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RESEARCH ARTICLE

QUALITATIVE SCREENING OF DEGRADING ENZYMES FROM MANGROVE DERIVED FUNGI

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Abstract

Mangrove habitat of Muthupet was selected in the present study. Water, sediment, sea foams and natural substrates were collected to isolate the fungi by plating, baiting and direct examination techniques. Screening and activity of fungal enzymes like pectate lyase, lipase, xylanase and lipase were studied.18 species of fungi were showed zone of clearance for laccase followed by xylanase (16 sp.), lipase (13 sp.) and pectate lyase (12 sp.).Maximum enzyme laccase activity was observed in *R. stolonifer* (0.173 U/ml), xylanase in *A. flavus* (5.805 U/ml), lipase in *Mucor* sp. 2.982 U/ml) and pectate lyase in *R. oryzae* (11.529 U/ml).

Keywords: Mangrove, fungal enzymes laccase activity.

Introduction

Fungi are eukaryotic, spore producing, acholorophyllous, heterotrophic organisms with absorptive nutrition that generally reproduce sexually and asexually. They secrete digestive enzymes outside their bodies and absorb the nutrients. They also produce valuable source of chemicals, antibiotics and enzymes.

The majority of manglicolous fungi are omnivorus and occur mostly on dead cellulosic substrates all around the tropics. Many important industrial products are now produced from fungi using fermentation technology. A wide range of enzymes are excreted by fungi and play an important role in the breakdown of organic materials and many of these enzymes now produced commercially. Qualitative screening of degrading enzymes in marine fungi was reported by Rohrmann and Molitoris (1992). The number of authors are investigated enzyme production especially pectate lyase (Raghukumar *et al*., 1994); Lipase (Yadav *et al*., 1997, Kamini *et al*., 1997); Xylanase (Nilsson, 1974) and Laccase (Safari *et al*., 1999). In the present study, 35 species of fungi were isolated from Muthupet mangrove ecosystem and screened for enzymes production.

Materials and methods

The study area comprises a stretch of 6km in the Muthupet mangroves up to its tail end. Totally, five sampling stations were selected. They are Koraiyar(S1) Korimunai (S2), Manakkattu (S3), Lagoon (S4) and Kadalmunai (S5). Water, sediment, sea foams and natural substrates of mangrove plants were collected to isolate the fungi. After sampling, the samples were subjected to agar plating technique using PDA, CZA, CMA and RBA with addition of mixture antibiotics. The plates were incubated at room temperature (28ºC) for 4-5 days. The semi permanent slides of the isolated fungi were prepared using Lactophenol Cotton Blue staining technique (Dring, 1976) and sealed with DPX mountant. The identification of fungal taxa was based on Hyphomycetes (Subramanian, 1971), Dematiaceous Hyphomycetes (Ellis, 1971) and A manual of soil fungi (Gilman, 1957, 1998). In this fungal enzyme studies, total of 35 species of fungi (most dominant) were selected and screened for the production of enzymes (Pectate lyase, lipase, xylanase and laccase). Screening of enzymes was done by employing plate assay technique using specific screening media such as Pectate lyase (Hankins's agar), Lipase (Tributyrin agar), Xylanase (Akiba and Horikoshi medium) and Laccase (Liquified basal medium). All the inoculated plated were incubated at room temperature and the zone of clearance was observed around the colonies.

All the fungi were inoculated into enzyme production media such as Hankin's medium for pectate lyase, Production medium C with coconut oil for lipase, Akiba and Horikoshi medium for Xylanase and Liquified basal medium for laccase enzyme. The entire inoculated medium was incubated at room temperature in rotary shaker and enzyme assay was performed.

Assay for Pectate lyase was studied according to Collmer *et al*.(1988). In this, 10 µl of enzyme solution was added to closed quartz cuvette containing 990 µl of substrate stock solution. The subsequent increase in absorbance at 230 nm was monitored as functions of time using spectrophotometer. One unit of enzyme forms 1 micromole of 4,5 unsaturated product/min.

Assay for Lipase was done with method of Safarik, (1999). In this assay method, 250 mg of olive oil was taken in test tube containing 2 ml of phosphate buffer (pH 6.3). From this 1 ml of enzyme sample was added and vortexed for 15 seconds. The mixture was incubated at 37ºC in a water bath under static conditions for 30 minutes. 2 ml of conc. HCl was added and vortexed for 10 minutes. 3 ml of benzene was added and vortexed for 90 seconds. 2 ml of aqueous solution of 5% cupric acetate (pH 6.2). It was vortexed for 90 seconds and centrifuged at 5000 rpm for 10 minutes.Clear organic phase of benzene layer was removed and used to estimate the liberated fatty acids by measuring the optical density at 715 nm.

