JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2012. Vol. **6**(1), p. 439-443

Characterization and Antifungal Activity of Siderophore Produced by Rhizospheric *Pseudomonas fluorescence* against Fungal Pathogen of Soybean and Groundnut

Syed Sajeed Ali and N.N. Vidhale

Department of Microbiology, Shri Shivaji Science College Amravati, Shivaji Nagar, Morshi Road, Amravati - 444 603, India.

(Received: 25 April 2011; accepted: 03 June 2011)

In the present study thirty six pseudomonas species were isolated and identified as Pseudomonas *fluorescence* from rhizospheric soil of different crops such as sugarcane, groundnut and soybean in different places of Parbhani District. All isolates were shown siderophore production on succinate medium. The maximum siderophore production was found in PFSP03 strain i.e 72%. The strain produced both hydroxamate and chatacholate type of siderophore which was maximum at 10mM iron concentration in the medium. Also antifungal activity was observed against phytopathogens viz., *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Macrophomina phaseolina.*

Key words: Antifungal Activity, Siderophore, *Pseudomonas fluorescence.*

Siderophore are low molecular weight iron chelating compound synthesized by microorganism under iron limited conditions[1,2]. Siderophore have an extreme affinity for ferric iron, which chelate ferric iron and transport it into microbial cell. It is secondry metabolites and are assembled by nonribosomal cytoplasmic peptide synthases[3]. There are two main siderophore classes, the catechol-type and the hydroxamate-type[4]. Iron is a vital element require by all living organisms for many cellular processes such as electron transport chain and acts as a cofactor for many enzymes[5]. However at the biological pH and under aerobic condition, iron is oxidized to insoluble oxyhydroxide polymers, which are unavailable to microorganism, to come over this problem various microorganism synthesize siderophore e.g. *Pseudomonas, Azotobacter, Bacillus, Enterobacter, Serratia, Azospirillum* and *Rhizobium*[6,7].Currently siderophore produced by *Pseudomonas* spp., used as a biological control for various plant diseases caused by fungal pathogen to reduced application of chemical pesticides and fungicides[8,9] and the possible use of Pseudomonas in detoxifying chemical wastes through a wide range of enzymatic metabolic activities[10].The aim of present investigation was to isolate and screen most promising siderophore producing strain of *Pseudomonas fluorescence* from rhizospheric soil of different crop in Parbhani District and their antifungal activity against fungal pathogen of soybean and groundnut.

* To whom all correspondence should be addressed.
E-mail: obaide2002@yahoo.com

MATERIAL AND METHODS

Thirty six samples of different rhizospheric soil from different field were collected along with root of different crop out of which 12 of Sugarcane, 12 of Soybean and 12 of Groundnut from different field of Parbhani District. The details of isolates were given in Table-1. Isolation of Pseudomonas species from soil was done by serial dilution method on King's B medium by plating method. Also grown on citramide agar and well spread individual colonies were selected for identification and characterization. The identification of *Pseudomonas fluorescence* was carried out by studying several biochemical tests. Pure culture of *Pseudomonas fluorescence* species was obtained by exposing the plate to ultraviolet light illumination to detect fluorescence.

Siderophore Production

Siderophore production was study by inoculating different isolate *Pseudomonas fluorescence* in conical flask containing 250 ML succinate medium [11], containing of gm/l K_2HPO_4 6.0, KH_2PO_4 3.0, $MgSO_4$ 0.2, $(NH_4)_2SO_4$ 1.0 and succinic acid 4.0, pH 7.0. It was incubated for 24-30h at 28°C with constant shaking at 120 rpm on rotator shaking incubator. After incubation the fermented broth were centrifuge at 10,000 rpm in cooling centrifuge at 4°C for 10 minute and cell free supernatant was then mixed with 0.5 ml CAS solution and 10ul shuttling solution (Sulfosalicyclic acid). The color obtained was determined using the spectrophotometer at Absorbance 630 nm after 20 min of incubation with blank (Succinate medium). The percentage of siderophore units was estimated as the proportion of CAS color shifted using the formula $[(A_r - A_s)/A_r] \times 100$, where A_r is the $A630_{nm}$ of reference sample (medium + CAS assay solution + shuttle solution) and A_s is the $A630_{nm}$ of the sample (supernatant + CAS assay solution + shuttle solution).

