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Evaluation of CMC-induced cellulase activities of a wide variety of foliar fungi isolated from mangrove plants of Sundarbans

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Twenty eight fungi were isolated from infected leaves of mangrove plants of Sundarbans, India and their cellulase activities and mycelial biomass production in culture were compared using carboxymethyl cellulose (CMC) as inducer. *Neocosmospora vasinfecta* var. *vasinfecta* and *Pestalotiopsis disseminata* showed highest (R.S. 0.90 mg/ml) enzyme activity while *Exserohilum psidii* and *Curvularia clavata* exhibited lowest (R.S. 0.14 mg/ml) activity in 15-day-old culture filtrates. Mycelial biomass production was maximum in *Paecilomyces lilacinus* and minimum in *Cladosporium* sp. Apparently, there was no correlation between enzyme activity and mycelial biomass production in culture. In most cases cellulase activity increased with incubation time up to 15 days but mycelial biomass decreased after 9 days. Results suggest that foliar fungi isolated from infected mangrove plants are poor sources of extracellular cellulases.

Key words: Mangrove, Sundarbans, foliar fungi, enzymes, cellulases

INTRODUCTION

Many phytopathogenic fungi and bacteria produce cellulases which play an important role in the disintegration of cell wall components and colonization of host tissues by pathogens (Wood, 1967; Barras *et al.*, 1994; Hoshino *et al.*, 1997; Pardo and Forchiassin, 1998). A large number of fungi were isolated from the infected leaves of mangrove plants of Sundarbans (India). It was presumed that these fungal species might secrete cellulases also like pectolytic enzymes during plant pathogenesis. Most of the fungi mentioned in this paper have shown their pectolytic enzyme activities in culture earlier (De *et al.*, 1999). In this study an attempt has been made to identify the potent cellulase producing fungi, if any, among those isolated from mangrove plants and to compare their biomass production in culture. Cellulolytic enzymes have many potential industrial and agricultural uses apart from its significant role in soft rot diseases.

MATERIALS AND METHODS

Fungal cultures

Twenty eight fungi were isolated from mangrove plants of Sundarbans, India. These cultures were maintained in potato-dextrose-agar medium (PDA).

Assessment of mycelial growth

Fungi were grown separately in Petri dishes (10 mm diam.) containing PDA medium and an agar block (4 mm diam.) with 4-day-old mycelia was transferred to each flask (50 ml/250 ml flask) containing sterilized liquid medium (NH_4NO_3,1.0 g; KH_2PO_4, 1.0 g; $MgSO_4$. $7H_2O$, 0.5 g; Peptone, 1.0 g; carboxymethyl cellulose, 10 g; distilled water, 1litre). The inoculated flasks were incubated at 30 ± 1°C for a desired period. At the end of experimental period, the mycelia were collected, dried at 60°C for 96 h, cooled and weighed. Culture filtrates were used for enzyme assay.

Enzyme assay

Celluloytic enzyme activities were assayed following the method of Trigiano and Fergus (1979) with modifications. One ml of cell-free culture filtrate, (1 ml of non-inoculated medium for control) was taken after a desired period of incubation and was mixed thoroughly with 9 ml buffered substrate (1% CMC in 0.01M citrate buffer; pH 5.2) and incubated for 1 h at 40°C. Cellulolytic enzyme activity of the fungus was estimated by measuring the reducing sugar produced from carboxymethyl cellulose. After incubation, 3 ml of 1% 3,5-dinitrosalicyc acid (DNS) colour reagent was added and boiled for 15 min, cooled and volume

made up to 50 ml and its optical density was measured by colorimeter using a green filter (at 250 nm). The enzyme activity present in 1 ml culture filtrate was calculated from released reducing sugar which was measured by comparing with a standard curve of maltose. The results are presented in Table 1.

Table 1: Growth and cellulase activities of foliar fungi isolated from mangrove plants of Sundarbans

