SIDEROPHORES PRODUCTION AND ANTAGONISTIC BEHAVIOUR OF RHIZOSPHERIC MICROORGANISMS IN RELATION TO IRON AVAILABILITY

ABSTRACT

Variable magnitudes of siderophores production were qualitatively and quantitatively determined amongst 64 representatives of four groups of rhizospheric microorganisms. Generally, fluorescent pseudomonads and phytopathogenic fungi were effective siderophores producers as compared with soybean root nodulators. Azotobacters, as asymbiotic N_2- fixers, appeared to occupy an intermediate rank. While all bacterial representatives produced catechol type siderophores, the phytopathogenic fungi produced the hydroxamate type. In iron limited media, no antagonism was reported between root nodule bacteria or azotobacters and phytopathogenic fungi. The phenomenon was particularly associated with fluorescent pseudomonads but was affected by the composition of culture medium and tested organisms. While available iron generally enhanced the growth of *Ps. fluorescens* and phytopathogenic fungal strains, their siderophores production capacities were concomitantly retarded. SDS-PAGE analysis showed that high levels of available Fe (5 and 10 mg Fe-EDTA / ml medium) repressed the expression of 4 protein bands in *Ps. fluorescens* B outer membrane which were disinctly observed in cells grown in iron starved medium. Available iron also induced variable degrees of reduction in antagonistic activities of fluorescent pseudomonads against phytopathogenic fungi or root nodule bacteria.

Keywords: Siderophores, Fluorescent pseudomonads, N_2-fixers, Phytopathogenic fungi, Iron availability, Relative power of antibiosis

INTRODUCTION

Iron is an essential nutrient for all living cells. It is the fourth most abundant element in the Earth's Crust. However, under aerobic conditions and neutral pH, ferrous ions are converted to their oxidized forms, which tend to form highly insoluble ferric hydroxides that are unavailable to living organisms **(Barash, 1990)**. Under iron limited condition, microorganisms evolved high-affinity systems mediated by siderophores (Greek for "iron-bearers"), which are low-molecular weight (0.5 to 1.5 KDa), highly specific Fe^{+3} chelating agents. Many studies have examined the chemistry and kinetics of iron transport by siderophores **(Neilands, 1995 and Terano *et al.* 2002)**, but the role of siderophores in competition for iron in plant / microbe and microbe / microbe interactions is receiving increased attentions **(Bhattarai and Prasad 2003)**.

Systems such as siderophores, involved in the acquisition of iron under iron-limited conditions, may play a role in microbial interactions. Many rhizosphere *Pseudomonas* species are plant-pathogenic, but it has been shown that some pseudomonads promote plant growth **(Kloepper *et al.* 1980 and Manwar *et al.* 2000)**, and inhibit pathogenic bacteria and fungi **(Manwar *et al.*, 2000)** by producing siderophores. Siderophore production by many *Pseudomonas* species has been clearly demonstrated in the control of phytopathogenic fungi **(Goel *et al.* 2000 and Manwar**

***et al.*, 2004)**. However, the dynamics of iron competition in the rhizosphere in relation to antagonism against phytopathogens are often complex.

This study aimed to verify the qualitative and quantitative siderophores producing abilities of a range of rhizospheric microorganisms along with their antagonistic interactions under limited and non- Fe limited conditions.

MATERIALS AND METHODS

Microorganisms

A range of rhizospheric microorganisms belonging to fluorescent pseudomonads, azotobacters, root nodule bacteria and soil borne pathogenic fungi were used in this study. The number and identity of strains or isolates belonging to each of the above-mentioned group are given in Table (1).

Iron supplements

Fe-Ethylene-diamine-tetraacetic (Fe-EDTA) contained 13% Fe and Ferrous sulphate ($FeSO_4$. $7H_2O$) contained 20.11% Fe, were used as sources of iron in laboratory experiments.

Experimental techniques

Examination of siderophore producing abilities of the tested microorganisms

Two indicators were used for qualitative siderophore production i.e., the formation of orange colour around microbial growth against the blue background of Chrome Azurol S agar medium **(Schwyn and Neilands, 1987)** and the ability of the organism to grow in 10% Tryptic Soya agar medium supplemented with 50 mg 8-hydroxyquinoline/l **(Alexander and Zuberer, 1991)**. The quantitative assessment of siderophore was determined by modified CAS assay method **(Alexander and Zuberer, 1991)**.

Verifying the chemical structure of siderophores produced by tested microorganisms

The method of **Arnow (1937)** modified by **Carson *et al.* (1992a)** was used for detection of catechol-type siderophores, while those of **Atkin *et al.* (1970)** and **Marrier and Boulets (1958)** were used for detection of hydroxamate type siderophores and citric acid, respectively.

Evaluation of antagonistic interactions among siderophores producing microorganisms

The relative power of antibiosis was used to measure the inhibitory effect of an organism against the other based on the formation of inhibition zone around the antagonistic organism. The parameter was quantified according to the formula proposed by **Ibrahim *et al.* (1987)** as follows:

$$\text{Relative power of antibiosis (RPA)} = \frac{\text{Diameter of inhibition zone}}{\text{Diameter of spotted antagonistic organism}}$$

Table 1. Microbial isolates or strains used in this study and their sources.

