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Characterization and Evaluation of Siderophore Producing Rhizospheric *Pseudomonas fluorescens* as *R. oryzae* and *R. solani* Antagonists

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To detect the potential influence of siderophores produced by *Pseudomonas fluorescens* on control of pathogens *viz. Rhizoctonia oryzae* and *Rhizoctonia solani* causing Sheath blight of paddy and Black scurf of potato respectively and impacts of cultural conditions on growth and siderophore production, *P. fluorescens* strains were isolated from various were isolated from various rhizospheric soils, identified by biochemical assays, and cultured in King B medium and these tested positive for siderophore production. The succinate medium, supplemented with 10 μM iron, was employed to study the effect of iron on siderophore production. Maximum catechol-type siderophore production at pH=7 was obtained by ML-I (88.6 μg/ml) and hydroxamate by SH-IV (15.6 μg/ml) while growth in terms of optical density by BM-II (OD_{600nm} 1.84). The effects of pH (5-9) and time (0-24 hrs) were also studied. Among the carbon and nitrogen sources, glucose (0.4%) and L-Lysine and L-Arginine (0.1%) were found to increase siderophore production as well as growth. Dual culturing of the isolates also proved to be an effective control measure for also showed decreased growth of *R. solani* (upto 56.17%) and *R. oryzae* (upto 56.17%). The siderophore producing microbes can be used in association with a variety of crop plants to satisfy their iron thirst and help in fighting against plant pathogens.

Key words: Biocontrol, *Pseudomonas fluorescens*, Rhizosphere.

Rhizosphere is a dynamic environment, which harbours diverse groups of microbes. Some of the bacteria, which directly or indirectly stimulate the plant growth, have been referred as cultural conditions on growth and siderophore producing *P. fluorescens* strains were isolated from various. Plant Growth Promoting Rhizobacteria (PGPR) (Bloemberg and Lugtenberg 2001[1]). Various species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth by mechanisms like nitrogen assimilation, iron, phosphorus uptake, synthesis of phytohormones, and by controlling plant diseases (Joseph *et al*. 2007[2]). Recently, *Pseudomonas fluorescens*, a Gram-negative rod shaped bacterium that inhabits soil, plants, and water surfaces, is emerging as a potent group of PGPR. These organisms are significantly important due to the fact that they reduce the rhizospheric population of phytopathogenic fungi and bacteria. Under aerated conditions at neutral to alkaline pH, inorganic iron is extremely insoluble and its concentration is less than optimal for bacterial growth (Gupta *et al*. 2008[3]). Iron is one of the most important micronutrients used by bacteria, being required as

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a cofactor for a large number of enzymes and iron-containing proteins (Rachid and Ahmed 2005[4]). To fulfill their iron requirement, these have evolved sophisticated iron uptake systems which usually involve the excretion, by iron-starved cells, of low-molecular-mass iron-sequestering compounds known under the generic name of siderophores (Neilands 1981[5]). Siderophores (Greek for iron carrier) are low molecular weight (<10 KD) iron binding compounds synthesized by microbes in large quantities under iron limited conditions (Lankford 1973[6]; Neilands 1981). Siderophores chelate the ferric ions with a high specific activity and serve as vehicles for the transport of iron (Fe^{3+}) into the microbial cell. The major groups of siderophores include the catecholates (phenolates), hydroxamates, carboxylates (e.g. derivatives of citric acid) and mixed ligands. (Modi *et al*. 2012[7]). *Pseudomonas fluorescens* is one of the fluorescent pseudomonads that secrete siderophores called pyoverdins which assist in sensing and eventual uptake of iron from the medium (Gupta *et al*. 2008). Siderophores produced by *Pseudomonas* spp. Have been employed efficiently as biocontrol agents against soil-borne plant pathogens. Production of secondary metabolites like antibiotics, Fe-chelating siderophores, and cyanide are most often associated with fungal suppression by fluorescent pseudomonads in the rhizosphere of several crops (Battu and Reddy 2009 [8]). Because microbes recognize and utilize only certain siderophores, such conjugates are anticipated to have selective antimicrobial activity (Ali and Vidhale 2011 [9]). Siderophore production by *Pseudomonas* spp., is thus of great interest because of its possibilities in the substitution of chemical pesticides.

