International Journal of Advanced Multidisciplinary Research (IJAMR)
ISSN: 2393-8870
www.ijarm.com
Coden:IJAMHQ(USA)

Research Article

SOI: http://s-o-i.org/1.15/ijarm-2-11-6

Preliminary screening of enzymes from Marine fungi

S. Priya[1*] and T.Sivakumar[2]

[1]Research and Development Centre, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India
[1]Department of Microbiology, Sri Akilandeswari Women's College, Vandavasi, T.V.Malai, Tamil Nadu, India.
[2]Department of Microbiology, Kanchi shri Krishna College of Arts and Science, Kilambi -631 551, Kancheepuram, Tamil Nadu, India
*Corresponding Author: *priyakumaravalli@gmail.com*

Keywords

Marine fungi
Enzymes
Activity

Abstract

The study area comprises a stretch of 16 kilometers in the coastal region of Thiruvarur, pudukottai, and Ramanathapuram districts which were selected for present study.Totally11 sampling stations are as follows Muthupettai (S1),Iyampattinam (S2), Kumarapattinam (S3), Gopalapttinam (S4), R.Pudupattinam (S5),Arasangaripattinam (S6), Muthukadu (S7), Sethadimudi (S8), Sundarapandipattinam (S10),and Therthandathanam (S11) were selected based on the richness of natural substrates availability. They were collected to isolate the fungi. All the collected samples were plated, incubated and the fungal colonies were identified. The baits samples were regularly observed under aseptic conditions using stereoscopic dissection microscope. Maximum growth rate of fungi was observed in PDA than other media. Maximum fungal growth was observed in pH 8, 30ºC (temperature), 5% (salinity), $FeSO_4$ (metal), carboxy methyl cellulose (carbon source) and ammonium nitrate (nitrogen source) after 8 days of growth in liquid medium. 18 species of fungi showed zone of clearance for amylase and protease followed by cellulase with 5 species,laccase (4sp.), xylanase (3 sp.), and pectate lyase (6 sp.). Enzyme assays were also done. From this investigation, we have concluded that the fungal biodiversity in Muthupet mangrove ecosystem, *Aspergillus* and *Penicillium* was the common fungal genera among the isolated from the study period. Fungi play an important role in decomposition of natural substrates in mangrove ecosystem. The fungi isolated from mangroves are mainly used in enzyme technology, biochemical, agricultural, pharmaceutical, molecular biology and other applied research fields.

Introduction

Fungal biotechnology has become an integral part of human welfare. Nature represents a formidable pool of bioactive compounds and is more than ever a strategic source for new and successful commercial product. Among the microorganisms, fungi are well recognized to produce a wide variety of most valuable pharmaceutical chemicals, agrochemicals and industrial products. Recent advances made in genomics, proteomics and combinatorial chemistry show that nature maintains compounds that are the essence of bioactivity, within the host and environment. So the major challenging task is to explore the unexplored fungal wealth in our country and reveal their potential applications.

The screening of marine fungi for novel bioactive compounds has yielded several novel metabolites, some of which are being commercially developed for medicinal or agricultural use. Sadly the data generated by pharmaceutical companies in screening for bioactive compounds if often 'lost' to science due to the need for industrial secrecy. Fungal enzymes are widely used in industry and, many vitamins and food supplements rely on fermentation processes using terrestrial fungi. Due to their slow growth rates it is unlikely that marine fungi will replace their faster – growing terrestrial counterparts in this respect.

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 are now produced commercially. Most of these

enzymes are used in food processing. Fungi are good candidate for employing them in degrading refractory substrates, cellulose, lignin, chitin, keratin and other substrates. Fungi like *Aspergillus niger* and *A. oryzae* are regarded as safe by the food and drug administration.

Microbial cells produce a variety of enzymes and help in microbial growth and respiration including other cellular activities. At times, these enzymes may themselves become fermentation products, so that one of them is specifically interested in obtaining high level of the enzymes (Bell *et al*., 1972). Qualitative screening of degrading enzymes in marine fungi was reported by Rohrmann and Molitoris (1992).

The use of enzymes in food preservation and processing predates modern civilization. Fermentation of common substrates such as fruits, vegetables, meat and milk provide a diverse array of food in the human diet. Beer, wine, pickles, sausage, salami, yogurts, cheese and buttermilk are all fermented products. Irrespective of their origin, these fermented food products are, in fact, result of the enzymatic modification of constituents in the substrate. The use of enzymes in food industry also involves a range of effects including the production of food quality attributes such as flavors and fragrances and control of colour, texture, and appearance besides affecting their nutritive value.

Materials and Methods

Screening and Assay of Fungal Enzymes

In this fungal enzyme study, 18 species of fungi (most dominant) were selected and screened for the production of 4 microbial enzymes (cellulase, pectate lyase, xylanase, and laccase).

Screening of Fungal Enzymes

Screening of enzymes were done by employing plating technique with specific media such as cellulase (Czapek - minimal salt agar), pectate lyase (Hankin's medium), xylanase (Akiba and Horikoshi medium), and laccase (Liquefied basal medium). All the inoculated plated were incubated at room temperature (28ºC) and the zone of clearance were observed around the colonies and noted.