Fluorescent Pseudomonads	Source	Azotobacters	Source	Root nodule bacteria	Source	Phytopathogenic fungi	Source
Isolates A7 – 9 - 10 13 – 18 D- T	Agric. Microbiology Dept.,Desert Research Center, Matariya, Cairo, Egypt	38 Isolates	Agriculture Microbiology Department, Fac. Agric., Ain Shams Univ., Cairo, Egypt.	*Bradyrhizobium japonicum* USDA 123 *Bradyrhizobium japonicum* E	Microbiology Dept., National Research Center, Dokki, Giza, Egypt.	*F. solani* *F. oxysporum* f.sp. *glycine*	Plant Pathology Dept., National Research Center, Dokki,Giza, Egypt
Strains *Ps. fluorescens* N6	Unit of Biofertilizer, Fac. Agric., Ain Shams Univ., Cairo, Egypt.			*B. japonicum* USDA110 *B. japonicum* ARC 501 *Sinorhizobium fredii* H.H.303	Culture collection of Biofertilizers Production Unit, Agric. Research Center, Giza, Egypt.	*Rhizoctonia solani*	Plant Pathology Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt
Ps. fluorescens 47 *Ps.fluorescens* 48 *Ps.fluorescens* 49 *Ps. putida* 50	Plant Pathology Dept.,Fac.Agric., Ain Shams Univ., Cairo, Egypt			*B. japonicum* B1	Unit of Biofertilizer, Fac. Agric., Ain Shams Univ., Cairo, Egypt.		
Ps. putida 52 *Ps. fluorescens* B *Ps. fluorescens* 1	Cairo MIRCEN, Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt			*Sinorhizobium fredii* N5	ARS Culture Collection (NRRL), USA		
Ps. fluorescens N2	ARS Culture Collection (NRRL), USA						

Effect of available iron on growth and siderophores production by *Ps. fluorescens* and phytopathogenic fungi

One hundred ml Erlenmeyer flasks contained 25 ml of sterilized (121°C/15min) Fe-deficient modified M9 liquid medium or 50 ml of Zapek's liquid medium were supplemented with increased concentrations of Fe-EDTA (0.0, 0.6, 1.25, 2.5, 5 mg/ml) or $FeSO_4.7H_2O$ (0, 20, 40, 60, 80, 160 μM Fe), respectively. The Fe-EDTA and $FeSO_4.7H_2O$ solutions were sterilized by filtration before supplementation. Media with different iron concentrations were inoculated with either 1 ml of cell suspension containing about 10^8 cells/ml of *Ps. fluorescens* strain B or 1 or agar disk of *F. solani*, *F. oxysporum* f.sp. *glycine* or *Rh. solani*. Inoculated flasks were incubated at 28 – 30 °C for 48 h on rotary shaker (160 rpm) for *Ps. fluorescens* strains and 6 days for fungal strains. After incubation period, bacterial and fungal dry weights were recorded following oven drying at 70 °C untill reaching a constant weight. The siderophores concentration was also determined in culture filtrates as mentioned above.

Verifying the protein banding pattern of *Ps. fluorescens* B outer membrane as influenced by increased concentrations of available iron.

For this purpose, the outer membrane receptor protein were isolated from *Ps. fluorescens* B grown in modified M9 broth without and with increased available iron concentrations (0.5, 2.5, 5.0, 10.0 mg/ml medium) as Fe-EDTA according to the method described by **Champomier-Verges et al. (1996)**. The protein banding pattern of outer membrane receptors collected from cells grown under different conditions were identified according to **Laemmli (1970)** using SDS-PAGE and protein marker with approximately 116, 66.2, 45.0, 35, 18.4 and 14.4 KDa.

Effect of available iron on the antagonism between fluorescent pseudomonas and phytopathogenic fungi or root nodule bacteria.

For this purpose, increased concentrations of Fe-EDTA (0.0, 0.6, 1.25, 2.5 and 5 mg / ml) were incorporated into a 1:1 (V:V) mixture of King's medium B and potato dextrose agar (for phytopathogenic fungi) or glucose yeast extract peptone agar (for root nodule bacteria) and the antagonistic behaviour was monitored as mentioned above.

RESULTS

Production and identity of siderophores produced by tested microorganisms

Data in **Fig. (1)** present the number of isolates or microbial strains categorized according to the diameter of siderophore production zone on Chrome Azurol S (CAS) or their growth on Tryptic Soy agar media. It is obvious that siderophores production is a common phenomenon amongst those representatives of rhizospheric microorganisms. On CAS agar, azotobacters gave a higher number of isolates capable to produce the lower rank (5 – 15 mm zone) of siderophores, but fluorescent pseudomonads and phytopathogenic fungi dominated higher ranks (16 – 30 and 31 – 45 mm), respectively **(Fig. 2 A)**. This finding was also true for azotobacters tested on Tryptic Soya agar. However, soybean root nodulators showed the lowest ability of siderophores production **(Fig. 2 B)** on the two tested media compared with other groups of microorganisms.