The main objectives of the present project were:

1. To investigate siderophore production by the *Pseudomonas* isolates from various rhizospheric soils.
2. Regulation of incubation time, pH and carbon/nitrogen sources to observe their effect on siderophore production.

MATERIALS AND METHODS

Characterization of Isolates

The isolates obtained from various rhizospheric soils (vegetables and cereal crops) over different locations in Punjab were identified on the basis of their morphology (Gram staining), colony characteristics and standard biochemical reactions (MRVP, citrate utilization, starch hydrolysis, nitrate production).

Qualitative detection of siderophore was done by using Chrome-Azurol S (CAS) agar medium (Schwyn and Neilands 1987 [10]) devoid of iron. The isolates were grown in succinate medium (with 10 µM iron and without iron) for 24 h on a rotary shaker at an incubation temperature of 30±2°C, followed by centrifugation and the cell free supernatant was thus applied to CAS plates containing wells of 3 mm diameter made with cork borer.

Quantitative methods

Catechol-type siderophores were assayed using the method of Arnow (1937 [11]). To study the siderophore formation, the bacterial isolates were grown in succinate broth and incubated at 28°C for 72 hours. The culture supernatant was separated by centrifugation at 10,000 g for 15 minutes. Ethyl acetate extracts were prepared by extracting supernatant twice with an equal volume of solvent at pH 2. For the assay, one volume of sample was added to one volume of Hathway's reagent and absorbance was measured at 560 nm. The quantity of siderophore present in the sample was calculated from standard curve prepared by using different concentrations of sodium salicylate.

Hydroxamate siderophores were detected in culture medium supernatants according to Atkin *et al*. 1970 [12]. The assay was carried out by mixing 0.5 ml of culture supernatant, with 2.5 ml 5 mM $FeCl_3$–0.1 M $HClO_4$ (pH 2) followed by the measurement of the maximum optical density (OD_{480nm}), with hydroxylamine N as standard.

Effect of pH on Siderophore Production

Succinate medium with different pH (in the range 5-9) were inoculated with the culture to test the effect of various pH levels on growth and siderophore production by *Pseudomonas* spp.

Siderophore Production as a Function of Time

The culture was grown in 50 ml of succinate medium with constant shaking on rotary shaker (120 g) at 30±2°C for 24 h. Samples were drawn every 4 h and monitored for growth (OD_{600nm}) and siderophore concentration.

Effect of Carbon and Nitrogen Sources

In order to study the effect of carbon sources (0.4%) on growth and siderophore production, succinate in the succinate medium was replaced with D-Glucose and D-Galactose. The effect of nitrogen sources (0.1%) was also studied by replacing ammonium sulphate in the succinate medium with L-Lysine and L-Arginine. Growth and siderophore production were measured after requisite incubation.

***P. fluorescens* as fungal antagonists**

The *Pseudomonas* isolates were evaluated against *Rhizoctonia solani* and *Rhizoctonia oryzae* causing black scurf of potato and sheath blight of rice respectively by dual culture technique. To analyze, a streak of the Pseudomonads from the margin of 7 days-old culture avin g 5mm block of the pathogen were placed on the opposite of the plate at equal distance from the periphery. The experimental design used was a completely randomized with three Petri dishes for each isolates. Inoculated plates were incubated at 25 ± 1°C until the end of the incubation period (7 days after inoculation). At the end of seventh day of incubation period, radial growth of pathogenic isolates was measured. Percent inhibition of the fungal growth was measured by the formul.

Formula: 100× (C-T)/C,

Where C= Control plate; T= Test plate

RESULTS

Characterization of Isolates

Based on their morphology, colony characteristics and biochemical reactions, isolates were found to be belonging to genus *Pseudomonas* and species *fluorescens*. The identification of bacteria was done according to Bergey's Manual (Jordan 1984 [13]).

