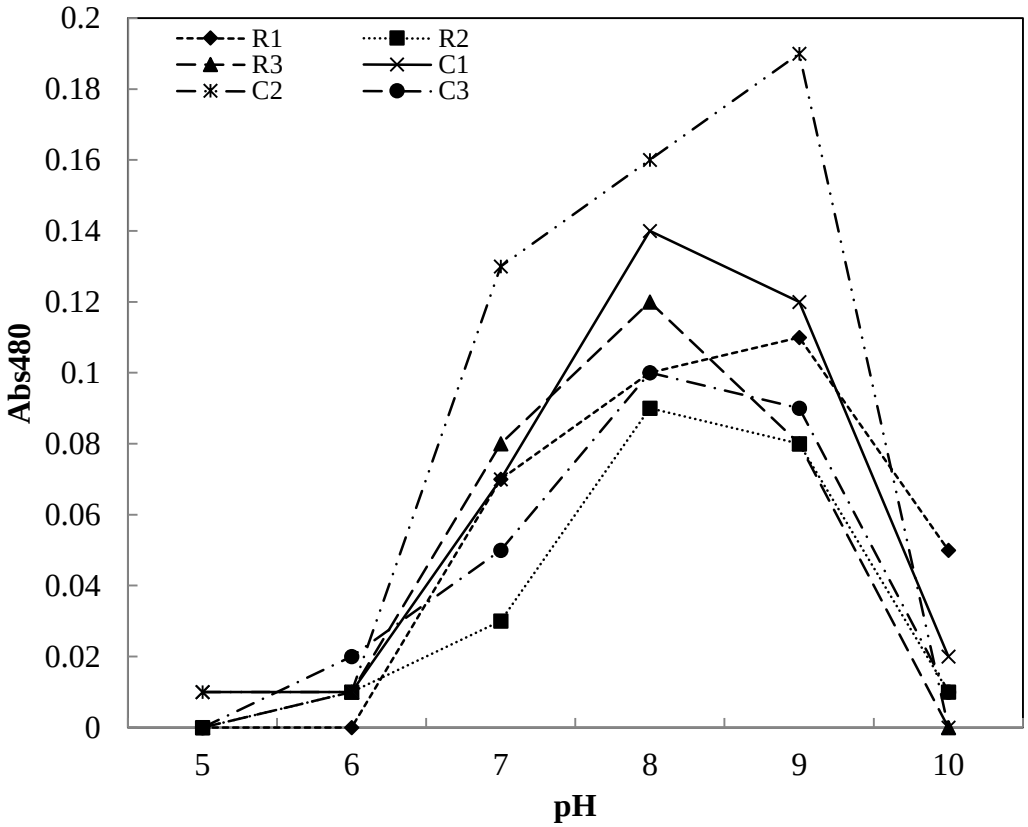

Figure 2: Effect of pH on siderophore production

Depending on optical density at 480 nm of media after centrifugation it was noted that, majority of the isolates of fluorescent Pseudomonads showed maximum siderophore production at pH 8.0 (isolates R2, R3, C1 and C3); while the isolates R1 and C2 required pH 9.0 for maximum siderophore production.
Generally the range of pH 7.0 to pH 9.0 is optimum for siderophore production among rhizospheric bacteria; maximum siderophore production within the pH range 7.0-7.5 has been reported from earlier studies from Sangli region (Supanekar *et al.*, 2013a,b; Supanekar and Sorty, 2013). At pH 10.0 there is rapid decrease in siderophore production was recorded. This confirmed the fact that siderophore production is highly dependent on the pH of the surrounding environment. Isolate C2 showed maximum siderophore production among all isolates, while the isolate R2 showed lowest one (Figure 2).

Conclusion

The isolated fluorescent *Pseudomonads* were found to produce siderophores in iron deficient conditions. The isolate R1 and C2 gave the higher siderophore production at pH 9.0 while others gave at pH 8.0 concluding that they are different from each other. The all isolates showed maximum siderophore production at temperature 37^0C, among them C1 is dominant. Further characterization of the isolates is needed for determination of their plant-growth promoting potential.

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