ISOLATION OF SIDEROPHORE- PRODUCING STRAINS OF RHIZOBACTERIAL FLUORESCENT *PSEUDOMONADS* AND THEIR BIOCONTROL AGAINST RICE FUNGAL PATHOGENS

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ABSTRACT: Rice blast caused by *Magnaporthe grisea* and sheath blight caused by *Rhizoctonia solani* are the major diseases affecting the rice production. Application of beneficial bacteria as seed or seedling root dip to protect these diseases may be an alternative strategies to chemical control. In this study, fluorescent Pseudomonads isolated from rice seedlings were used to screen for their antagonistic ability and siderophore mediated antibiosis under *in-vitro* conditions against these pathogens. Among 10 isolates, strain P.f 003 gave significantly higher inhibition of mycelial growth of *M. grisea* and *R. solani*. Strains of P.f 001, P.f 003, P.f 005 and P.f 007 produced siderophores when grown on Fe deficient and Fe fortified King's B medium. These strains again tested for their *in-vitro* antagonistic activity against *M. grisea* and *R. solani* on King's B media with or without $FeCl_3$. Our results showed that all these strains significantly reduced the growth of *M. grisea* and *R. solani* with $FeCl_3$ in the media compared to without $FeCl_3$. Strain P.f 003 activity was superior compared to other strains evaluated.

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

INTRODUCTION

Blast caused by *Magnaporthe grisea* and sheath blight caused by *Rhizoctonia solani* are the major diseases affecting rice cultivation. Though, these diseases are being managed through fungicides, their adverse effects on environment and beneficial soil micro-organisms are quite evident. Biocontrol approach for managing these diseases is considered to be a practical and economical alternative. Fluorescent Pseudomonads are known to inhibit several plant pathogenic fungi (Shanahan, P, et al, 1992). But, their activity varied among the strains (Deweger, L. et al, 1986). Production of secondary metabolites such as antibiotics, Fe-chelating siderophores, and cyanide are most often associated with fungal suppression by fluorescent Pseudomonads in the rhizosphere of several crops (Rabindran, R, et al, 1996). In view of this, we have evaluated several strains of fluorescent Pseudomonads isolated from rice seedlings for their antagonistic ability with or without iron supplement against *M. grisea* and *R. solani.*

MATERIALS AND METHODS

Isolation and *in-vitro* screening of *Pseudomonas fluorescens* against rice pathogens

Rhizosphere samples were collected from different rice growing areas in Andhra Pradesh. Strains of *P. fluorescens* were isolated using King's B medium. Colonies that have shown fluorescence at 365 nm were selected, purified and used for our studies. These strains were screened for their *in-vitro* antagonistic ability against rice blast pathogen, *M. grisea* and sheath blight pathogen, *R. solani* by dual culture technique (Rabindran, R, et al, 1996). Bacterial isolate was streaked at one side of petri dish (1 cm away from the edge) containing PDA. Five mm mycelial plug from seven-day-old PDA cultures of rice pathogens were placed at the opposite side of petri dishes perpendicular to the bacterial streak. Petri dishes were then incubated at 28 $\pm 2^0$C for 5 days. Petri dishes inoculated with fungal discs alone were served as control. There were three replications for each isolate against each pathogen. Observations on width of inhibition zone and mycelial growth of test pathogens were recorded and percent inhibition of pathogen growth was calculated.

Four effective strains resulted from the above dual culture studies were selected and tested for siderophore production and for their siderophore mediated antifungal metabolite production. Strains were initially grown on King's B medium with or without $FeCl_3$ (100 µM) (Radheshyam, K, et al, 1990). An agar plug (9 mm dia) taken from actively growing fungal culture was placed on the surface of the plate-enhancing medium. Simultaneously *P. fluorescens* strain streaked 3 cm away from the agar plug at both sides towards the edge of petriplates (Kumar, G, et al, 2000). Plates inoculated with fungal agar plugs alone were used as control.

The plates were incubated at 28^0C until fungal mycelium completely covered the agar surface in control plate. Observations on mycelial growth of test pathogens were recorded and percent inhibition of pathogen growth was calculated.

$$\% I = \frac{100 (C-T)}{C}$$

where I= inhibition of mycelial growth, C= growth of pathogen in the control plate (cm) and T= growth of pathogen in dual cultures (cm).

RESULTS AND DISCUSSION

Ten bacterial strains were isolated from rhizosphere soil samples collected from rice seedlings grown from Andhra Pradesh. All the strains were gram negative; rod shaped and produced yellowish green pigment on King's B medium. All were gelatin liquifiers and oxidase and arginine dihydrogenase positive and were identified as *P .fluorescens*. Dual culture studies of these strains against rice pathogens revealed that the inhibition of sheath blight pathogen, *R. solani* and blast pathogen, *M. grisea* ranged from 17-58% and 3-50% respectively. Among the strains, P.f 003 was found to be highly effective in controlling both the pathogens with inhibition ranging from 50 to 58%. The other effective strains are P.f 001, P.f 005 and P.f 007 with inhibition of test pathogens in the range of 33 to 42% (Table 1).

Table 1. *In-vitro* antagonistic effect of *P. fluorescens* on mycelial growth of rice blast and sheath blight pathogens.

Strain	*Rhizoctonia solani*		*Magnaporthe grisea*	
	Mycelial growth (mm)	Inhibition over control (%)	Mycelial growth (mm)	Inhibition over control (%)
P.f 001	58	42[b]	52	42[b]
P.f 002	75	17[g]	76	15[e]
P.f 003	38	58[a]	45	50[a]
P.f 004	71	21[f]	66	27[d]
P.f 005	56	38[c]	59	34[c]
P.f 006	59	34[d]	72	20
P.f 007	52	42[b]	60	33[c]
P.f 008	63	30[e]	87	3[f]
P.f 009	74	18[g]	78	13[e]
P.f 010	72	20[g]	80	11[e]
Control	90	-	90	-

Values are the means of three replications. Means followed by a common letter within a column are not significantly different by Duncan's Multiple Range Test (DMRT) at ($P \leq 0.05$).