Qualitative detection on the CAS plates indicated that *Pseudomonas* isolates showed more siderophore production (9-33%) in the presence of iron (10 µM) in the succinate medium, than without it, showing orange to yellow colour halo around the well (Table 1). Only one isolate i.e., SH-I did not show any siderophore production in iron containing medium, which may be due to the fact that siderophores are iron-specific compounds which are secreted under low iron stress. This observation was in support of Budzikiewicz (1993) [14].

Effect of pH on Siderophore Production

While detecting quantitatively the siderophores, by the method of Arnow (1937), it was observed that four of the isolates (WH-I, KK-I, LP-I and CL-I) showed a decrease in catechol-type siderophore production from lower to higher pH, which may be due to the fact that alkaline pH helps in excess solubilization of iron, which increases the iron content of the medium and ultimately results in decrease in siderophore production (Schwyn and Neilands 1987). Some others such as LS-II, BM-I, RN-I, T_1R_2 and $T_{15}R_1$ increased its production with increase in pH. Few others i.e. (WH-II, LS-I and PH-I) produced enhanced levels at neutral pH. Isolates BM-II, BM-III, SH-I, SH-IV, JSF-I, ML-I, $T_{10}R_1$, Lal Pot and T_2R_3 decreased their siderophore production ability at pH=7, as compared to pH=6 and pH=8 (Table 2). A different trend was shown by the isolates in the production of hydroxamate-type siderophores from that of catechol-type siderophores. Few isolates like BM-III, LP-I, RN-I, KK-I and T_2R_1 were not producing any hydroxamate siderophore at any

Table 1. Effect of Iron on siderophore producing ability of *P. fluorescens*

Isolates	Size of halo on CAS agar medium (cm)	
	Without Iron	With Iron
WH-I	1.4	1.6
WH-II	1.5	1.7
LS-I	1.9	2.0
LS-II	1.4	1.5
BM-I	1.3	1.4
BM-II	0.9	1.2
BM-III	1.1	1.2
SH-I	1.1	0
SH-IV	1.4	1.5
KK-I	1.6	1.7
JSF-I	1.4	1.4
ML-I	1.5	1.4
$T_{15}R_1$	1.3	1.7
$T_{10}R_1$	1.3	1.1
LP-I	1.1	0.9
RN-I	1.1	1.1
T_1R_2	1.2	1.3
T_2R_1	1.5	1.6
PH-I	1.0	1.0
CL-I	1.6	2.1

Values indicate mean of three replicates

Table 2. Growth and siderophore production by *P. fluorescens* isolates as a function of pH