Characterization of Siderophore

The hydroxamate and catecholate type of siderophore was determined by Neilands Spectrophotometric assay[12]. A cell free supernatant of different isolate of *Pseudomonas fluorescence* were harvested by centrifuging the culture broth at 10,000 rpm in cooling centrifuge at 4°C for 10 minute. To a 1 ml of supernatant 2 % aqueous solution of FeCl3 was added and spectral scan was performed on Systronics Double beam UV-VIS Spectrophotometer. The graph obtained from Spectrophotometer shown two peak one at (400nm) and second (490nm) which indicate presence of Hydroxamate and catecholate type siderophore.

Effect of iron concentration on Siderophore production

Siderophore are iron-specific compounds which are secreted under low iron stress and which capture iron from the environment. On the other hand, the biosynthesis and secretion of siderophore are strictly regulated by environmental factors of which iron concentration is the most important. Taking into account this factor, we have studied siderophore production on different iron concentration. To determine the effect of iron concentration the maximum siderophore producing strain of *Pseudomonas fluorescence i.e* PFSP03 were grown in succinate medium containing $Fecl_3$ in increasing amount i.e. 1-100µM. The flask was incubated for 24-30h at 28°C with constant shaking at 120 rpm on rotator shaking incubator.

Antifungal activity of siderophore

Plant pathogenic fungi i.e, *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Macrophomina phaseolina*, were isolated from the diseased seeds of groundnut and soybean by blotter technique[13] and identified based on vegetative and spore morphology[14]. The Identified fungal plant pathogens were grown on potato dextrose agar media and incubated for 8 days to get profuse growth of selected fungi, thereafter 10mm diameter dish of each fungi was obtained by cork which was placed at the center of plate containing spread siderophore supernatant of PFSP03strain. Then the plates were incubated for 36h at 32°C and the liner growth in the form of diameter of the fungal growth was measured and compared with control. Percent inhibition was determined by comparing reduction in fungal growth in relation to control.

RESULTS AND DISCUSSION

Thirty six Pseudomonas species isolated from rhizospheric soil of different crop in Parbhani District were identified as a *Pseudomonas fluorescence*. They produced a fluorescent yellow

green pigment on King B medium. All isolates were positive for catalase, lipase, arginine dhiydrolase, gelatinase, urease and did not hydrolyse starch. Also shown growth at 4°c but not at 41°c. Among the thirty six isolate the *Pseudomonas fluorescence* strain obtained from rhizospheric soil of sugarcane, groundnut and soybean of Pedgaon shown highest percentage of siderophore production while the strain obtained from of Hatta and Gangakhed shown moderate and the strain of Bori lowest percentage of siderophore production(Fig. 1,2,3). As cited in literature biosynthesis and secretion of siderophore are strictly regulated by environmental factors and availability of free iron. Hence the above finding revealed that sugarcane rhizospheric soil of Pedgaon and their environment is suitable for *Pseudomonas fluorescence* to produce maximum percentage of siderophore. As the

Table 1. *Pseudomonas fluorescence* isolated from Rhizospheric soil of different crop in different place of Parbhani District

S. No	Isolates	Origin	Number
Pseudomonas fluorescence of Sugar Cane			
01	PFSP01	Pedgaon District Parbahni (M.S)	03
02	PFSP02		
03	PFSP03		
04	PFSB01	Bori District Parbahni (M.S)	03
05	PFSB02		
06	PFSB03		
07	PFSH01	Hatta District Parbahni (M.S)	03
08	PFSH02		
09	PFSH03		
10	PFSG01	Gangakhe District Parbahni (M.S)	03
11	PFSG02		
12	PFSG03		
Pseudomonas fluorescence of Soybean Crop			
13	PFSYP01	Pedgoan District Parbahni (M.S)	03
14	PFSYP02		
15	PFSYP03		
16	PFSYB01	Hatta District Parbahni (M.S)	03
17	PFSYB02		
18	PFSYB03		
19	PFSYH01	Bori District Parbahni (M.S)	03
20	PFSYH02		
21	PFSYH03		
22	PFSYG01	Gangakhe District Parbahni (M.S)	03
23	PFSYG02		
24	PFSYG03		
Pseudomonas fluorescence of Groundnut Crop			
25	PFGP01	Pedgaon District Parbahni (M.S)	03
26	PFGP02		
27	PFGP03		
28	PFGB01	Bori District Parbahni (M.S)	03
29	PFGB02		
30	PFGB03		
31	PFGH01	Hatta District Parbahni (M.S)	03
32	PFGH02		
33	PFGH03		
34	PFGG01	Gangakhe District Parbahni (M.S)	03
35	PFGG02		
36	PFGG03		

Pseudomonas strain PFSP03 isolated from sugarcane rhizospheric soil of Pedgaon showed maximum siderophore production further studies were extended by employing it.