Fungi isolated	Mangrove plants	Incubation days			
		9		15	
		Mycelial dry wt. (mg)	Reducing sugar (mg/ml)	Mycelial dry wt. (mg)	Reducing sugar (mg/ml)
(1)	(2)	(3)	(4)	(5)	(6)
Alternaria infectoria E.G. Simmons	*Rhizophora mucronata* Lamk.	25.00	0.31	15.33	0.33
Bipolaris hawaiiensis (M.B. Ellis) S. Uchida & Aragaki	*Meliotropium curassavicum* L.	33.33	0.27	19.66	0.36
Cladosporium sp.	*Ceriops decandra* (Griff.) Ding Hou	19.66	0.29	8.33	0.31
Colletotrichum gloeosporioides (str. 3)	*Excoecaria agallocha* L.	18.00	0.25	21.66	0.52
C. gloeosporioides (str. F 744) (Penzig) Penzig & Sacc.	*Rhizophora mucronata* Lamk.	22.00	0.68	20.66	0.74
C. gloeosporioides (str. 756b)	*Nypa fruticans* (Thunb.) Wurumb.	24.33	0.39	23.33	0.58
Coniella musaiaensis var. *hibisci* B. Sutton	*Excoecaria agallocha* L.	48.00	0.44	12.33	0.36
Coniothyrium sp.	*Sonneratia apetala* Buch. - Ham.	26.33	0.63	20.66	0.75
Curvularia clavava B.L. Jain	*Bruguiera parviflora* W. & A.	28.00	0.09	27.00	0.14
C. lunata (Wakker) Boed.	*Acanthus illicifolius*	42.66	0.68	35.66	0.81
Exserohilum psidii A. Sivanesan	*Xylocarpus granatum* Koen.	23.00	0.03	19.66	0.14
Fusarium equisetii (Corda) Sacc.	*Acanthus illicifolius* L.	30.00	0.59	23.00	0.70
Neocosmospora vasinfecta var. *vasinfecta* E. F. Sm.	*Excoecaria agallocha* L.	29.00	0.90	20.50	0.90
Paecilomyces lilacinus (Thom.) R.A. Samson	*Heritiera fomes* Buch. - Ham.	61.33	0.61	44.60	0.74
Pestalotiopsis agallochae Pal & Purkayastha	*Excoecaria agallocha* L.	32.00	0.70	17.33	0.80
P. apetalae Purkayastha & Pal	*Sonneratia apetala* Buch. - Ham.	13.00	0.43	34.33	0.57
P. caseolaris Purkayastha & Pal	*Sonneratia caseolaris* (L.) Engl.	28.66	0.53	31.00	0.46
P. disseminata (Thum.) Stey *corniculatum* (L.)	*Aegiceras corniculatum* (L.) Blanco	28.33	0.67	22.00	0.90
P. moluccens Purkayastha & Pal	*Xylocarpus moluccensis* (L.) Roem.	13.66	0.16	17.33	0.27

Table 1 continued

(1)	(2)	(3)	(4)	(5)	(6)
Petalotiopsis palmarum (Cooke) Stey.	*Phoenix paludosa* Roxb	26.30	0.27	14.66	0.25
Phoma herbarum Westend.	*Avicennia alba* Bl	12.00	0.37	18.33	0.31
P. nebulosa (Pers. : Fr.) Berk.	*Sarcolobus carinatus* Wall.	35.00	0.22	29.60	0.20
Phomopsis sp.	*Avicennia alba* Bl.	30.00	0.86	21.50	0.83
P. clerodendrumii Ali et Saikia	*Clerodendron inerme* (L.) Gaertn.	23.30	0.44	8.66	0.44
Phomosis sp. cf. *commelinae* E. Punithalingam	*Bruguiera gymnorrhiza* (L.) Lamk.	21.33	0.19	13.66	0.33
P. rhizophorae Batistsa & Maia	*Rhizophora mucronata* Lamk.	22.00	0.48	12.66	0.40
P. sonneratiae Purkayastha & Pal	*Sonneratia apetala* Buch. -Ham.	15.66	0.62	12.66	0.62
P. thespesiae Luke & C. N. Reddy	*Thespesia lampus* (Cav.) Dalx. & Gibs.	20.66	0.21	16.00	0.31
Sporothrix sp.	*Rhizophora mucronata* Lamk.	13.66	0.27	11.66	0.42
Trichothecium roseum (Pers.) Link.	*Phoenix paludosa* Roxb.	24.33	0.62	22.66	0.57

RESULTS AND DISCUSSION

All fungi isolated from infected leaves of mangrove plants showed variable cellulase activity in their respective culture filtrates. Differences in some cases were significant. Variation in activities were also noted among 3 strains of *C. gloeosporioides* growing on different mangrove plant species. Highest enzyme activity (0.90 mg/ml) was recorded for *N.vasinfecta* var. *vasinfecta* and *P. disseminata* while lowest (0.14 mg/ml) for *E. psidii* and *C. clavata in* 15-day-old culture filtrates. Apparently there was no correlation between mycelial biomass production and enzyme activity *in vitro*. Similar observation was made by Nakazawa (1974) who studied cellulolytic enzyme activities of 9 higher fungi. In the present study enzyme production increased with increasing incubation period up to 15 days but mycelial biomass production decreased after 9 days in most cases. Amadioha (1997) reported that C_x cellulase and C_1 cellulase activities of *Rhizoctonia bataticola* in culture medium increased with incubation period and attained maximum values between 4th and 6th day. Acidic medium was favourable for the production of C_x and C_1 cellulases. Although cellulases are constitutively secreted by certain fungi their rate of synthesis could be enhanced by using specific substrates. Results of present study reveal the cellulase activities of a wide range of foliar fungi but its role,if any, in pathogenesis of mangrove plants remain yet to be investigated.

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