Isolates	pH=5			pH=6			pH=7			pH=8			pH=9		
	OD600nm	Catechol (µg/ml)	Hydroxamate (µg/ml)	OD600nm	Catechol (µg/ml)	Hydroxamate (µg/ml)	OD600nm	Catechol (µg/ml)	Hydroxamate (µg/ml)	OD600nm	Catechol (µg/ml)	Hydroxamate (µg/ml)	OD600nm	Catechol (µg/ml)	Hydroxamate (µg/ml)
WH-I	-	-	-	1.58	54.8	6.4	1.07	44.5	9.7	0.81	32.9	4.4	0.78	-	-
WH-II	-	-	-	1.76	0	6.5	1.23	31.4	12.6	1.10	0	46.8	0.74	-	-
LS-I	1.02	-	-	1.96	3.7	5.9	1.37	82.7	3.1	1.13	43.6	12.9	1.08	-	-
LS-II	-	-	-	1.67	24.1	-	1.29	26.0	12.2	1.28	52.9	-	0.91	-	-
BM-I	-	-	-	1.47	19.8	9.3	1.63	88.3	3.4	1.67	112.8	13.1	1.05	-	-
BM-II	-	-	-	1.41	44.4	-	1.84	27.1	2.1	1.56	34.8	-	0.95	-	-
BM-III	-	-	-	1.66	15.7	-	1.30	6.3	-	0.75	30.2	-	0.75	-	-
SH-I	-	-	-	1.95	88.4	19.4	1.34	19.1	3.4	0.61	106.1	24.5	0.78	-	16.4
SH-IV	-	-	-	1.75	62.4	16.1	1.50	9.3	15.6	1.31	50.9	13.5	1.08	-	28.0
KK-I	-	-	-	1.05	55.2	-	1.29	39.3	-	1.17	12.4	-	0.78	-	-
JSF-I	-	-	-	1.75	83.0	3.5	1.25	39.1	0.10	1.38	87.2	10.1	0.99	-	-
ML-I	-	-	-	1.55	132.9	6.9	1.32	88.6	9.7	0.78	200.9	24.9	0.87	-	-
$T_{15}R_1$	-	-	-	1.89	52.1	-	1.20	68.8	6.8	1.19	62.4	3.5	1.02	-	25.9
$T_{10}R_1$	0.86	-	-	1.70	93.9	6.7	1.54	66.3	-	1.15	101.4	28.7	0.91	-	30.6
LP-I	-	-	-	1.41	21.9	-	1.33	8.2	-	1.13	10.4	-	0.86	-	-
RN-I	-	-	-	1.51	7.8	-	1.35	20.3	-	1.37	21.7	-	1.13	-	-
T_1R_2	0.93	-	-	1.73	3.4	16.6	1.32	26.5	-	0.75	78.3	28.5	0.63	-	-
T_2R_1	-	-	-	1.01	25.1	-	0.73	6.2	-	0.50	23.6	-	0.60	-	-
PH-I	-	-	-	1.83	12.8	3.3	1.28	15.9	14.0	1.26	12.9	18.1	0.79	-	-
CL-I	-	-	-	1.73	42.4	-	0.76	40.1	13.4	0.77	33.5	21.8	0.87	-	-

Values indicate mean of three replicates

pH, while on the other hand, BM-II and LS-II could produce it only at neutral pH (2.1 and 12.2 µg/ml, respectively). Three isolates, namely, WH-II, PH-I and CL-I showed increased in its production from pH=6 to pH=9, while SH-IV showed continuous decrease pH=6 onwards. Only one isolate, WH-I showed enhanced production at pH=7, as compared to pH=6 and 8 (9.7, 6.4 and 4.4 µg/ml respectively). Rest of the isolates were observed to make more of it at pH of 6 and 8/9 as compared to 7 pH. Unlike Arnow's test, 4 isolates showed positive Atkin's test at pH of 9, but none at pH=5.

Effect of pH on Growth

It was found that only three isolates i.e., LS-I, $T_{10}R_1$ and T_1R_2 were able to grow at a pH of 5. It was observed that most of the isolates showed maximum growth at pH=6, which then decreased up to 9. Only three isolates, BM-I, BM-II and KK-I showed enhanced growth at neutral pH (OD_{600nm}=1.63, 1.84 and 1.29 respectively), whereas the isolate KK-I presented a bell shaped curve (Table 2).

Siderophore Production and growth as a Function of Time

As illustrated in Table 4, both the parameters were studied at every four hour interval for 24 hours. During siderophore production, a lag phase of 4-5 hours was observed. Its production initially started after 8 hours of incubation. As depicted in fig. 1, two types of growth patterns were shown by the isolates. First group of isolates, which included isolates RN-I, BM-I, BM-II, KK-I, L-I, WH-I, LP-I and PH-I, showed first a decrease in OD from 0^{th} to 4^{th} hour followed by a slow increase till 8^{th} hour and then a rapid increase at the 24^{th} hour. In second group of isolates, which included $T_{15}R_1$, $T_{10}R_1$, T_1R_2, T_2R_1, LS-I, LS-II, BM-III, WH-II, JSF-I, SH-I, SH-IV and ML-I, first showed a lag period from 0^{th} to 8^{th} hour and then a rapid increase till 24^{th} hour.

