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Evaluation of Antifungal Activity of Siderophore Produced by Fluorescent *Pseudomonas* sp. Isolated from Paddy Rhizosphere

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Fluorescent *Pseudomonas* sp. isolated from paddy rhizosphere was biochemically confirmed and screened for siderophore production. The isolate was able to produce two types of siderophores ie, hydroxamates and catecholates and the production were assayed at different pH and different time intervals. The production of hydroxamate type siderophore was high at pH 8 (246 μg ml^{-1}) and on 3rd day while the production of catecholate type siderophore at pH 7(146 μg ml^{-1}) and on 3rd day. The bacterial isolate and their crude extract of siderophore were screened for antifungal activity against two phytopathogens *Fusarium oxysporum* and *Alternaria* sp. and the isolate showed good inhibition against both the pathogens tested. The crude extract (hydroxamate and catacholate) showed higher inhibition at a concentration of 0.6 ml.

Key words: Fluorescent *Pseudomonas* sp. siderophores, antifungal effect.

Paddy, *Oryza sativa* is the staple food crop of Asians and its cultivation in large quantity is very important. Damage to paddy plants are caused by insects pests, rates, nematodes, fungi, bacteria and viral pathogens. The fungal pathogens could be controlled using biocontrol agents such as bacteria fluorescent *Pseudomonas* sp. is a Gram-negative bacteria which is chemoheterotropic, motile rods with polar flagella and are grouped in rRNA homology group I, as defined by Palleroni *et al* (1973).

In order to facilitate iron to the cell, bacteria secrete iron binding ligands called siderophores, which can bind the ferric iron and make it available to the host organism. Siderophores are "low molecular mass, virtually ferric specific ligands, that is carefully regulated by iron and their function is to supply iron to the host cell" said Neilands, (1981). The structure of siderophores depends on the microorganism that produces the siderophores, a common feature of all the siderophores is that they form six coordinate octahedral complexes with ferric iron (Raymond *et al* 1984). Most known siderophores can be grouped into hydroxamate, and phenolate/ catacholate type structures and they have different affinities for ferric iron (Neilands, 1981).

The present study was carried out on the evaluation of antifungal activity of siderophore produced by fluorescent *Pseudomonas* sp. isolated from paddy rhizosphere.

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MATERIAL AND METHODS

Sample collection

The rhizosphere soil was collected from root zone of paddy crop (ATD45 variety) in the cultivation land, at Athoor village, Dindigul district, Tamil Nadu, South India.

Isolation of fluorescent *Pseudomonas* sp

The fluorescent *Pseudomonas* sp. was isolated by serially diluting the sample (10-5) and plating on the King's B medium and they were characterized based on Gram's staining and various biochemical tests such as IMViC, Starch hydrolysis, Casein hydrolysis, Gelatin hydrolysis, Arginine dehydrolase activity, Nitrate reduction and Catalase test.

Screening of fluorescent *Pseudomonas* sp for siderophore production

The selected fluorescent *Pseudomonas* sp was further screened for siderophore production. The medium used to screen the bacterial cells is glucose aspargine minimal medium. (Bultreys & Gheysen, 2000). The siderophore production was determined by mixing the culture supernatant with 2 percent $FeCl_3$ solution. The appearance of orange red brown color was taken as an indication for the production of siderophore.

Estimation of siderophore at different pH and at different day intervals

The culture supernatant of fluorescent *Pseudomonas* sp was collected and estimated for the two types of siderophores at different pH ranging from 3 to 13 at every 24 hours interval from first day to seventh day using Atkin's assay for hydroxamate type siderophore (Atkins *et al,* 1970) and Arnow assay for catecholate type siderophore (Arnow, 1937). Salicylic acid and catechol were used as standards to characterize hydroxamate and catecholate siderophores respectively.

Analysis of antifungal activity of crude siderophore

The phytopathogens *Fusarium oxysporum* and *Alternaria* sp were collected from Tamil Nadu Agricultural University, Madurai and these fungal isolates were sub cultured in the laboratory and maintained on potato dextrose agar (PDA) slants. Antifungal activity of fluorescent *Pseudomonas* sp was determined on PDA medium, 1 ml of the bacterial isolate was swabbed on the PDA plates and the fungal culture was transferred at the centre of the petriplate. Another set of PDA plates were also swabbed with 0.2, 0.4, 0.6 ml of the culture supernatant and the fungal culture was placed at the centre and the antifungal activity of both the culture and the culture supernatant (crude siderophores) were determined.

RESULTS AND DISCUSSION

The fluorescent *Pseudomonas* sp isolated from the paddy rhizosphere (ATD 45 variety) soil was identified based on various biochemical tests (Table 1). The bacterial cells were able to grow in glucose aspargine minimal medium and the culture supernatant of fluorescent *Pseudomonas* sp also formed orange red brown colour when it was mixed with 2 percent $FeCl_{3;}$ this observation indicates that the fluorescent *Pseudomonas* sp is able to produce siderophore. Atkin's assay for hydroxamate type siderophore and Arnow's assay for catacholate type siderophore indicates that the fluorescent *Pseudomonas* sp was able to produce both the type of siderophores.