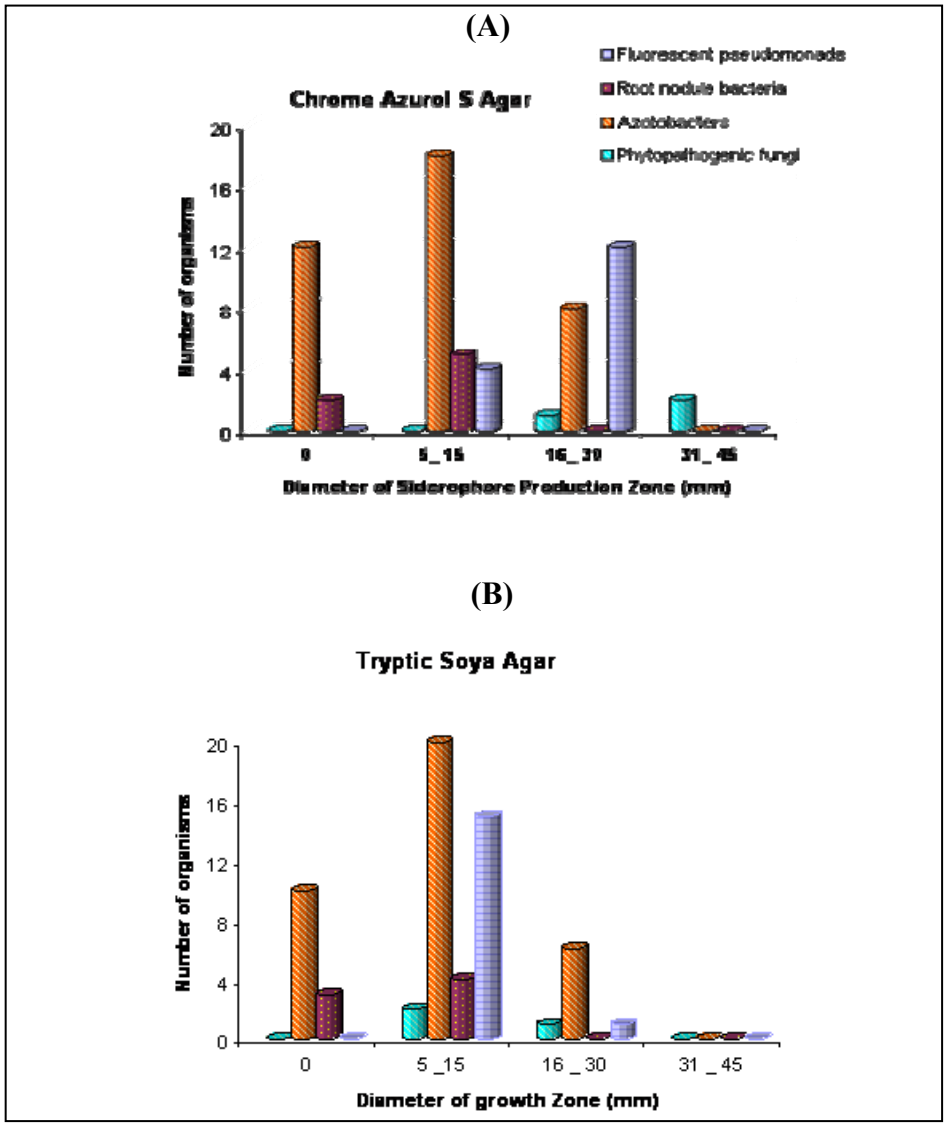
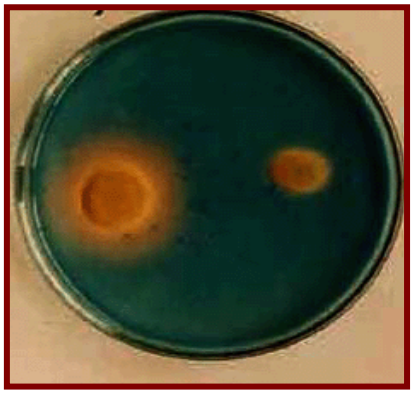


Fig. (1): Frequency distribution of siderophore production by four rhizospheric groups of microorganisms grown on Chrome Azurol S agar (A) or Tryptic Soya agar (B) media.



A **B**

Fig. (2): Feature of siderophore production as indicated by the formation of orange colour around bacterial growth on Chrome Azurol S medium .

A- ***Pseudomonas fluorescens*** **B**

B- ***Bradyrhizobium japonicum*** **ARC501**

Variable amounts of siderophores were produced by microbial representatives showed positive reactions with qualitative detection. Data presented in **Table (2)** showed that *Ps. fluorescens* B & 1 and isolate T along with the 2 strains of *Fusarium* were highly siderophores producers as they gave 105, 90, 115, 120 and 130 μM DFOM, respectively. Again the tested strains of soybean root nodulators appeared to be low siderophores producers. *Azotobacter* isolates, on the other hand, occupied an intermediate rank amongst the tested microbial groups as they produce 50 – 70 μM DFOM.

Verification of chemical nature of released siderophores showed that all tested bacteria produce catechol-type siderophores, while those of fungal origin are belonging to hydroxamate type **(see Fig. 3)**. Citric acid, on the other hand, was not detected in culture filtrate of any of the tested organisms.

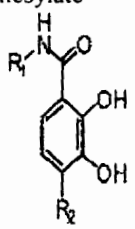
Table (2). Amount of siderophores produced (as μM DFOM* equiv.) by some selected isolates or strains of fluorescent pseudomonads, symbiotic and asymbiotic N_2 – fixers as well as phytopathogenic fungi.

Organisms	Amount of siderophore (as uM DFOM)	Organisms	Amount of siderophore (as uM DFOM)
Fluorescent pseudomonads		***Bradyrhizobium japonicum***	
Isolates		USDA 110	22
A7	52	ARC 501	20
9	79	USDA 123	22
10	87	***Sinorhizobium fredii***	
13	80	H.H. 303	22
18	82	N5	20
D	73	**Phytopathogenic fungi**	
T	115		
Strains		*Fusarium solani*	120
Ps. fluorescens N6	30	*Rhizoctonia solani*	69
Ps. fluorescens 47	79	*Fusarium oxysporum*	130
Ps. fluorescens 48	88	f. sp. *glycine*	
Ps. fluorescens N2	86		
Ps.fluorescens 49	76	**Azotobacter Isolates**	
Ps. fluorescens B	105	8	60
Ps. fluorescens 1	90	9	70
Ps. putida 50	30	14	55
Ps. putida 52	60	47	65
		A3	60
		Z3	65
		Z2	50

* DFOM = deferoxamine mesylate

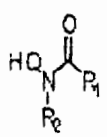
A

Catecholate



B

Hydroxamate



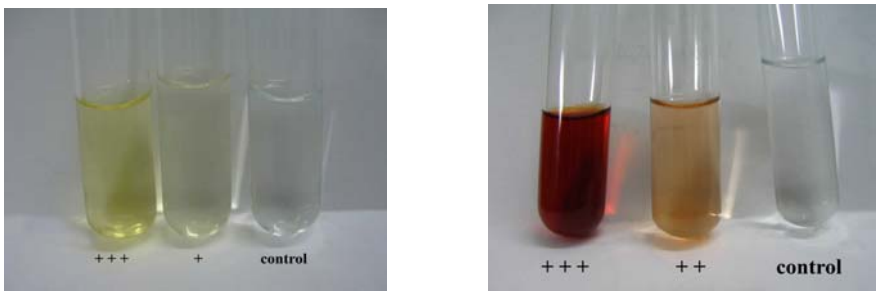
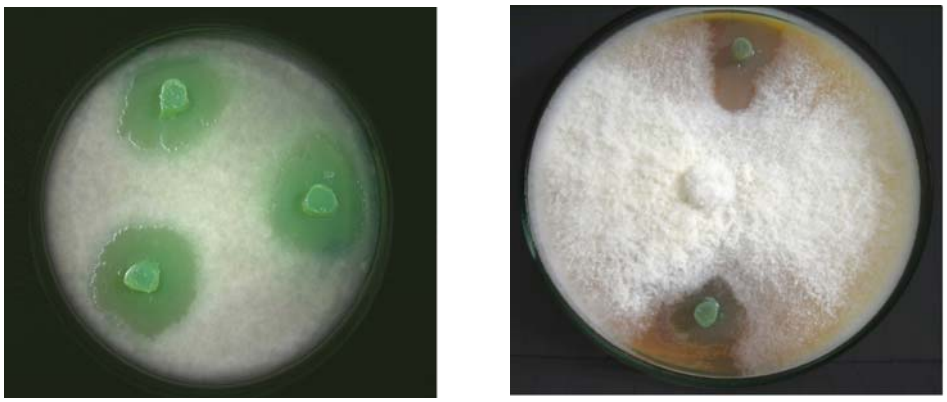