Effect of Carbon and Nitrogen Sources

Among the carbon sources tested, glucose at 0.4% concentration promoted both growth and siderophore production. Both the parameters were shown by all the isolates in glucose-medium (Table 4), while only 10 isolates (BM-III, ML-I, SH-I, LS-II, T_2R_1, LP-I, $T_{15}R_1$, RN-I, BM-II, $T_{10}R_1$) were able to grow in galactose-containing medium, out of which RN-I did not produce significant growth and siderophore (OD_{600nm} = 0.09 and 11.7 µg/ml respectively). Among the nitrogen sources tested, maximum growth was observed in L-Lysine, as compared to L-Arginine (Table 4). Growth was found to enhance in the presence of L-Lysine as an N-source, with respect to ammonium sulphate by isolates T_2R_1 and CL-I, while rest of the isolates showed a decrease in growth. The isolates when grown in the presence of L-Arginine showed a decrease in growth with respect to ammonium sulphate containing medium, although increasing pigment production. Siderophore concentration was also observed to increase in both the media, except LS-I, SH-I, $T_{15}R_1$, $T_{10}R_1$ (L-Lysine) and LS-I, $T_{15}R_1$, $T_{10}R_1$ and W-II (L-Arginine) (Table 3).

***P. fluorescens* as fungal antagonists**

A comparison of the data presented in Table 5 indicate that out of the 20 Pseudomonads isolates 9 were tested *in vitro*. A total of 6 isolates (BM-I, BM-II, KK-I, RN-I, PH-I, CL-I) were effective in suppressing *R. solani*. These isolates inhibited pathogen growth by 42.64-56.17 % within 7 days of inoculation. Seven isolates (LS-I, LS-II, KK-I, JSF-I, RN-I, PH-I, CL-I) were promising against *R.*

Table 3. Catechol- type siderophore production as a function of time

Isolates	Siderophore production (µg/ml)	
	8 h	24 h
WH-I	1.06	14.85
WH-II	-	10.66
LS-I	3.78	27.33
LS-II	-	8.72
BM-I	5.21	31.00
BM-II	-	9.33
BM-III	-	1.65
SH-I	-	6.19
SH-IV	-	3.98
KK-I	0.67	12.66
JSF-I	1.01	12.34
ML-I	5.97	37.66
$T_{15}R_1$	3.92	23.01
$T_{10}R_1$	2.76	22.95
LP-I	-	2.67
R-I	-	6.40
T_1R_2	-	8.61
T_2R_1	-	2.02
PH-I	-	4.44
CL-I	-	13.66

Values indicate mean of three replicates

Table 4. Growth and siderophore production by *P. fluorescens* isolates in the presence of different nutrients

Isolates	Glucose		Galactose		L-Lysine		L-Arginine	
	OD600nm	Catechol(µg/ml)	OD600nm	Catechol(µg/ml)	OD600nm	Catechol(µg/ml)	OD600nm	Catechol(µg/ml)
WH-I	1.25	47.4	-	-	1.07	61.0	0.48	78.1
WH-II	0.60	32.7	-	-	0.91	-	0.77	6.8
LS-I	0.87	49.2	-	-	0.63	16.9	0.226	36.9
LS-II	0.51	34.6	0.40	27.6	1.07	-	0.53	59.7
BM-I	0.71	31.1	-	-	0.78	42.2	0.27	37.1
BM-II	0.74	-	0.41	26.5	0.98	66.1	0.79	43.2
BM-III	0.56	31.7	0.55	33.6	0.34	28.0	0.68	43.5
SH-I	0.27	42.6	0.09	22.4	0.31	10.5	0.42	64.9
SH-IV	0.84	42.4	-	-	1.00	32.9	0.38	43.7
KK-I	0.22	30.9	-	-	0.40	-	0.0.38	44.3
JSF-I	0.46	14.6	-	-	1.01	44.5	0.75	60.2
ML-I	1.12	-	0.82	34.0	0.93	42.8	0.70	42.4
$T_{15}R_1$	0.74	53.4	0.31	31.5	0.91	36.9	0.40	37.7
$T_{10}R_1$	0.84	55.4	0.18	45.3	0.91	17.7	0.66	43.7
LP-I	0.83	34.4	0.15	49.0	1.19	43.5	0.85	40.0
RN-I	0.72	25.9	0.09	11.7	1.07	57.3	0.54	38.3
T_1R_2	0.65	45.9	-	-	1.09	53.6	0.71	69.6
T_2R_1	0.81	47.0	0.34	55.6	1.16	33.4	0.68	59.7
PH-I	0.46	31.7	-	-	0.93	45.5	0.64	71.1
CL-I	0.48	55.6	-	-	1.07	69.4	0.15	57.1