Assay for xylanase was studied according to Nanmori *et al*. (1990). In this assay, Enzyme solution (0.5 ml) was added to 2% xylan suspension (0.5 ml) in 100 mM tris HCl buffer (pH 7.0) and the mixtures were incubated at 55ºC for 30 minutes. After incubation the mixtures were cooled rapidly on ice water, the insoluble xylan was removed by centrifugation. To the resulting supernatant (0.5%) solution was added and the mixture was heated in boiling water bath. Colour development was measured using a spectrophotometer at 535 nm. The enzyme activity was expressed as micromole of xylose released per ml/min.

Laccase activity was assessed in 1.0 ml reaction mixture containing catechol as substrate in 50 mM sodium phosphate buffer (pH 5.0), to which, 0.2 ml enzyme extract was added. The progress of the reaction monitored at 440 nm for 10 minutes. One unit of laccase activity is defined as a change of A440 of 1.0 ml in 1 minute (Ruttimann *et al.*, 1992).

Results and discussion

Totally, 200 species of fungi were isolated and enumerated from all the five sampling stations by plating, baiting and direct observation techniques. Among these, maximum fungal diversity was observed in Manakattu (S3) with represented by 90 species and least at S5 with 70 species. From the mangrove samples, maximum fungal diversity was observed in sediment samples with 128 species. Among the fungal isolates, *Aspergillus* was the common genus represented by 39 followed by *Alternaria, Curvularia* and *Penicillium.*

Among the fungal isolates, 35 species were most dominant and selected for enzyme studies. In this study, 18 species of fungi were showed zone of clearance for laccase followed by xylanase (16 sp.), lipase (13 sp.) and pectate lyase (12 sp.) (Table.1 & Figure 1).

Table 1. Screening of enzymes from most dominant fungi isolated from Mangroves

Name of the fungi	**Pectate Lyase**	**Lipase**	**Xylanse**	**Laccase**
Rhizopus nigricans	-	+	+	-
R. oryzae	+	+	-	-
R. stolonifer	-	+	-	+
Mucor sp.	-	+	+	-
Neurospora crassa	-	+	+	-
Aspergillus candidus	-	-	+	-
A. carbonarius	+	-	-	+
A. clavatus	+	+	+	+
A. conicus	+	-	-	-
A. erythrocephalus	-	-	-	-
A. flavus	+	-	+	+
A. fumigatus	-	-	+	-
A. funiculosis	-	-	+	-
A. koningi	-	+	+	+
A. luchensis	-	-	+	-
A. nidulans	+	+	-	+
A. niger	+	+	+	+
A. ochraceus	-	-	-	-
A. oryzae	+	+	-	+
A. quercinus	-	-	+	-
A. sacchari	-	-	-	+
A. sulpuhreus	-	-	-	-
A. sydowi	-	-	+	+
A. terreus	-	-	-	+
A. terricola	-	-	+	+
A. ustus	-	-	-	+
A. variecolor	-	+	+	-
A. versicolor	+	-	+	+
A. wentii	-	-	-	+
Aspergillus sp.1	-	-	-	-
Pencillium citrinum	-	+	-	-
P. janthinellum	+	+	-	-
P. rubrum	-	-	-	+
Cladosporium britannicum	+	-	-	+
F. semitectum	+	-	-	+
Total No.of Fungi	12	13	16	18

(+) - Presence of Zone formation (-) - Absence of Zone formation

Table 2. Enzyme activity of fungi isolated from mangroves
(The values are represented in U/ml)