Fig. (3): The chemical formula and detection reaction of catecholate (A) and hydroxamate (B) siderophores

Evaluation of antagonistic interactions among siderophores producing microorganisms

No antagonism was observed between the tested soybean root nodulators or azotobacters and phytopathogenic fungal strains. Similar finding was reported regarding the antagonistic interaction between soybean root nodulators and azotobacters. The phenomenon was generally associated with fluorescent pseudomonads **(Fig. 4)** and depended upon the used culture medium and tested organisms. The fluorescent pseudomonad isolate 9 and *Ps. fluorescens* strains N2, 49 and B were strongly antagonistic to *F. oxysporum* f.sp. *glycine* on King's B / potato dextrose agar (1:1) and tryptic soya agar as well **(Table 3)**. While isolate 13 and 18 gave similar depressive effect against *F. oxysporum* f.sp. *glycine* on the 3 tested media, isolate T was particularly effective on King's / potato dextrose agar (1:1). Wider antagonistic potential was encountered for isolate 18 as it effectively inhibited the growth of *F. solani* and *F. oxysporum* f.sp. *glycine* on King's B / potato dextrose agar (1:1).



A **B**

Fig. (4): *In vitro* antagonism as indicated by the growth inhibition zone of the fungus around the antagonist .

A: between *Pseudomonas fluorescens* B and *F. oxysporum* f.sp. *glycine.*

B: between *Pseudomonas fluorescens* B and *Rhizoctonia solani*

Table (3) Relative power of antibiosis (RPA) of fluorescent pseudomonads against 3 strains of phytopathogenic fungi on King's B / Potato dextrose (1 : 1), Potato dextrose and 10% Tryptic soya agar media.

Fluorescent pseudomonads	King's B / potato dextrose agar (1:1)			Potato dextrose agar			Tryptic soya agar		
	Fusarium solani	*Fusarium oxysporum* f.sp. *glycines*	*Rhizoctonia solani*	*Fusarium solani*	*Fusarium oxysporum* f.sp. *glycines*	*Rhizoctonia solani*	*Fusarium solani*	*Fusarium oxysporum* f.sp. *glycines*	*Rhizoctonia solani*
Isolates									
A7	1.87	1.70	1.50	1.20	2.00	1.54	1.88	2.00	1.13
9	1.50	2.07	0.00	1.20	1.23	0.00	1.28	2.01	1.90
10	1.57	1.92	0.00	1.20	1.33	0.00	1.35	1.80	0.00
13	1.99	2.40	1.22	1.40	2.30	1.20	1.07	2.00	0.00
18	2.00	2.33	1.44	1.60	2.60	0.00	1.05	2.00	1.34
D	2.90	1.89	1.21	1.80	2.70	1.13	1.21	1.80	0.00
T	1.50	2.00	1.81	1.10	1.70	0.00	1.55	1.76	1.40
Strains									
Ps. fluorescens									
N6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47	0.00	2.00	1.80	0.00	1.60	0.00	1.33	1.70	1.73
48	1.28	1.44	0.00	1.20	0.00	0.00	1.11	1.31	0.00
N2	1.38	2.30	1.20	1.30	1.40	0.00	1.11	2.00	1.10
49	0.00	2.50	0.00	0.00	0.00	0.00	1.77	2.10	1.13
B	1.40	2.40	1.82	1.30	1.44	1.31	1.72	2.01	1.35
1	1.14	1.55	1.20	1.10	0.00	1.20	1.20	1.33	0.00
Ps. putida									
50	0.00	1.30	0.00	0.00	0.00	0.00	0.00	1.10	0.00
52	0.00	1.14	0.00	0.00	0.00	0.00	0.00	1.10	0.00

Relative power of antibiosis (RPA) = $\frac{\text{Diameter of inhibition zone}}{\text{Diameter of spotted antagonistic organism}}$

However, the antagonistic activities of fluorescent pseudomonads isolates or *Ps. fluorescens* strains against *Rh. solani* were generally lower than those recorded against the 2 other fungal strains. However, the 2 tested strains of *Ps. putida,* on the other hand, showed the lowest records of Relative Power of Antibiosis against all fungal strains on the 3 culture media.

No antagonism was observed between all tested isolates of fluorescent pseudomonads and *B. japonicum* ARC 501 on tryptic soya agar or glucose yeast extract peptone agar medium (**Table 4**). The same observation was recorded between the seven tested strains of *Pseudomonas fluorescens* and *B. japonicum* USDA 123 on tryptic soya agar medium. *S. fredii* N5 was only antagonized by isolate 18 or *Pseudomonas fluorescens* strains 47 and 49 on tryptic soya agar but the 4 isolates, A7, 9, 10 and 13 along with 6 out of 7 strains of *Pseudomonas fluorescens* showed antagonistic effects on glucose yeast extract peptone agar. The 2 isolates D and T showed antagonistic activities against 2 out of the 5 tested strains of root nodule bacteria on the 2 above mentioned media. Similar observation was also reported for *Pseudomonas fluorescens* strains N6, N2 and B or *Pseudomonas putida* 50 but on tryptic soya agar only.

Concentration of available iron in relation to growth and siderophores production by *Ps. fluorescens* and phytopathogenic fungi.