Values indicate mean of three replicates

Table 5. Percentage inhibition in fungal growth

Isolates	*Rhizoctonia solani*	*Rhizoctonia oryzae*
LS-I	0	50.29
LS-II	0	42.35
BM-I	53.76	0
BM-II	56.17	0
KK-I	51.76	41.17
JSF-I	0	51.76
RN-I	52.94	43.25
PH-I	42.64	44.11
CL-I	48.82	58.23

Values indicate mean of three (P≤ 0.05)

oryzae and they reduced the growth of the pathogen by more than 41.17-58.23 % within 7 days of inoculation. The inhibitory potential of pseudomonad isolates also differed significantly. Against *R. solani*, *Pseudomonas fluorescens* isolates BM-II exerted the maximum inhibitory effect as evidenced by the widest inhibition zone (7.5 mm). CL-I was the most inhibitory isolates against *R. oryzae* (11.75 mm in PDA). The biocontrol potential of fluorescent pseudomonads against R. solani (Rini and Sulochana 2007 [15]; Bautista *et al*. 2007 [16]) have been previously reported.

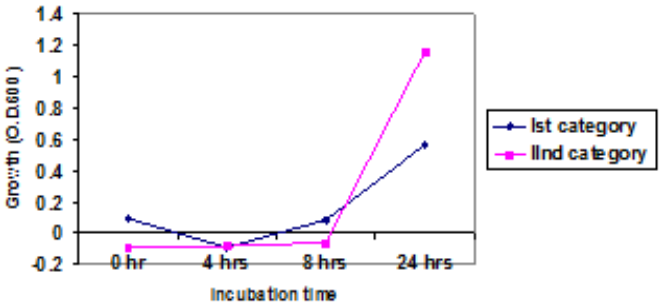

Fig. 1. Growth produced by rhizobacterial isolates as a function of time

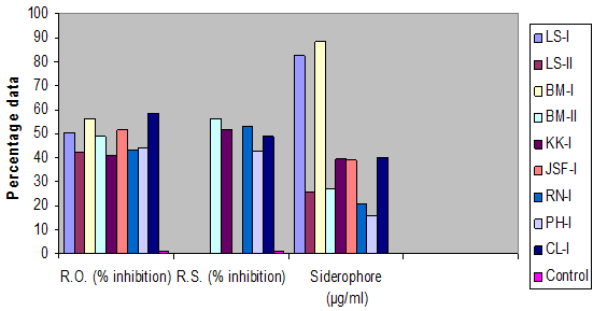

Fig. 2. Comparison between amount of siderophore production and percent growth inhibition

