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Antagonistic potentials of mangrove soil fungi against plant pathogens

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ABSTRACT

The culture filtrate of mangrove soil fungi *viz, Aspergillus flavus, A.fumigatus, A.luchensis, A.niger, A.ochraceous, A.terreus, Penicillium citrinum, Penicillium janthinellum, Trichoderma harzianum* and *T.pseudokoningii* were tested against plant pathogens of *Drechslera ellisii, Fusarium oxysporum* and *Rhizoctonia solani*. The maximum inhibition of growth of test fungi was observed with staling products of the antagonistic fungi *A. niger* and *P. janthinellum*. The pH 6 and salinity 30 ppt were optimum for the production of antimicrobial compounds.

Keywords: Culture filtrate, *Fusarium oxyporum* Antagonistic fungi, Mangrove soil, Plant pathogens

Introduction

In the recent years considerable interest has been shown in the secondary metabolites of marine fungi, because of their importance (Kirk and Catalfoma, 1970) in medicine (Cuomo *et.al.*, 1996; Varoglu *et.al.*, 1997; Crews, 1998; Jenkins, 199; Hoeller *et.al.*, 1999; Byun *et.al.*, 2003). Therefore it is proved that the ocean can provide an inexhaustible source of water, metabolically derived products, food materials and nutrients. Over the past three decades fungi in different mangrove environs have been described (Pugh, 1968; Rai *et.al.*, 1969; Lee and Baker, 1972; Kohlmeyer and Kohlmeyer, 1979; Dunn and Baker, 1983). But studies on their potential utilization for various purposes have received little attention. In the present study, antagonistic activity of some soil fungi against plant pathogens has been investigated *in vitro* with cell-free culture filtrate of fungi amended in medium.

Materials and methods

Isolation of soil fungi

The soil samples were collected from Muthupet mangrove environs situated at Thiruvarur district, Tamil Nadu. Random samples of soil were collected from sampling stations in sterile polytene bags and pooled together. For the isolation of fungi from the soil, dilution plate technique *et.al.*, 1960) was used. Sea water corn meal agar medium (SWCMA) (Corn meal powder 20 g, dextrose 20 g, peptone 20 g, agar 20 g, aged sea water 500 ml and 500 ml of distilled water) was used in isolation of fungi.

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Antagonistic activity

The plant pathogens *Drechslera ellisii, Fusarium oxysporum* and *Rhizoctonia solani.* were obtained from A.V.V.M. Sri Pushpam College culture collection centre, Poondi, Thanjavur district, Tamil Nadu and maintained in potato dextrose agar medium (PDA). The antagonistic activity of 10 isolaed soil fungi (Table 1) were performed by cell-free culture filtrate method (food poisoning technique) as proposed by Grover and Moore (1962).

Optimization of pH and salinity

In order to determine suitable pH and salinity that favour the antibiotic production, those fungi *Aspergillus niger* and *Penicillium janthinellum* that showed more activity against pathogens were selected and experiments were designed as follows.

(a) pH

Seawater corn meal liquid broth was prepared and pH was adjusted to 4, 5, 6, 7, 8 and 9 using 0.1 N HCL and NaOH. The sterilized medium was inoculated at 28 ± 2°C for 15 days. After 15 days of incubation the staling substances were filtered through filter paper (Whatman No.1) and then through Sietz filter (G5). The filtrate was transferred aseptically into the conical flasks and stored at 4°C for further use.

(b) Salinity

NaCl and freshwater were used to increase and decrease the salinity (10-60 ppt) at 10 ppt interval respectively in Sea Water Corn Meal (SWCB) broth. The fungi were inoculated and growth in the different salinity broth and filtrate collected and stored as described earlier.

The prepared culture filtrates were added separately to the cooled PDA medium to give the concentration of 5, 10 and 25 per cent. The filtrate

