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Effect of some fluorescent Pseudomonads on some plant pathogenic fungi and bacteria *in vitro*

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Nine isolates of fluorescent Pseudomonads were tested against some plant pathogenic fungi and bacteria (*Macrophomina phaseolina; Fusarium monoliforme, Aspergillus niger, Pythium aphanidermatum, Rhizoctonia solani, Xanthomonas campestris* pv. *oryzicola* and *Pseudomonas solanacearum*) *in vitro* for their inhibitory effect. Results indicated that some of the fluorescent bacterial isolates had antifungal and antibacterial properties against the above mentioned five fungi and two bacteria. Four out of nine isolates would inhibit all the fungus and bacteria.

Key words : Fluorescent Pesudomonads, pathogenic fungi, biocontrol

INTRODUCTION

Antagonism shown by different strains of fluorescent bacteria against different organisms particularly some plant pathogens have increased interest regarding this group of bacteria in different workers. Zaspel (1989) has isolated some strains of fluorescent pseudomonads and tested *in vitro* their antagonism towards *Gaeumannomyces graminis* isolates and 36% of these isolates cause medium to strong inhibition of the fungus. Livens *et al.* (1989) have isolated *P. fluorescence* from roots and leaves of different crops and found antifungal properties against a set of phytopathogenic fungi.

Hebber *et al.* (1991), Digat (1992), Andreoli *et al.* (1993), and Sarath Chandra *et al.* (1993), have tested *Pseudomonas fluorescence* against different pathogenic fungi. Barbosa *et al.* (1995), Gomes *et al.* (1996), Wilson et al. (1992), and Silva *et al.* (1998) have observed the application of commercial formulation of *Pseudomonas fluorescence* in control or reduction of pathogen per plant. We have taken this experiment to find out the effect of fluorescent Pseudomonas on different pathogenic fungi and bacteria *in vitro*.

MATERIALS AND METHODS

Nine isolates of fluorescent Pseudomonads were tested against 5 different pathogenic fungi like *Macrophomina phaseolina, Fusarium moniliforme, Rhizoctonia. solani, Aspergillus niger, Pythium aphanidermatum* and 2 different bacterial like *Xanthomonas campestris* pv. *oryzicola* and *Ralstonia salanacearum.* Screening was done in PDA medium at 30-32^{0}C by inoculating both end of the plate by equal amount of fungal plug and at the middle of the plate by fluorescent bacteria. The inhibitory action of the becteria were measured by the formula cited below.

Zone of inhibition = $\frac{a+b+c}{3}$ cm ; Bacterial growth area = $\frac{b_1+b_2+b_3}{3} \times L$ sq. cm; Fungal growth area = $f_1 \times f_2$ sq. cm,

To study the inhibition of bacteria by fluorescent Pseudomonads, firstly, plates containing PDA medium were divided by glass strip and on one side of the strip two bacteria (one test bacteria and other fluorescent Pseudomonads) were placed side by side and on the other side only the test bacteria was inoculated (control). The plates were incubated for 72 h and the growth of test bacteria were observed in comparison to the control side. Twenty one days Kings' broth medium was inoculated with fluorescent bacteria and the cell free culture filtrate was used for the inhibition study. All the cultures were

shaked in a shaker for 2 h and then centrifuged at 1000 rpm for 20 minutes and supernatants were collected. The supernatants were then kept in sterilized inoculation chamber along with formalin in separate plate for 6-8 h. Simultaneously un-inoculated broth was also kept with culture filtrates. The killing of the bacterial cells was done by formalin vapour and evaporated the extra absorbed formalin by heating. This cell free culture filtrates were used in this experiment.

Inhibition of fungi through bacterial culture filtrates were observed on PDA medium plate, where fungal plug was placed at the middle of the plate. Four cups were prepared on the four side of the plug at different distance, measuring the distance properly, and 0.2 ml culture filtrates were placed in each cup. The plates were incubated at 28-32°C for four days. Observations were taken by measuring the inhibition zone. Uninoculated control broth was also used in one case instead of culture filtrate for comparison. The same experiment followed for different fungi.

To observe the inhibition of bacteria by this method, the PDA medium containing plates were flooded with 0.5 ml test bacteria and in the middle of the plate 0.2 ml fluorescent bacterial culture filtrate was placed in cup and incubated for 48 h. After that zones of inhibition were measured.