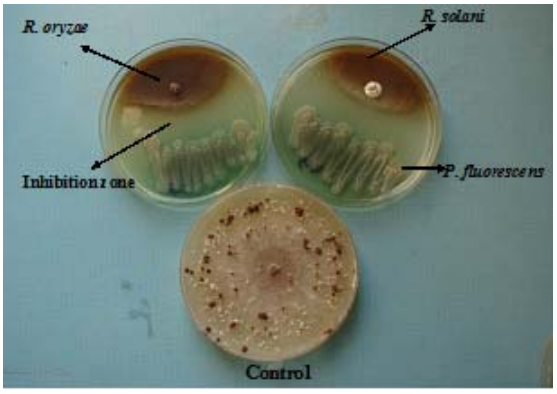

Fig. 3. Inhibition of the fungal pathogen by the rhizobacterial isolate

DISCUSSION

Iron is important for all life forms, especially for processes such as respiration and DNA synthesis. Inspite of being one of the most abundant elements in the Earth's crust, the bioavailability of iron in the environments like the soil or sea is limited due to very low solubility of the Fe^{3+} ion, which is the most dominant state of iron on the planet. It accumulates in common mineral phases such as iron oxides and hydroxides (the minerals that are responsible for red and yellow soil colours) hence cannot be readily utilized by organisms. The ability to sequester iron provides a competitive advantage to microorganisms. To acquire iron, certain microorganisms have been found to produce specific high affinity iron binding compounds, termed siderophores. In this study, we examined 20 strains of *P. fluorescens* isolated from rhizospheric soils for production of siderophores. All strains examined produced siderophores detectable by CAS assay. Conversion of medium from blue to golden yellow colour after growth of rhizobacteria with CAS reagent confirmed production of siderophores. The siderophore of *P. fluorescens* was water soluble with a yellow-green fluorescence. Siderophore production increased markedly with added iron. These results agree with previous studies that siderophore production is iron regulated (Neilands 1981; Ali and Vidhale 2011). Two methods were used to determine the chemical class of the siderophores. The present study indicated that *P. fluorescens* produced siderophores of both the catecholate and hydroxamate type. Culture supernatants subjected to Arnow's test gave positive reaction, indicating the presence of the catecholate group of siderophores. Rare growth and no siderophore production was detected at pH=5. While studying the growth and siderophore production as a function of time, it was found that both the processes came into effect only after 8 hours of incubation and reached a maximum after 24 hours. These results are similar to those of Sridevi *et al*. (2008) [17] in *Rhizobium*. The production of siderophores varied with the type of carbon sources was also reported in *Pseudomonas* sp. by Sayyed *et al*. (2005) [18]. In the present study enhanced growth and iron-chelating compounds production was observed when grown in glucose-medium by most of the isolates, which may be due to the fact that glucose is more favorable nutrient for the bacteria. Only 10 isolates could utilize galactose as the carbon source, probably due to the lack of galactosidase enzyme activity by other isolates. The results for L-Lysine and L-Arginine media are in accordance with Deelip *et al*. (1998) [19], who found that arginine and lysine are supportive for growth, fluorescence and siderophore production in *Pseudomonas* sp. The isolates showed antagonistic properties, *in vitro,* against the pathogens ranging from 41.17-58.23 % (*R. oryzae*) and 42.64 -56.17 % (*R. solani*) under *in vitro* conditions, which were comparable with the siderophore producing abilities of the respective isolates (Fig 2 and 3).

Several workers have studied the distribution and activity of siderophore producing fluorescent pseudomonads from the viewpoint of plant disease control since the production of pyoverdines scavenges available iron in the rhizosphere thus, creating an environment unfavourable to the growth of phytopathogens. Early reports of siderophores from growth promoting fluorescent pseudomonads relate to production under *in vitro* conditions (Dave and Dubey 2000) [20]. In subsequent work from this group, rhizobacteria from groundnut and soybean were found to secrete trihydroxymate type siderophores under iron deficient conditions (Yeole *et al*. 2001) [21]. Kumar and Dubey (1993) [22] also demonstrated that siderophore production by a plant growth promoting fluorescent *Pseudomonas sp*. RBT 13 was effective against several fungal and bacterial pathogens. Rao *et al*. (1999) [23] reported that five strains of fluorescent pseudomonads exhibited growth promotion of lentil and biocontrol of wilt caused by *F. oxysporum* f. sp. *lini* with siderophore production as the main biocontrol mechanism. Similarly, Kumar *et al*. (2000) [24] reported that the *Pseudomonas fluorescens* produced siderophores and antifungal metabolites which are involved in the control of phytopathogenic fungi. These microbes can be used in association with crop plants to satisfy their iron thirst and help in fighting against plant pathogens.

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