Table 1. Antifungal activity of mangrove soil fungi against plant pathogens

Name of the culture filtrate	Contn. (%)	*D.ellisii*		*F.oxysporum*		*R.solani*	
		Growth rate (mm/day)	% inhibition	Growth rate (mm/day)	% inhibition	Growth rate (mm/day)	% inhibition
Control		10.0		9.		9.33	
Aspergillus flavus	5	7.6	23	7.0	22	9.0	3
	10	6.0	40	6.66	25	8.66	7
	20	4.33	56	6.66	25	8.33	10
A.fumigatus	5	6.0	40	6.66	25	9.0	3
	10	6.0	40	6.0	33	8.66	7
	25	6.0	40	5.0	44	8.0	14
A.luchuensis	5	9.33	6	6.66	25	9.33	3
	10	8.0	20	6.66	25	9.33	7
	25	6.33	36.6	6.66	25	8.33	10
A.niger	5	6.33	36.6	3.33	62	5.0	46
	10	6	40	3.33	62	4.66	50
	25	4.33	56	2.66	70	4.0	57
A.ochraceous	5	8.33	16.6	4.66	48	7.33	21
	10	6.33	36.6	4.33	52	6.66	28
	25	5.33	46.6	4.0	55	5.66	39
A.terreus	5	8.0	20	6.0	33	9.0	3
	10	7.0	30	5.33	40	8.33	10
	25	6.33	36	5.0	44	8.0	14
Penicillium citrinum	5	8.0	20	7.66	14.8	8.0	14
	10	7.0	30	7.33	18.5	5.66	25
	25	5.66	43	5.33	40	4.33	53
P.janthinellum	5	7.0	30	4.33	52	7.0	25
	10	5.66	43	3.66	59	6.0	35
	25	4.0	60	3.33	62	5.66	39
Trichoderma harzianum	5	9.33	6.	6.0	33	7.33	21
	10	8.33	16.6	5.66	37	6.66	28
	25	4.33	56	5.33	40	6.0	35
T.pseudokoningii	5	7.33	26.6	5.0	44	8.0	14
	10	7.0	30	4.33	52	7.33	21
	25	5.66	43	3.33	62	6.66	28

able 2. Effect of culture filtrate of *Aspergillus niger* and *Penicillium janthinellum* grown in fferent salinity against plant patthogens

Salt oncen ration	Conc. used (%)	*D.ellisi*		*Aspergillus niger*						*Penicillium janthinellum*			
				F.oxysporum		*R.solani*		*D.ellisii*		*F.oxysporum*		*R.solani*	
		Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)
10	5	6.0	40	2.66	65	8.33	10.7	9.0	10	6.66	13	7.33	21
	10	5.66	43	2.0	73.9	7.0	25	8.0	20	6.33	17.3	6.66	28
	25	5.0	50	0.33	95.6	6.33	32	7.0	30	5.66	26	6.33	32
20	5	5.66	43	2.33	69.5	5.0	46	8.66	13	5.33	30	2.66	17
	10	5.0	50	1.66	78.2	4.66	50	8.0	20	4.66	39	7.0	25
	25	4.66	53	0.33	95.6	4.0	57	7.66	23	3.66	52	5.66	39
30	5	4.33	56	1.33	8.26	4.0	57	666	33	4.66	39	6.0	35
	10	3.66	63	0.33	95.6	3.33	64	5.66	43	4.0	47	5.33	42.8
	25	2.66	73.3	Nil	100	2.66	71	3.0	70	2.66	65.2	4.33	53
40	5	5.0	50	1.66	78.2	5.0	46	7.0	30	5.66	26	6.66	28
	10	4.33	56	1.0	86.9	4.66	50	6.33	36	5.0	34.7	6.0	35
	25	3.66	63	0.66	91	3.66	60	5.66	43	4.33	43	5.66	39
50	5	5.0	50	2.0	73.9	5.33	42	7.33	26	6.0	21	7.0	25
	10	4.66	53	1.33	82.6	4.66	50	6.66	33	5.33	30	6.33	32
	25	4.0	60	10	86.9	4.0	57	6.33	36	4.66	39	5.66	39
60	5	5.66	43	1.66	78.2	5.0	46	7.66	23	6.33	17.3	6.66	28
	10	5.0	50	1.33	82.6	4.33	53	7.0	30	5.66	26	6.0	35
	25	4.66	53	0.66	91	4.0	57	6.33	36	4.33	43	5.33	42.8

able 3. Effect culture filtrate of *Aspergillus niger* and *Penicillium janthinellum* grown in fferent pH against plant pathogens

pH sed	Conc. used (%)	*D.ellissi*		*Aspergillus niger*						*Penicillium jathinellum*			
				F.oxysporum		*R.solani*		*D.ellissi*		*F.oxysporum*		*R.solani*	
		Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)	Growth rate (mm/ day)	inhibit ion (%)
pH4	5	6.33	36	2.66	65	5.0	46	9.33	6	6.66	13	7.33	21
	10	6.0	40	1.0	86.9	4.66	50	9.0	10	5.66	26	6.66	28
	25	5.33	46.6	0.33	95.6	4.33	53	8.0	20	5.0	34	6.33	32
pH5	5	6.0	40	2.0	73.9	5.0	46	9.33	6.6	5.0	34.7	7.66	17
	10	5.66	43	1.33	82.6	4.66	50	7.66	23	4.33	43	6.66	28
	25	5.0	50	0.33	95.6	4.0	57	4.33	56	3.33	56	6.0	35
pH6	5	4.33	56	2.0	73.9	4.66	50	7.66	23	4.66	39	7.0	25
	10	3.66	63.3	1.33	82.6	4.0	57	6.66	33	3.66	52	6.0	35
	25	3.0	70	Nil	100	3.33	64	3.66	63	2.66	65	5.0	46
pH7	5	4.66	53	2.66	65	4.33	53	8.33	16	5.66	26	7.0	25
	10	4.0	60	1.66	78.2	4.0	57	8.0	20	4.66	39	5.66	39
	25	3.66	63.3	0.33	95.6	3.33	64	6.66	33	3.66	52	5.0	46
pH8	5	4.66	53	2.33	69.5	4.66	50	7.66	23	6.0	21	7.0	25
	10	4.33	56	1.66	78.2	4.33	53	6.66	33	5.0	34	5.66	39
	25	3.33	66	1.0	86.9	4.0	57	6.33	36	4.66	39	5.0	46
pH9	5	4.33	56	4.33	43.4	5.0	46	7.66	23	5.0	34.7	6.66	28
	10	4.0	60	1.66	78.2	5.0	46	7.33	26	4.33	43	5.0	46
	25	3.66	63	1.0	86.9	4.66	50	6.66	33	3.33	56	5.0	46