RESULTS AND DISCUSSION

From the Table 1 it was evident that all the fluorescent bacteria were not equally effective against all the fungi tested, though BRSA, KKA, BBA and in most cases KBGA showed potential results against all most all the fungi. Against *M. phaseolina* KBGA showed the highest zone of inhibition i.e., 2.0 cm where as KBGL gave the lowest i.e. 0.99 where as against *F. moniliforme* KEL and KBGL showed the highest zone i.e., 2.32 cm and BSA showed only 1.23 cm. Again, BSEA gave the best result against *R. solani* i.e., 2.2 cm and KMA with the lowest zone i.e., 0.92 cm but KBGA showed the highest zone i.e. 2.54 cm against *P. aphanidermatum* and BRSA with the lowest i.e. 0.67 cm against the same. Though maximum fluorescent bacteria failed to inhibit *A. niger*, BBA gave the best result i.e., 1.82 cm and KEL with the lowest i.e. 1.13 cm.

Table 1 : Inhibitory effect of fluorescent Pseudomonads towards different fungal pathogens on solid media

Name of bacterial isolates	Zone of inhibition (cm)				
	M. phaseolina	*F. moniliforme*	*R. salani*	*P. aphanidermatum*	*A.niger*
Control (without bacteria)	-	-	-	-	-
KBGA	2.0*	2.05	1.58	2.54*	-
KKA	1.99	2.12	1.77	1.85	1.54
BRSA	1.75	1.88	1.87	0.67*	1.5
KEL	1.55	2.32*	-	2.45	1.13
KBGL	0.99*	2.32*	1.02	0.9	-
BSA	-	1.23*	1.29	1.19	-
BSEA	1.1	1.65	2.2*	0.77	-
KMA	1.27	1.63	0.92*	0.78	-
BBA	1.02	2.17	1.53	1.12	1.82

From Table 2 it was evident that all the bacteria free culture filtrate of the fluorescent bacteria did not effectively inhibited the fungi. But the cell free culture filtrates of KBGA, BRSA and BSEA were mostly effective. Except those, the culture filtrates of BSA, KMA, BBA produced effective inhibition zone against *P. aphanidermatum* though they could not inhibit *M. phaseolina* and *F. moniliforme*. Again, BSA and BBA showed effective inbibitory zones also against *R. solani*.

Table 2 : Inhibitory property of culture filtrates of fluorescent Pseudomonads against fungal pathogens

Name of bacterial isolates	Zone of inhibition (cm) due to culture filtrate			
	M. phaseolina	*F. moniliforme*	*R. salani*	*P. aphanidermatum*
Control (uninoculated broth)	-	-	-	-
KBGA	1.58	1.28	1.58	1.25
KKA	-	-	-	-
BRSA	-	1.2	1.53	2.1
KEL	-	-	1.35	-
KBGL	-	-	-	Nt
BSA	-	-	1.38	1.48
BSEA	1.48	1.48	1.6	1.33
KMA	-	-	1.48	NT
BBA	-	-	1.63	1.33

It was concluded from Table 3 that the cell free culture filtrate of KBGA showed the best result against *X. campestris* pv. *oryzicola* and *R. solanacearum* i.e. 4.5 cm and 4.03 cm respectively. The cell free culture filtrate of KMA gave no zone against *X. campestris* pv. *oryzicòla* where as KKA also

showed no zone against *R. solanacearum*.

Table 3 : Inhibitory effect of culture filtrate of the isolates of fluorescent Pseudomonads against some bacterial plant pathogen

Name of bacterial isolates	Zone of inhibition (cm) due to culture filtrate	
	X. campestris pv. *oryzicola*	*R. solanacearum*
Control (uninoculated broth)	-	-
KBGA	4.5*	4.03
KKA	1.27	-
BRSA	2.47	1.92
KEL	2.3	0.72
KBGL	4.2	1.18
BSA	3.2	1.12
BSEA	4.5*	2.25
KMA	-	0.70
BBA	3.8	0.98

The results of the present study confirmed that some of fluorescent bacterial isolates had antifungal and antibacterial properties against the above mentioned five fungi and two bacteria which echoed the observation of Yoshikawa *et al.* (1995) and Bustamante *Gallardo and Ciampi* (1989).

Isolates like KBGA, BRSA, BSEA could inhibit almost all the fungal isolates. It helped to identify the isolates of *P. fluorescens* with broad spectrum inhibitory activity. KBGA, BRSA, BSEA, BBA, KBGL, were also inhibitory to both the bacterial pathogens.

To understand primarily the nature of inhibitory principle of the fluorescent bacteria, it appeared that it was water soluble but possibly large molecule of chemical. Some of the isolates remained unaffected due to formalin treatment i.e. KBGA, BRSA, BSEA. Some of the culture filtrates were heat labile but mostly not so.

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