Name of the fungi	Pectate lyase	Lipase	Xylanse	Laccase
Rhizopus nigricans	-	2.44	4.804	-
R .oryzae	11.529	1.67	-	-
R. stolonifer		1.318	-	0.1738
Mucor sp.	1.2153	2.982	3.712	-
Neurospora crassa	-	0.4820	4.0178	-
Aspergillus candidus	-	-	3.732	-
A. carbonarius	1.6470	-	-	0.0416
A. clavatus	1.3513	0.690	5.232	0.0169
A. conicus	3.0193	-	-	-
A. erythrocephalus	-	-	-	-
A. flavus	0.6956	-	5.805	0.0366
A. fumigatus	-	-	4.162	-
A. funiculosis	-	-	5.125	-
A. koningi	-	0.771	2.6125	0.0510
A. luchuensis	-	-	4.85	-
A. nidulans	2.2857	0.6643	-	0.0189
A. niger	3.3557	1.341	3.688	0.0258
A. ochraceus	-	-	-	-
A. oryzae	-	1.021	-	0.0103
A. quercinus	-	-	3.389	-
A. sacchari	-	-	-	0.0608
A. sulpuhreus	-	-	-	-
A. sydowi	-	-	4.123	0.0212
A. terreus	-	-	-	0.0207
A. terricola	-	-	3.980	0.0504
A. ustus	-	-	-	0.0264
A. variecolor	-	0.3415	4.179	-
A. versicolor	1.999	-	5.424	0.0113
A. wentii	-	-	-	0.0151
Aspergillus sp.1	-	-	-	-
Penicillium citrinum	-	0.7638	-	-
P. janthinellum	1.8151	1.1933	-	-
P. rubrum	-	-	-	0.0861
C. britannicum	-	-	-	0.0366
F. semitectum	1.085	-	-	0.0149

Figure 1. Number of fungi showed zone of clearance on screening media

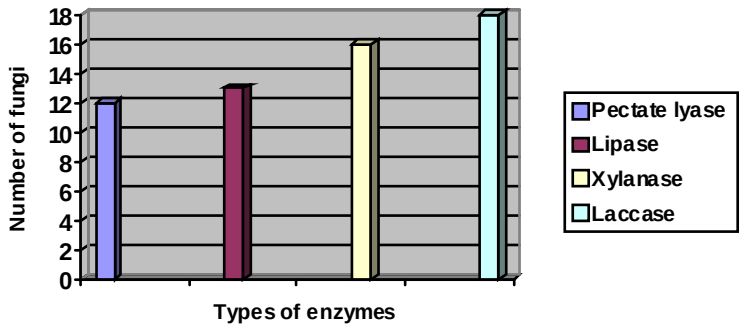
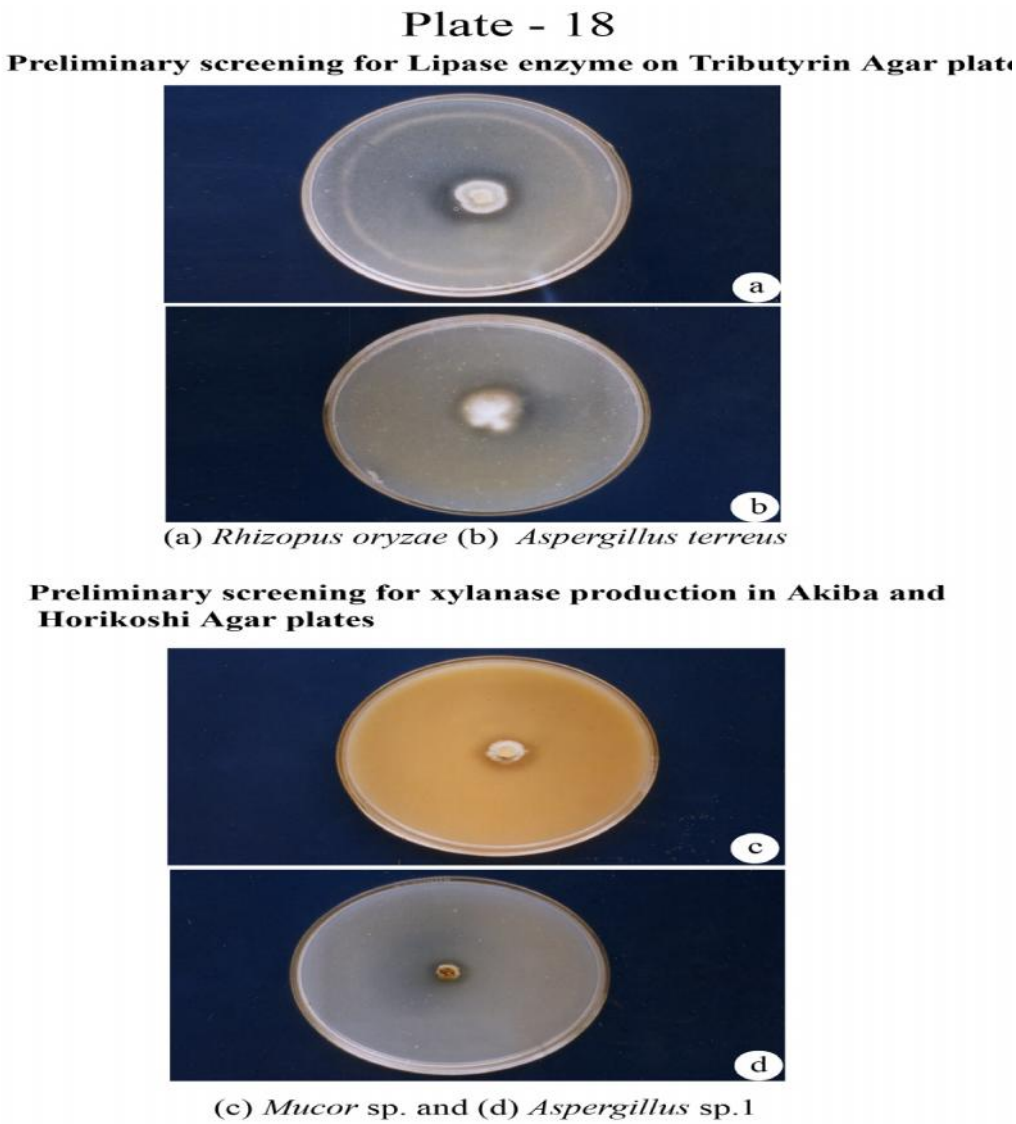