The effective isolates (P. f 001, 003, 005 and 007) were tested for their production of siderophores under *in-vitro* conditions and also for the siderophore mediated antibiosis against rice blast and sheath blight pathogens. Of the four *P. fluorescens* strain P.f 003 completely inhibited the mycelial growth of the rice pathogens both in presence and absence of $FeCl_3$ (Figures. 1 & 2).

Fig. 1. Enhanced antifungal activity of *P. fluorescens* strains in iron supplemented King's B medium against rice sheath blight pathogen, *Rhizoctonia solani.*

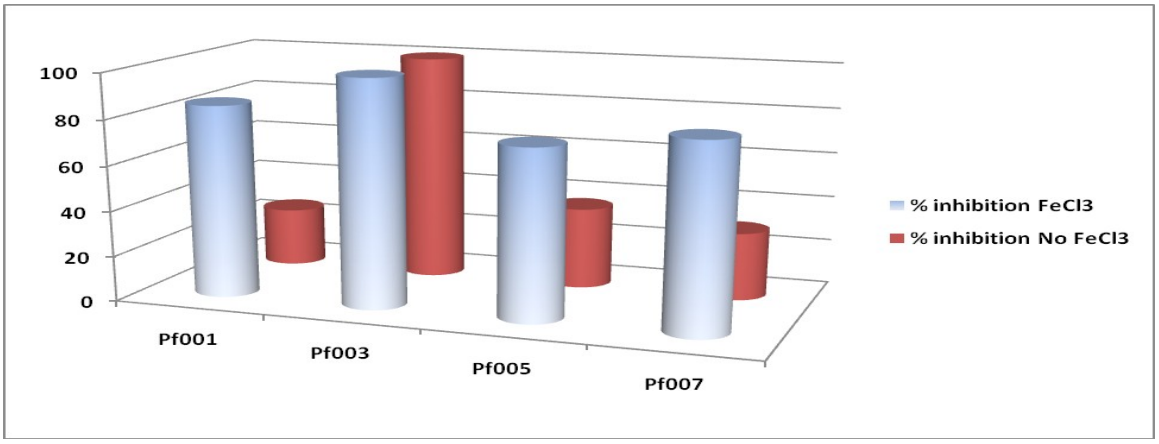
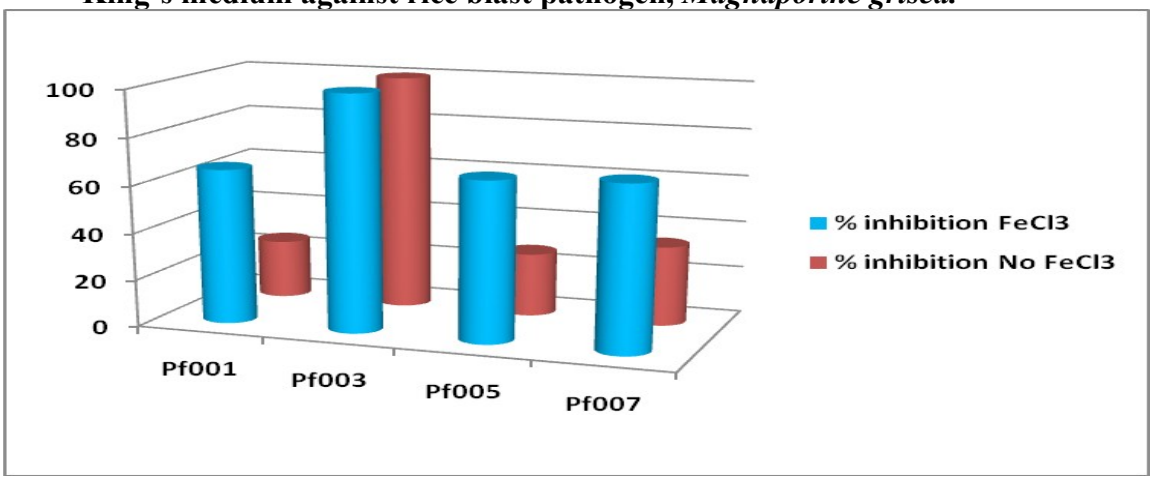


Fig. 2. Enhanced antifungal activity of *P. fluorescens* strains in iron supplemented King's medium against rice blast pathogen, *Magnaporthe grisea.*



Siderophore production was observed on reverse side of petri plates as green dots and also as color change of the medium to fluorescent green. With regard to other three strains, increased mycelial inhibition of test pathogens was observed in $FeCl_3$ fortified media compared to in media not amended with $FeCl_3$. The inhibitions of test pathogens for these three strains are in the range of 25 to 36% in the absence of $FeCl_3$ whereas the inhibition ranged from 66 to 85% in presence of $FeCl_3$. Based on these results it can be concluded that the antifungal metabolites produced by the strain P.f 003 was different from other strains and the mechanism of siderophore mediated antibiosis was evidenced with this strain.

Disease suppression by PGPR is by iron sequestration, production of antibiotics or through induction of systemic resistance (Liu, L., Kloepper, J. W, 1995). The results obtained here pointed out the possible use of *P. fluorescens* strains commercially in rice fields for blast and sheath blight disease suppression. However, further research is needed to elucidate in detail the mechanism of action of these strains and their compatibility with other components in integrated management of rice diseases.

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