The two effective siderophores producers *Ps. fluorescens* B and *Ps. fluorescens* 1 were selected for further studies on siderophores iron interactions. Growth enhancements were observed with increased Fe concentration up to 1.25 mg Fe-EDTA /ml medium for *Pseudomonas fluorescens* strains and 60, 80 and 160 μM Fe (as $FeSO_4.7H_2O$) for *F. solani, Rh. solani* and *F. oxysporum*, respectively (**Fig. 5A &6A**). On the other hand, maximum amounts of siderophores were produced in iron deprived media (control treatments). Incorporation of increased concentrations of Fe into culture media parallely decreased siderophores production. Thus, the lowest amounts of produced siderophores were reported with the highest concentration of added Fe (see **Fig. 5B & 6B**).

Table (4). Relative power of antibiosis (RPA) of fluorescent pseudomonads against 3 strains of *Bradyrhizobium japonicum* and 2 strains of *Sinorhizobium fredii* .

Fluorescent pseudomonads	10% Tryptic Soya agar					Glucose yeast extract peptone agar				
	S.fredii		*B. japonicum*			*S.fredii*		*B. japonicum*		
	H.H303	N5	ARC 501	USDA110	USDA123	H.H303	N5	ARC 501	USDA110	USDA123
Isolates										
A7	1.00	0.00	0.00	1.20	0.00	1.08	1.20	0.00	1.29	3.10
9	1.60	0.00	0.00	1.66	0.00	1.66	1.78	0.00	1.66	0.00
10	0.00	0.00	0.00	1.00	1.21	0.00	2.30	0.00	1.40	1.11
13	1.30	0.00	0.00	1.50	0.00	1.27	2.60	0.00	1.50	0.00
18	1.10	1.77	0.00	0.00	1.31	1.09	0.00	0.00	0.00	3.00
D	1.10	0.00	0.00	0.00	1.71	1.14	0.00	0.00	0.00	2.70
T	1.10	0.00	0.00	0.00	1.70	1.07	0.00	0.00	0.00	1.70
Strains ***Ps. fluorescens***										
N6	2.00	0.00	0.00	0.00	0.00	2.62	3.50	0.00	0.00	1.70
47	1.13	1.36	1.20	1.30	0.00	1.11	1.67	1.16	1.45	0.00
48	0.00	0.00	0.00	1.20	0.00	0.00	1.22	0.00	1.19	0.00
N2	1.00	0.00	0.00	0.00	0.00	1.15	1.78	0.00	0.00	1.80
49	1.20	2.50	1.30	0.00	0.00	1.26	2.00	1.47	1.05	0.00
B	0.90	0.00	0.00	0.00	0.00	1.20	1.14	0.00	0.00	0.00
1	1.10	0.00	1.30	1.41	0.00	1.42	0.00	1.49	1.50	0.00
Ps. putida										
50	1.10	0.00	0.00	0.00	0.00	1.16	2.70	0.00	0.00	2.10
52	2.10	0.00	1.00	2.00	0.00	2.75	0.00	1.50	2.50	1.05

$$\text{Relative power of antibiosis (RPA)} = \frac{\text{Diameter of inhibition zone}}{\text{Diameter of spotted antagonistic organism}}$$

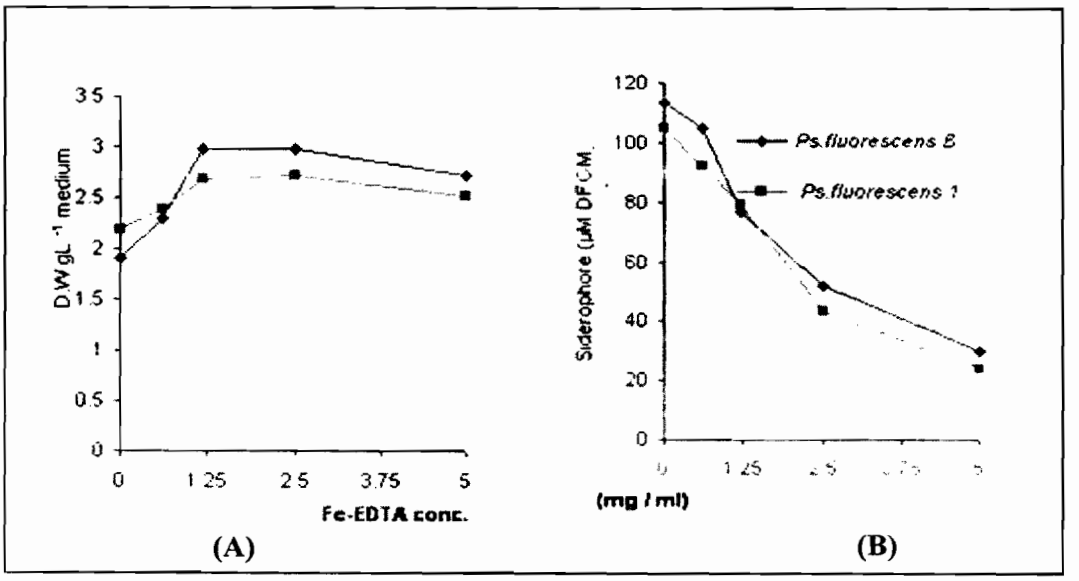


Fig.(5): Effect of increased concentrations of available iron as Fe-EDTA on growth (A) and siderophore production (B) by *Pseudomonas fluorescens* B & 1.