Plate 1: Preliminary screening for Lipase on Tributyrin agar plates



Laccase activity was observed in maximum in *Rhizopus stolonifer* (0.173 U/ml) and minimum activity was observed in *Aspergillus oryzae* (0.010 U/ml) (Tbale.2). It was well accepted with earlier reports of lignin is an amorphous high molecular – mass composed of phenylpropane subunits interconnected by variety of non – hydrolysable bonds. The relatively few groups of microorganisms that can degrade the macromolecule. The most efficient degraders are the white rot fungi (Orth and Tien, 1995; Paul and Clark, 1989). Safari Sinegani *et al*. (1999) assessed the production of lignin – degrading enzymes by the imperfect fungi *Aspergillus terreus* and *Trichoderma reesei* and yeast in the N-ethyl alanine, benzyl alcohol and benzaldehyde.

Maximum xylanase activity was observed in *Aspergillus flavus* with 5.805 U/ml followed by *A. versicolor* (5.424 U/ml), *A. funiculosis* (5.125 U/ml), *A. clavatus* (5.232). The minimum enzyme activity was observed in *A. koningi* with 2.612 U/ml (Table.2). This results was correlated with previous studies of most microbial hemicellulolytic system contain beta xylosidase, which has been purified and characterized from many fungi *Aspergillus niger* (Rodonova *et al*., 1983), *A. fumigatus* (Kitpreechavanich *et al*., 1986), *Trichoderma viride* (Matsuo and Yasui, 1984b), *Emericella nidulans* (Matsuo and Yasui, 1984a) and *Chaetomium trilaterale* (Uziie *et al*., 1985). Screening and production of xylanase enzyme required in the hydrolysis of different xylan was investigated using strains of 35 species of fungi isolated from mangrove samples (Sivakumar and Ravikumar, 2006).

Lipase activity was maximum observed in *Mucor* sp. with 2.982 U/ml followed by R. nigricans (2.440 U/ml) and *R. oryzae* (1.67 U/ml) and minimum activity was observed in *A. variecolor* with 0.341 U/ml and *F. semitectum* with 0.482 U/ml (Table. 2). The result was accepted with studies of Lazer and Schroder (1992) investigated fungal lipases, which degrade lipids from palm oil. Among Mucorales, the lipolytic enzymes of the moulds *Mucor hiemalis, Mucor miehei, Mucor lipolyticus, Mucor pusillus, Rhizopus japonicus , R. arrhizus, R. delear, R. nigricans, R. microsporus* and *R. chinesis* have been studied.

Prabhakar *et al.* (2002) reported the effect of cultural conditions on the production of lipase by Fungi. They found that selected organism *Aspergillus niger, A. flavus, A. japonicus* and a fungi isolated from the contaminated ghee belonging to the genus, *Aspergillus* spp. were tested for the production of lipase on four different media by submerged fermentation technique.

Maximum pectate lyase activity was recorded in *R. oryzae* (11.529 U/ml), *A. niger* (3.355 U/ml), *A. conicus* (3.019 U/ml) and minimum of *A. oryzae* with 0.695 U/ml (Table 2). It was agreed with earlier report of Raghukumar *et al*. (1994) have investigated degradative enzyme pectin lyase production by fungi isolated from detritus of the leaves of the mangrove *Rhizophora apiculata*. Twenty-one higher filamentous fungi isolated from *Spartina alterniflora* and other salt march substrata were shown to capable of degrading cellulose, lipids, starch including pectin compounds (Gessner, 1980).

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