The type of siderophore produced by PFSP03 strain were studied by Spectral analysis which showed one peak at (400nm) and second (490nm) which indicate presence of Hydroxamate and catecholate type siderophore[15] (Fig. 4). The study of effect of iron on siderophore production revealed that 10mM iron concentration is beast for siderophore synthesis further increase of iron in the medium decrease siderophore production (Fig. 5). This indicates that siderophore synthesis is iron restricted mechanism in bacteria[1]. De Villegas *et al*., 2002[16] also reported that iron concentration

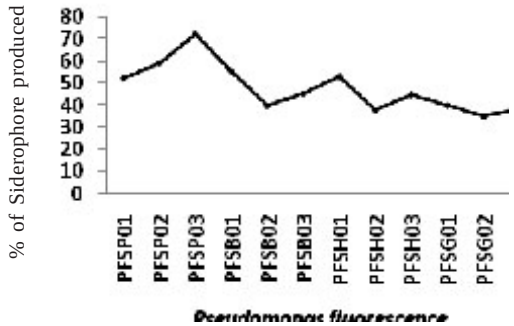

Fig. 1. Siderophore production by Sugarcane Rhizospheric *Pseudomonas fluorescence*

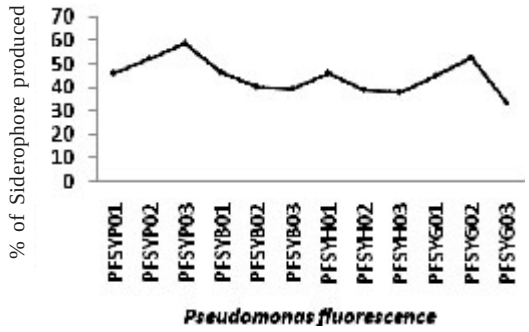

Fig. 2. Siderophore production by Soybean Rhizospheric *Pseudomonas fluorescence*

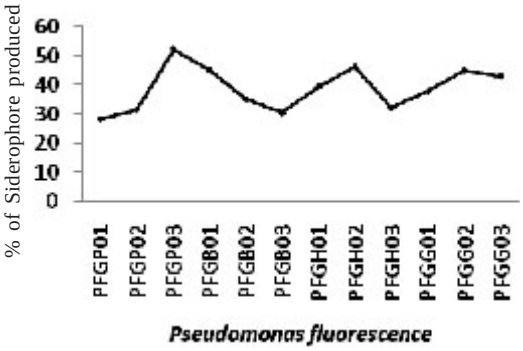

Fig. 3. Siderophore production by Groundnut Rhizospheric *Pseudomonas fluorescence*

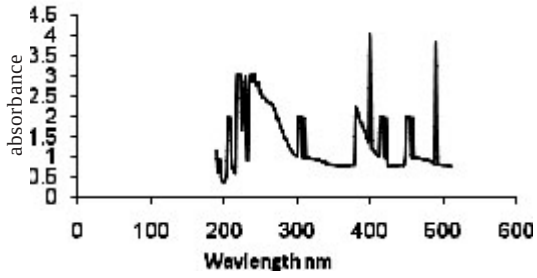

Fig. 4. Characterization of siderophore produced by Strain PFSP03

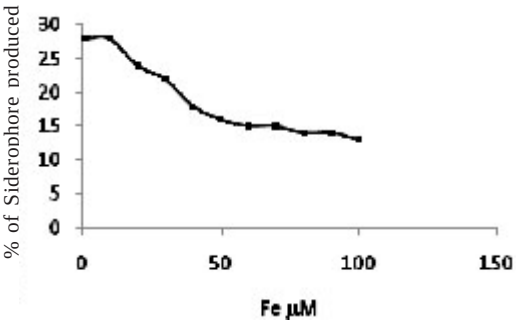

Fig. 5. Effect of iron concentration on Siderophore production

in medium greater than 10mM suppressed the siderophore production. The cell free supernatant of strain PFSP03 shown antifungal activity against all plant fungal pathogen on agar plate assay. The % inhibition were different with different fungi its maximum with *Fusarium oxysporium* and *Macrophomina phaseolina* i.e. 70% and 72% as compared to *Alternaria alternate*, *Aspergillus flavus*, and *Aspergillus niger* showed in (Table 1). Similar finding has be reported by Ahmadzadeh *et al*, 2006[17];Rajappan and Ramaraj, 1999[18];Patil *et al*, 1998[19].