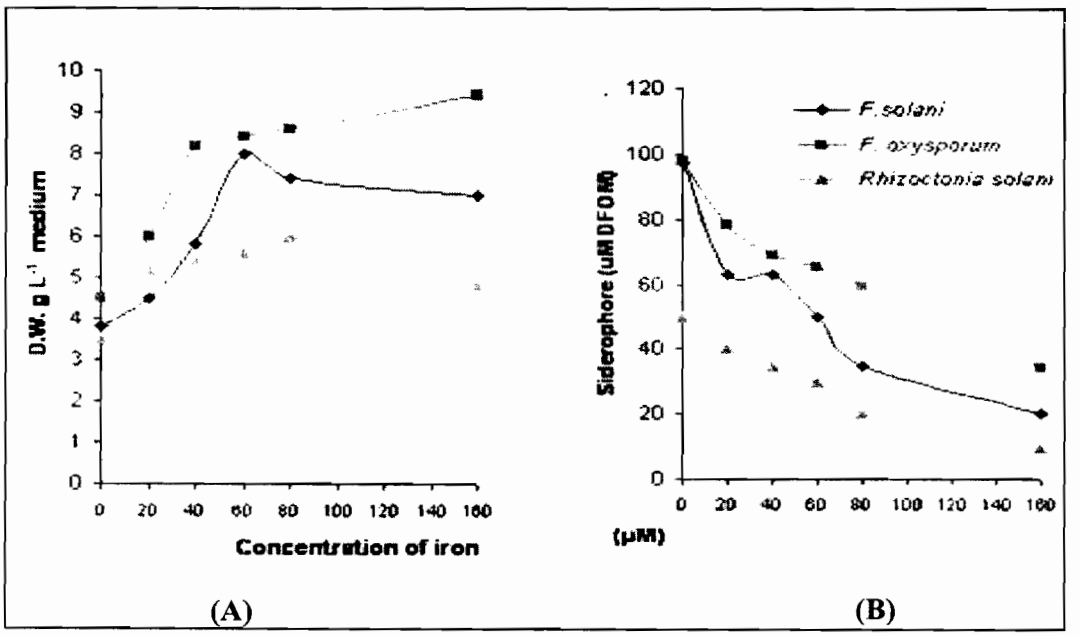


Fig. (6): Effect of increased concentrations of available iron as ferrous sulphate on growth (A) and siderophore production (B) by 3 strains of phytopathogenic fungi.

Protein banding pattern of *Ps. fluorescens* B outer membrane as influenced by increased concentrations of available iron

The results of SDS-PAGE of protein banding pattern of *Ps. fluorescens* B outer membrane are shown in **Fig. (7)** and **(Table 5)**. Results revealed a total of 7 bands with molecular weight ranging from about 19.65 – 118.07 KDa. The outer membrane of *Ps. fluorescens* B cells grown in medium without iron or suplemented with 0.6 and 2.5 mg Fe-EDTA / ml medium was charactarized by four specific bands with molecular weight (MW) of about 118.07, 99.21, 90.15 and 19.65 KDa. These bands were absent from the treatments of higher iron concentrations i.e., 5 and 10 mg Fe-EDTA/ ml medium. Therefore, protein banding pattern of *Ps. fluorescens* B outer membrane grown without Fe could be considered as positive markers for the depressive effect of available iron on the development of these proteins.

Effect of available iron on the antagonistic activity of *Ps. fluorescens* against phytopathogenic fungi and root nodule bacteria.

Due to their varied siderophores mediated an antagonism against phytopathogenic fungi and root nodule bacteria, the 2 strains *Ps. fluorescens* B and 1 wre selected to study the effect of available Fe on that phenomenon. Therefore, the antagonism between *Pseudomonas fluorescens* B against *F. oxysporum* f.sp. *glycine* or *Rh. solani* and *Pseudomonas fluorescens* 1 against *B. japonicum* ARC 501 or USDA 110 were retested on the appropriate media supplemented with increased concentrations of Fe-EDTA.

Data showed that the relative power of antibiosis of *Ps. fluorescens* strains against phytopathogenic fungi was gradually reduced by increasing Fe-EDTA concentration in the culture media **(Fig. 8 A)**. Incorporation of 5 mg Fe-EDTA / ml medium resulted in complete depression of antibiosis induced by *Ps. fluorescens* B against the 2 fungal strains. However, the antagonism of *Ps. fluorescens* 1 against *B. japonicum* strains was slightly affected by available iron incorporation in the culture medium **(Fig. 8 B).**

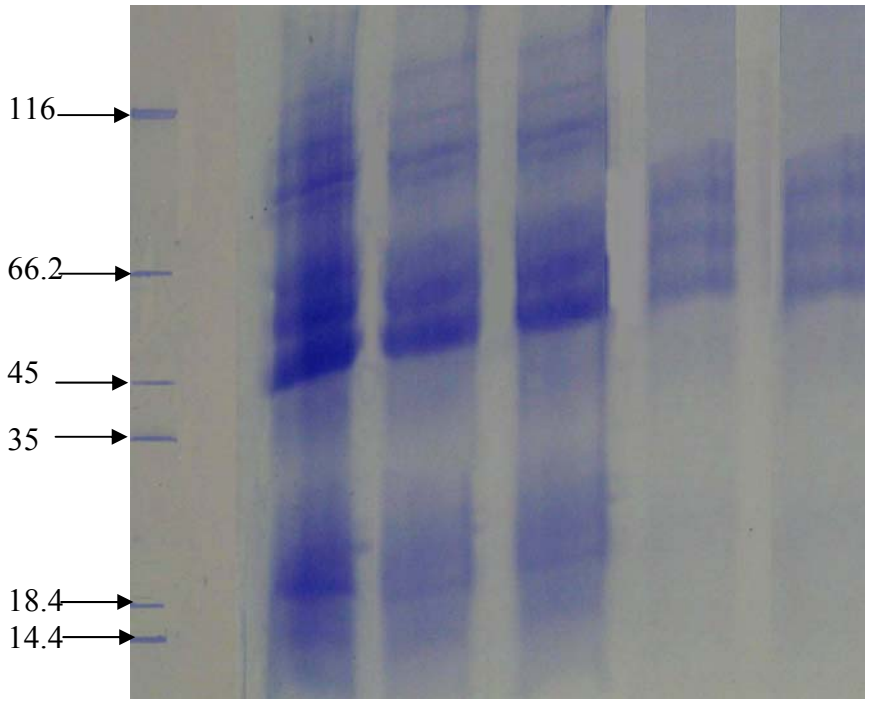


Fig. (7): Protein banding patterns of *Ps. fluorescens* B outer membrane grown in different concentrations of Fe-EDTA. *(M) refers to marker proteins

Table (5): Molecular weights of protein bands and their pattern of distribution in *Ps. fluorescens* B outer membrane as affected by increased concentration of Fe – EDTA in the culture medium.

