

Siderophore-mediated Antibiosis of rhizobacterial fluorescent *Pseudomonads* against Rice fungal pathogens.

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ABSTRACT:Fifteen rhizobacterial fluorescent *pseudomonas* isolates obtained from rice in the region of Andhra Pradesh, India In all 10 strains of *Pseudomonas fluorescens* were selected based on preliminary screening of all these isolates for antifungal activity against rice fungal pathogens (*P.oryzae* and *R.solani*)., inhibited the growth of rice fungal pathogens in Fe deficient King's B medium that varied from (3 to 58 % inhibition). Among these Pf 003 strain completely inhibited the mycelial growth of two rice pathogens (*P.oryzae* and *R.solani*) both in presence and absence of FeCl₃ which indicated the siderophore mediation along with antifungal metabolites.

Key words: Siderophores, *Pseudomonas fluorescens*, Rice fungal pathogens

INTRODUCTION

Fluorescent *Pseudomonas* spp. are known to inhibit plant pathogenic fungi in the rhizosphere¹. Members of the *Pseudomonas fluorescens*, namely *Pseudomonas putida* species complex and *Pseudomonas aureofaciens* have exhibited in vitro antagonism towards several fungi, but with great variability among the strains². 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^{3,4,5}. The rhizosphere isolate of *Pseudomonas fluorescens* was able to produce a pigment which showed a strong inhibition in vitro against pathogenic fungi such as *Phytium* spp., *Fusarium* spp. and *Rhizoctonia solani*⁶. In this paper we describe the involvement *pseudomonas fluorescens* siderophores inhibition of rice fungal pathogens.

MATERIALS AND METHODS

Fifteen strains were isolated from one hundred soil samples collected from rhizosphere regions of rice in Andhra Pradesh, India, using King's B medium⁷. Colonies that showed fluorescence under UV light (365 nm) were selected and further purified on the same medium. Ten strains were selected based on preliminary screening of all these isolates for antifungal activity against rice fungal pathogens by dual culture technique⁸. The most effective strains were examine the role of the antifungal metabolites and/or siderophores further, these

effective strains were grown on King's B agar medium in presence and absence of FeCl₃ (100 μM)⁹. An agar plug (9mm dia) taken from actively growing fungal culture was placed on the surface of the plate-enhancing medium. Simultaneously *Pseudomonas fluorescens* strain streaked 3 cm away from the agar plug at sides towards the edge of petriplates¹⁰. Plates inoculated with fungal agar plugs alone were used as control. The plates were incubated at 28°C until fungal mycelia completely covered the agar surface in control plate. Pathogens used were *Pyricularia grasia* and *Rhizoctonia solani* obtained from Directorate of Rice Research, Hyderabad.

RESULTS AND DISCUSSION

In this study fifteen strains were tested against two pathogens of rice on dual cultures, out of which ten strains exhibited percent inhibition ranging from 3 to 58 percent. Among the ten strains the P.f 003 strain effectively inhibited the both the pathogens (50-58%) tested. The next best was P.f 001(42% inhibition) on both the pathogens (Table 1)

The most effective isolates (P.f 001, 003, 005 and 007) were tested in the presence and absence of FeCl₃. Among these P.f 003 strain completely inhibited the mycelial growth of the rice pathogens both in presence and absence of FeCl₃ (Table 2). Which indicated the siderophore mediation along with antifungal metabolites. Siderophore production was observed reverse side of

petri plates, as green dots and also the change of color of the medium to fluorescent green. However, three strains P.f 001, 005 and 007 partially inhibited fungal growth in the presence and absence of FeCl₃ ranging from 25-85 percent. This suggested that these three isolates produced antifungal metabolites were different from the other strains. Similarly Kumar *et al.*, (2000)¹⁰ reported that the

pseudomonas fluorescens produced siderophores and antifungal metabolites which are involved in the control of phytopathogenic fungi. Radheshyam *et al.*, (1990) also confirmed the antibiotic property of *pseudomonas fluorescens* using FeCl₃.

Table 1. Bioefficacy of *P. fluorescens* against rice fungal pathogens

Strain code	<i>Rhizoctonia solani</i>		<i>Pyricularia oryzae</i>	
	Radial growth (mm)	Inhibition over control (%)	Radial growth (mm)	Inhibition over control (%)
P.f 001	58	42	52	42
P.f 002	75	17	76	15
P.f 003	38	58	45	50
P.f 004	71	21	66	27
P.f 005	56	38	59	34
P.f 006	59	34	72	20
P.f 007	52	42	60	33
P.f 008	63	30	87	3
P.f 009	74	18	78	13
P.f 010	72	20	80	11
Control	90	-	90	-

Table 2. Antifungal activity of *P. fluorescens* strains on fungal pathogens (% inhibition) from rice in the presence and absence of FeCl₃

Strain code	<i>Rhizoctonia solani</i>		<i>Pyricularia oryza</i>	
	FeCl ₃	No FeCl ₃	FeCl ₃	No FeCl ₃
P.f 001	85	26	66	25
P.f 003	100	100	100	100
P.f 005	75	36	68	27
P.f 007	82	30	70	34

REFERENCES

1. Shanahan, P., D. J. O. Sullivan, P. Simpson, J. D. Glennon and F. O. Gara. Isolation of 2, 4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Appl. Environ. Microbiol.*, (1992), 58: 353- 358.
2. Deweger, L. A., R. Vanboxtel, B. Vanderburg, R.A. Gruters, F. P. Geels, B. Schippers and B. Lugtenberg. Siderophores and outer membrane proteins of antagonistic, plant growth-stimulating root colonizing *Pseudomonas* ssp. *J. Bacteriol.*, (1986), 165: 585-594.
3. Howell, C. R. and R.D. Stipanovic. Control of *Rhizoctonia solani* on cotton seedlings With *Pseudomonas fluorescens* and with an antibiotic Produced by the bacterium *Phytopathology* , (1980) ,69: 480-482.
4. Viosard, C., C. Keel, D. Haas and G. Defago. Cyanide production by *Pseudomonas fluorescens* Helps suppress black root rot of tobacco under Gnotobiotic conditions. *EMBO. J.*, (1989), 8: 351-358.
5. Thomashow, L. S., D. M. Weller, R. F. Bonsall and L. S. Pierson. Production of antibiotic phenazine 1-carboxylic acid by fluorescent pseudomonad species in the rhizosphere of wheat. *Appl. Environ. Microbiol.* (1990)., 56: 908-912.
6. Altamirano, F., N. Correa, E. Schroder and S. Rosas. Activated biocontrol de *Pseudomonas aurantica* bajo conditions *in vitro* XVIII. RELAR. Santa Cruz de la Sierra. Bolivia. (1996).
7. King, E.O., M.K. Ward and D.E. Raney. Two simple media for the demonstration of Pyocyanin and fluorescein. *J. Lab. Clin. Med.*, (1954), 44: 301-307.
8. Sivakumar G., Sharma, N., Thirumalai, A. and Guna sekharan, P., Biocontrol of banded leaf and sheath blight of Maize by peat based *Pseudomonas fluorescens* formulations., *Indian Phytopathology*, (2000), 190-192.
9. Radheshyam, K., A. J. Marcel, Fernandez and G. Ralph. Isolation and Characterization of a *Pseudomonas* strain that restricts growth of various Phytopathogenic fungi. *Appl. Environ. Microbiol.* (1990). ,1053-1058.
10. Kumar, G., R. C. Sharma and S. N. Rai. Biocontrol of banded leaf and sheath blight Of maize by peat based *Pseudomonas fluorescens* formulation. *Indian Phytopathology*, (2000). ,53: 190-192.