mixed Potato Dextrose Agar media were dispended in Petriplates and allowed to solidify. After solidification, 5 mm disc blocks were cut from actively growing margin of the test fungi *Drechslera ellisii, Fusarium oxysporum and Rhizoctonia solani.* and inoculated separately at the centre of the plates. The plates were incubated at 28 ± 2°C for five days. The radical growth was measured every day and the mean growth rate was calculated. A control was also maintained for each fungal filtrate assay. The per cent inhibition of growth was calculated as follows.

$$\% \text{ of inhibition of growth} = \frac{\text{Growth in Control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Results and discussion

Totally 10 species of fungi belonging to 3 genera (*Aspergillus, Penicillium* and *Trichoderma*) were recorded from the mangrove oil samples. Swart (1958) recorded the dominance of the species of *Aspergillus* and *Penicillium* in the mangrove swamps of Inhyaca Island. Rai and Chowdhery (1976) reported that the species of *Aspergilli* were dominated over Mucorales and Penicillia in the mud of mangrove swamps. Chowdhery (1983) studied the succession pattern of fungi colonizing wood in the mangrove swamps reported that species of *Aspergillus* and *Penicillium* rank among initial colonizers along with Mucoraceous fungi.

The staling products in the culture filtrates of the antagonistic fungi inhibited the growth of the pathogenic fungi. The fungi varied in their efficiency. The maximum percentage of inhibition of growth of pathogens was on the potato dextrose agar medium amended with 25% of the culture filtrate of *A. Niger* and *P. Janthinellum* compared with other fungi tested (Table 1). The fungus *Trichoderma* proved to be a good antagonistic fungi against plant pathogens by many workers includes Singh (1988), Gupta *et.al.*, (1991), Kumaresan and Manibhushan rao (1991), Mondal *et.al.*, (1995), Khan and Saxena (1997), Ahmed *et.al.*, (1999), Jeyalakshmi *et.al.*, (1999), Ambikapathy (2000), Patricio *et.al.*, (2001), Niknejad Kazempour *et.al.*, (2002), Deore and Sawant (2002). But in the present investigation *A.niger* and *P.janthinellum* were found to have antagonistic potential against plant pathogenic fungi compared with the other soil fungi tested.

The efficacy of an antagonist depends on the media composition, pH, salinity and other environmental condition in which the organism being grown (Weindling, 1934; Whipps, 1987; Maiese *et.al.*,, 1994; Gogoi and Roy, 1996). The optimization of conditions is essential not only for the growth of a particular organism but also obtain more extracellular compounds. In the present investigation the pH and salinity stress over the activity of antagonistic organism (*A.niger* and *P.janthinellum*) revealed that maximum percentage inhibition of pathogenic fungi observed with the extract of *A. niger, P.janthinellum*) that have been grown in pH 6 and 30% of salinity.

The culture filtrate of *A.niger* grown in 30 ppt showed maximum percentage of inhibition of growth of pathogenic fungi viz., *Fusarium oxysporum* (100%), *Drechslera ellisii* (73.3%) and *Rhizoctonia solani* (71%). The culture filtrate of *P.janthinellum* grown in 30 ppt showed maximum percentage of inhibition of pathogenic fungi tested such as *D.ellisii* (70%), *F.oxysporum* (65.2%) and *R.solani* (53%) (Table 2). The culture filtrate of *A.niger* grown in pH 6 showed maximum percentage inhibition of growth of the pathogenic fungi tested *F.oxysporium, D.ellisii* and *R.solani* were 100, 70 and 64% respectively. Where as *P.janthinellum* grown in pH 6 also showed maximum percentage inhibition of growth of the pathogenic fungi tested *F.oxysporum, D.ellisii* and *R.solani* were 65, 63 and 46% respectively (Table 3). The present study gives an idea on the mangrove soil fungi and their antifungal properties and optimization of media for the production of anti fungal properties.

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