Marker (M)	Band No.	M W (KDa)	Fe – EDTA Concentration (mg / ml medium)				
			0.0	0.6	2.5	5.0	10.0
116.0	1	118.07	+	+	+	-	-
66.2	2	99.21	+	+	+	-	-
45.0	3	90.15	+	+	+	-	-
35.0	4	66.59	+	+	+	+	+
18.4	5	61.65	+	+	+	+	+
14.4	6	46.75	+	+	+	+	+
	7	19.65	+	+	+	-	-

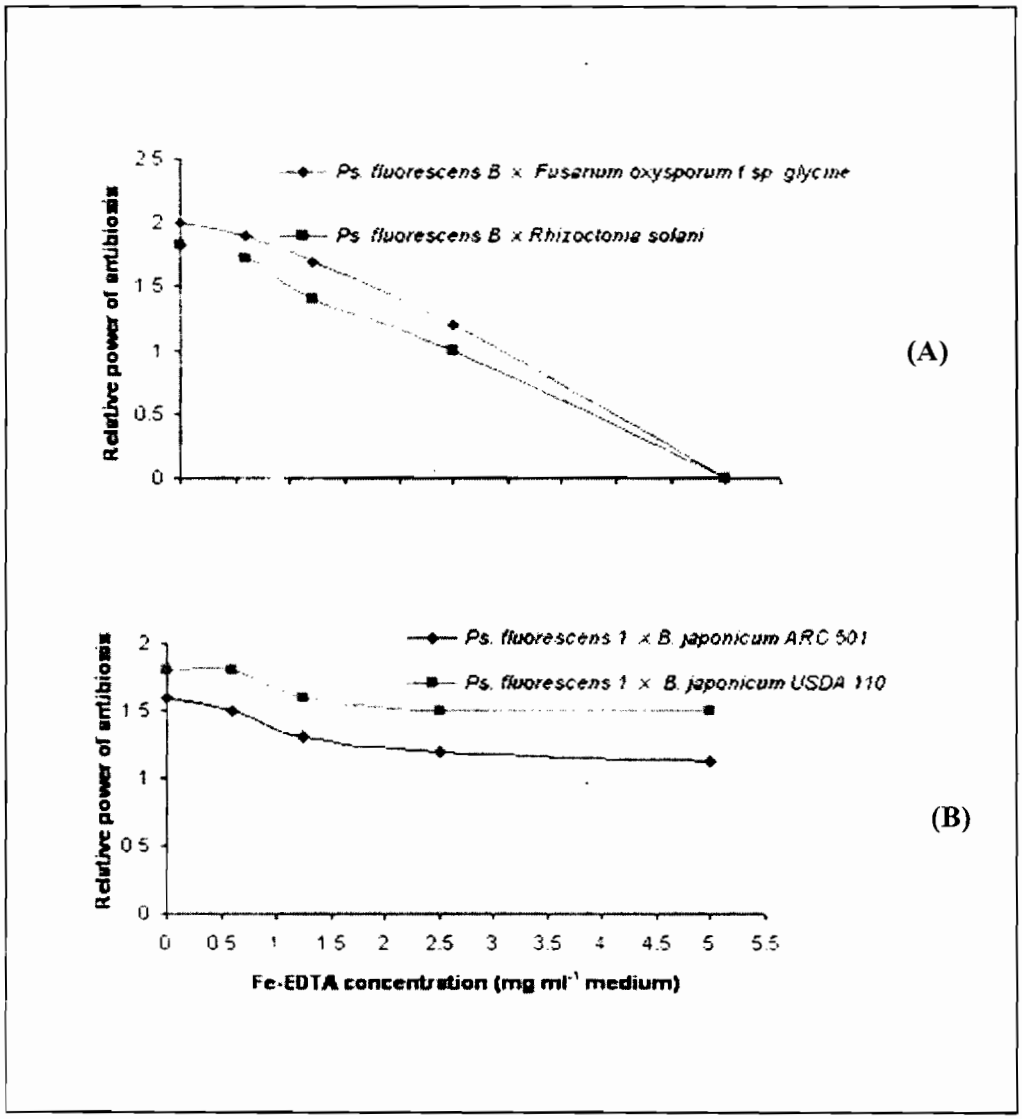


Fig.(8): Effect of increased concentrations of Fe-EDTA on relative power of antibiosis (RPA) induced by 2 strains of *Pseudomonas fluorescens* against 2 strains of soil borne pathogenic fungi (A) or root nodule bacteria (B)

DISCUSSION

Interactions among rhizosphere microorganisms based on siderophores producing abilities under iron limited condition are receiving increased interests in ecological studies. The Chrome Azurol S (CAS) Agar medium of **Schwyn and Neilands (1987)** is generally a useful tool to differentiate microbial populations according to siderophore producing ability.The qualitative reaction is based on the development of orange halos around colonies of the siderophore – producing microorganism as the siderophores remove Fe from the Fe-CAS dye complex which gives the medium its characteristic blue color. However, **Alexander and Zuberer (1991)** reported that CAS agar failed to support the growth of some microflora because it contains a large amount of hexadecyl trimethyl ammonium bromide (HDTMA) which is toxic to some microorganisms. Therefore, in this study, the 10% tryptic soya agar was used in addition to CAS agar to eliminate the above-mentioned interferring factor and also to enlarge the scope of siderophores qualitative assessment among the tested microorganisms. The 8 –hydroxyquinoline added to tryptic soya agar is proved to be an effective selective agent for siderophores producers. It has a high capability to scavenge any iron traces in the medium. Therefore, siderophores producing organisms are only able to grow under that condition **(Geels *et al.* 1985).**

The finding that fluorescent pseudomonads and phytopathogenic fungi were particularly active in siderophores production **(Teintze and Leong, 1981 and Wiebe, 2002)** and that soybean root nodulators are low producers **(Carson *et al.* 1992b and Van Rossum *et al.*, 1994)** seemed to be interesting findings for further studies. However, other researchers reported that bradyrhizobia differ in their ability to produce siderophores **(Sunita *et al.* 2000 and Khandelwal *et al.* 2002)** .