Studies on optimization of pH for siderophore production showed an increase in

Table 1. Biochemical characteristics of fluorescent *Pseudomonas* sp

Characteristics of the test isolate	Fluorescent *Pseudomonas* sp.
Fluorescence under UV light	+
Gram's staining	-
Biochemical tests·	
*Indole test	-
*Methyl Red	-
*Vogues Proskauer test	-
*Citrate utilization test	+
*Arginine dehydrolase test	+
*Starch hydrolysis	-
*Casein hydrolysis	+
*Gelatin hydrolysis	+
*Catalase test	+
*Nitrate Reduction test	+

(+)Positive (-)Negative

the rate of production with the increase in the pH from 3 to 13 (Fig. 1). The highest yield of hydroxamate type siderophore was observed at pH 8 (246 µg ml^{-1}) and catacholate type at pH 7 (146 µg ml^{-1}).

Studies on optimization of days for

Table 2. The antifungal activity of the fluorescent *Pseudomonas* sp strain and its supernatant at various concentrations

Antifungal activity of fluorescent *Pseudomonas* sp at various concentrations		Organisms tested	
		Fusarium oxysporum	*Alternaria* sp.
Fluorescent *Pseudomonas* sp culture	1 ml	+++	+++
Supernatant of Fluorescent *Pseudomonas* sp	0. 2 ml	+	+
Supernatant of Fluorescent *Pseudomonas* sp	0.4 ml	++	++
Supernatant of Fluorescent *Pseudomonas* sp	0.6 ml	+++	+++

\+ - low inhibition; ++ - medium inhibition; +++ - good inhibition

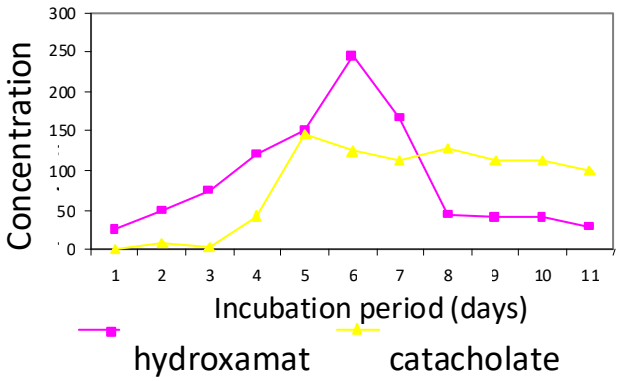

Fig. 1. Estimation of hydroxamate and catacholate siderophores produced by fluorescent *Pseudomonas* sp at different pH level

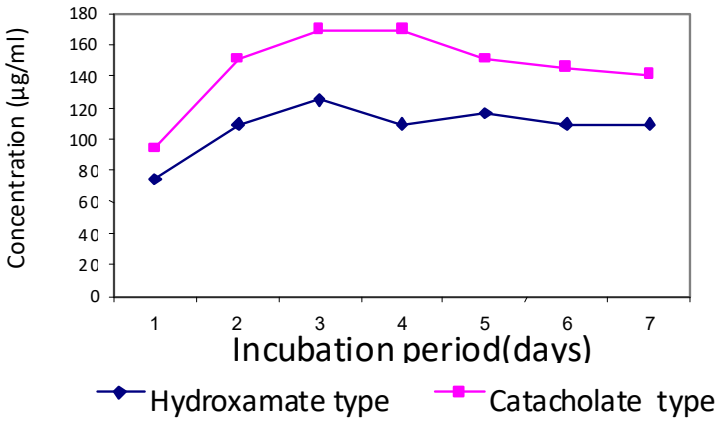

Fig. 2. Determination of hydroxamate and catacholate siderophore produced by fluorescent Pseudomonas sp. at different incubation period

hydroxamate type siderophore production showed increased production on 3 day (125 µg ml^{-1}) and increased rate of catacholate type of siderophore was noticed on 3 day (170 µg ml^{-1}) respectively (Fig. 2). Similar kind of work was done by Yang *et al*., (1990) and they have reported that Siderophore production was evident at late log phase after the culture reached stationary phase and the concentration of siderophore continued to increase slowly and the activity remained stable throughout the assay period of 72 h.

The results of the antifungal studies showed that the fluorescent *Pseudomonas* sp was able to inhibit the growth of fungal pathogens *Fusarium oxysporum* and *Alternaria* sp efficiently. The supernatant of the bacterial culture showed a higher growth inhibition at 0.6 ml (Table 2). Similar kind of work was carried out by Letty *et al*., (1985) and they have reported that the antifungal activity of the supernatant containing crude siderophore reacted positively in the assay for hydroxamate type siderophore and negatively for the phenolate type of siderophore.

In the present study, the fluorescent Pseudomonas sp from the rice rhizosphere exhibited the presence of siderophores and it also showed good fungicidal property. Further detail study is essential for standardizing the dosage of application through field trails.

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