Table 1. Antifungal activity of siderophore

S. No	Test Fungi	Percent Inhibition
1	*Fusarium oxysporium*	70%
2	*Macrophomina phaseolina*	72%
3	*Alternaria alternate*	60%
4	*Aspergillus flavus*	58%
5	*Aspergillus niger*	55%

CONCLUSION

It is concluded that the *Pseudomonas fluorescence obtained* from rhizospheric soil of sugarcane of Pedgaon District Parbhani can be used as efficient strain for biocontrol of varied rang fungal pathogen of groundnut and soybean. Further it can be employed on large scale siderophore production which have a vide application in medicine and agriculture.

REFERENCES

1. Lankford, C.E. Bacterial assimilation of iron. *Crit.Rev.Microbiol*. 1973; **2**: 273-331.
2. Neilands, J.B. Microbial iron transport compunds (siderophores)as chelating agents. In :Development of ironchelator for clinical use Martell, A.E., Anderson, W.J and Badman, D.G. (eds),Elsevier, North Holland, Amsterdam., 1981; pp. 13-31.
3. Wandersman, C. and Delepelaire, P. Bacterial iron sources: from siderophore to hemophores. *Annu.Rev. Microbiol*. 2004; **58**: 611-647.
4. Crosa, J.H., and Walsh, C.T. Genetics and assembly line. Enzymology of siderophore biosynthesis in bacteria. *Microbiol. Mol. Biol. Rev*., 2002; **66**: 223-249.
5. Litwin, C.M. and Calderwood, S.B. Role of iron in the regulation of virulence genes. *Clin. Microbiol. Rev*., 1993; **6**: 137-149.
6. Glick, B.R., Patten, C.L., Holguin, G. and Penrose D.M. Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria. *Imperial College Press. London.*1999.
7. Loper, J.E., Henkels, M.D. Utilization of heterologous siderophore enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. *Applied Environmental Microbiology*., 1999; **65**: 5357-5363.
8. Loper, J.E., and Lindow, S.E. Lack of evidence for in situ fluorescent pigment production by *Pseudomonas syringae* pv. Syringae on bean leaf surfaces. *Phytopathology*., 1987; **77**: 1449-1454.
9. Wilson, M. Biocontrol of aerial plant diseases in agriculture and horticulture: Current approaches and future prospects. *J. Ind. Microbial. Biotechnol*. 1997; **19**: 188-191.
10. Raaijmarkers, J.M., Sluis, I.V.D., Koster, M., Bakker, P., Weisbek, P.J., Shippers. Utilization of heterologosus siderophores and rhizospheres competence of fluorescent *Pseudomonas* Spp. *Can. J. Microbiol*., 1995: **41:** 126.
11. Meyer, J.M., Abdullah, M.A. The fluorescent pigment of *Pseudomonas fluorescence*: biosynthesis, purification and Physico-chemical properties. *J. Gen. Microbiol*., (1978); **107:** 319-328.
12. Jala, M.A.F., and Vander Helm, D. Isolation and spectroscopic identificatin of fungal siderophore. In :Handbook of microbial iron chelates, Winklemann, G. (ed), Pegamon Press, Oxford., 1990; pp. 235-269.
13. De Tempe.J. *Proceeding of International seed Testing Association*. 1933; 1963; 28.
14. Alexopoulos, C.J., Mims, C.W. and Blackwell, M. *Introductory mycology*. Fourth Edition, Johon Wiley&. Sons, Inc., New York. 1996.
15. Dhanya, M.K, Potty, V.P. Siderophore produciton *byPseudomonas fluorescens* isolated from the rhizosphere of *Solenostemon rotundifolius*. *J. Root Crop.*, 2007; **33**:138-140.
16. De Villegas, M.E.D., Villa, P., Frias, A. Evaluation of the siderophores production by *Pseudomans aeruginosa*. *Microbiologia.*, 2002; **44**:112-117.
17. Ahmadzadeh, M. H., Afsharmanesh, M., Javan-Nikkhah, A., Sharifi-Tehrani. Identification of some molecular traits in fluorescent pseudomonads with antifungal activity, *Iranian Journal of Biotechnology.*, 2006; **4**: 245-253.
18. Rajappan, K. and Ramarej, B. Evaluation of fungal and bacterial antagonists against *Fusarium moniliforme* causing wilt of cauliflower, *Annals of Plant Protection Sciences.*, 1999; **7**: 205-207.
19. Patil R., Jagadeesh, K., Krishnaraj, P. and Kulkarni, J. Bacterization of groundnut with *Pseudomonas fluorescens* for the control of collar rot caused by *Sclerotium rolfsii* Sacc.Karnataka, *Journal of Agricultural Sciences*, 1998: **11**: 423–425.