Among the tested siderophores-producing microorganisms, bacteria produced catecholate siderophores, whereas fungi produced the hydroxymate type. In this respect, **Jadhav and Desai (1992)** reported that different types of catechole siderophores were produced by bradyrhizobia. **Messenger and Ratledge (1985),** on the other hand, reported that hydroxamate type siderophores were produced by many soil fungi. However, both types of siderophores were reported to be produced by fluorescent pseudomonads **(Bezbaruah *et al.* 1996)** and that hydroxymate – along with other types of siderophores are produced by rhizobia **(Carson *et al.*, 2000)**. That siderophores production is detected only in the iron-starved conditions coincide with the findings of **Nasr *et al.* (2000)** and **Sharma and Johri (2003 a)**. Available iron, on the other hand, has an inhibitive effect on siderophore production. Synthesis of siderophores was almost completely depressed by addition of $\geq 10\mu$M Fe for fluorescent pseudomonads **(Nasr *et al.*, 2000)** and 7μM Fe for *F.venenatum* A3/5 culture media **(Wiebe 2002)**. Under iron deficiency condition, siderophores production by Gram-negative bacteria is associated by the appearance of one or more new major proteins in the outer membranes termed iron regulated outer membrane proteins (IROMPs) **(Neilands 1982)**. In this study, SDS – PAGE analysis of the outer membrane fractions from *Ps. fluorescens* B grown under iron – deficient condition clearly indicates the expression of 4 distinct outer membrane proteins which were completely repressed under high iron concentrations (5 and 10 mg Fe-EDTA / ml medium). It is very likely that these proteins are involved in siderophore transportation activity. The depressive effect of high concentrations of available iron on receptor protein development in outer membrane was also reported by **Jadhav and Desai (1994)** and **Terano *et al.* (2002)** .

Siderophores production is known to be involved in antagonistic mediated interactions. *In vitro* studies, fluorescent pseudomonads suppressed the growth of several *Fusarium* and *Rhizoctonia* pathogens through siderophores production **Schen and Baker (1982)**. However, the antagonistic activity differed according to the kind of culture medium and microbial strain as shown by **Benizri *et al.* (1995), Goel *et al.* (2002)** and **Sharma and Johri (2003 b). Benizri *et al.* (1995)** showed that *Pseudomonas* originated from maize rhizosphere antagonized *F. graminearum* (the agent of root rot of maize) *in vitro* and the inhibition depended on the agar medium used. They reported that the carbon source of the medium and its iron content is affecting the concentration of the antifungal agent produced by the bacterium and its distribution by diffusion in agar plates. **Goel *et al.* (2000)** also reported larger inhibition zones from *Pseudomonas* MRS16 antagonizing plant pathogenic fungi on nutrient agar and King's B media compared to potato dextrose agar. Mutants altered in fluorescent pigment production ability, derived by nitrosoguanidine mutagensis, showed variable inhibtory interactions. The degree of *B. japonicum* inhibition by *Pseudomonas fluorescens* also differed according to media composition and tested strain **(Sindhu *et al.* 1999)**. When high concentrations of Fe-EDTA were incorporated into King's medium B / malt agar plates, there was no antagonestic effect of *Pseudomonas* on the growth of *F. graminearum.* and the organism did not synthesize siderophores indicating that siderophore was the main factor responsible for the antagonism **(Benizri *et al.* 1995)**. The addition of 100 mM $FeCl_3$ to the nutrient agar medium also decreased the inhibition of fungal growth by *Pseudomonas* MRS16 **(Goel *et al.* 2000)**, suggesting the involvement of siderophores in the antagonestic effect.

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انتاج السيدروفورس والسلوك التضادى للكائنات الدقيقة بالريزوسفير وعلاقتهما بتيسير الحديد

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تم فى هذه الدراسة فحص إنتاج السيدروفورس وصفياً وكمياً لمدى من العزلات و السلالات التى تمثل أربعة مجاميع من الكائنات الدقيقة بالريزوسفير . وعموماً فقد كانت بكتريا السيدوموناس الفلورسنتيه والفطريات الممرضة للنبات ذات كفاءة فى إنتاج السيدروفورس مقارنة ببكتريا العقد الجذرية لفول الصويا ، واحتلت بكتريا الأزوتوباكتر المثبتة للأزوت الجوى مكانة متوسطة فيما بين المجاميع السابقة ، ومن الناحية التركيبية أنتجت كل الأنواع البكتيرية المختبرة النوع الكاتيوكولى من السيدروفورس ، فى حين أنتجت الفطريات النوع الهيدروكسيماتى ، ولم يلاحظ وجود تضاد بين بكتريا العقد الجذرية ، أو الأزوتوباكتر والفطريات الممرضة للنبات فى البيئات الفقيرة فى محتواها من الحديد، ولكن ارتبطت هذه الظاهرة ببكتريا السيدوموناس الفلوروسنتيه بصفة خاصة ، وتأثرت فى حدتها بتركيب البيئة المزرعية ونوع الميكروب المختبر . وقد أدى إضافة الحديد الميسر للبيئة الى تشجيع نمو سلالات *Pseudomonas fluorescens* والفطريات الممرضة للنبات فى حين ثبطت قدرتها على إنتاج السيدروفورس ، وأظهرت تحليلات الجل الكتروفوريسيس لبروتينات الغشاء الخارجى لبكتريا *Pseudomonas fluorescens* B ، أن المستويات العالية من الحديد الميسر (٥ ، ١٠ مجم Fe – EDTA/ مل بيئة) قد ثبطت ظهور أربع حزم بروتينية لوحظت فى أغشية الخلايا المنماة فى البيئات التى لاتحتوى على الحديد ، كما أدى الحديد الميسر إلى اختزال الأنشطة التضادية لبكتريا *Ps. fluorescens* ضد الفطريات الممرضة للنبات ، بكتريا العقد الجذرية بدرجات متباينة .