Production media employed for the enzymes

All the fungi were inoculated into enzyme production media such as Modified Czapek's – cellulose medium for cellulase, Hankin's medium for pectate lyase, Akiba and Horikoshi medium for xylanase, P and liquefied basal medium for laccase enzyme. All the media were incubated at respective temperatures and the enzyme assays were performed.

Assay methods for enzymes

After incubation, enzyme assay were performed for enzymes like cellulase (Denison and Koehn, 1977), pectate lyase (Collmer *et al*., 1988), xylanase (Nanmori *et al*., 1990), and laccase (Ruttimann *et al*., 1992).

Assay for Cellulase

0.5 ml of enzyme extract was added in test tube containing 0.45 ml of 15 Carboxy methy cellulose. This mixture was incubated at 55°C for 15 min. 0.5 ml of Dinitrosalicyclic acid reagent (DNS) was added and heated in a water bath for 5 min. 0.1 ml of potassium sodium tartrate was added and make up to 5 ml with distilled water. The optical density was measured at 540 nm. The enzyme activity was expressed as mg of glucose released per min $^{-1}$ mg of protein $^{-1}$.

Assay for pectate lyase

10 µl of enzyme solution was added to a closed quartz cuvette containing 990 microlitre of substrate stock solution (0.25% (w/ v) PGA**,** 50 mM Tris / HCl (pH.8.0), 0.6 mM $CaCl_2$). 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 Xylanase

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 55°C for 30 min. After incubation the mixtures were cooled rapidly on ice water, the insoluble xylan was removed by centrifugation. To the resulting supernatant (0.5 ml), 1 ml of 3, 5 dinitrosalicyclate (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.

Assay for Laccase

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 of enzyme extract was added. The progress of the reaction was monitored at 440 nm for 10 min. One unit of laccase activity is defined as a change of A 440 of 1.0 ml in 1min.

Results and Discussion

Screening and activity of fungal enzyme

Among the fungal isolates, 18 species were most dominant and selected for enzyme studies. cellulase with 5 sp., pectate lyase with 6 sp., xylanse with 3 sp., and laccase in3 sp. were showed zone of clearance around the colony on selective screening media *Aspergillus fumigatus* showed maximum zone of clearance in all the media employed for enzyme screening assay

Table 1 Screening of enzymes from most dominant fungi

Name of the fungi	Cellulase	Pectate	Xylanse	Laccase
Absidia	+	-	-	-
R. stolonifer	-	+	-	-
R. oryzae	+	-	-	-
R.oligosporum	-	+	-	-
A.clavatus	+	-	-	-
A.fumigatus	+	-	-	-
A. fumiculous	-	-	-	+
A.luchensis	-	-	+	-
A. cineriae	+	-	-	-
Alternaria brasicola	-	+	-	-
Verticillium longisporum	-	-	+	-
A. ustus	-	+	-	-
A. 0ryzae	-	+	-	-
F.oxysporum	-	+	-	-
P.frequentans	-	-	-	+
P.fumiculosm	-	-	-	+
P.rubrum	-	-	+	-
P.jamanthellam	-	-	-	+
	5	**6**	**3**	**3**

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

Table 17 Enzyme activity of fungi isolated

The values are represented in U/ml)

Name of the fungi	Cellulase	Pectate lyase	Xylanse	Laccase
Absidia	**11.412**	-	-	-
R. stolonifer	**3.0147**	-	-	-
R. oryzae	**0.7428**	-	-	-
R.oligosporum	-	**0.6936**	-	-
A.clavatus	**3.856**	-	-	-
A.fumigatus	**3.2066**	-	-	-
A. fumiculous	-	-	-	**0.0110**
A.luchensis	-	-	**5.765**	-
A. cineriae	**3.856**	-	-	-
Alternaria brasicola	-	**3.0569**	-	-
Vericillium longisporum	-	-	**5.126**	-
A. ustus	-	**3.3412**	-	-
A. 0ryzae	-	**1.235**	-	-
F.oxysporum	-	**1.698**	-	-
P.frequentans	-	-	-	**0.1635**
P.fumiculosm	-	-	-	**0.1635**
P.rubrum	-	-	**2.219**	-
P.jamanthellam	-	-	-	**0.1796**

Cellulase activity

Cellulase activity was maximum in *Absidia* with 11.412 U/ml followed by *A. clavatus* with 3.856 U/ml, *A. cineriae* with 3.306 U/ml *A. fumigatus* with 3.2066 U/ml, . Cellulase activity was minimum *in R.oryzae* (0.7428U/ml)

Pectate lyase activity

Maximum pectate lyase activity was observed in *A. oryzae* (1.235 U/ml), *A. ustus* (3.3412 U/ml), *A. brasicola* (3.0569 U/ml) R.stolonifer (3.0147) *F.oxysporum* (1.698 U/ml)and minimum in *R.oligosporum* (0.6936 U/ml)

Xylanase activity

Maximum xylanase activity was observed in *A. luchensis* (5.765 U/ml) followed by *V.longisporum (*5.126 U/ml). The minimum enzyme activity was observed in *P.rubrum* with 2.219 U/ml

Laccase enzyme activity

Laccase enzyme activity was maximum in P.jamnethallem (0.1796 U/ml),P.frequentans (0.1635U/ml),A.fumiculous and minimum enzyme activity was observed in *A. fumicuolous* (0.0110 U/ml)

Species of *Neurospora* and *Trichoderma* are known to produce cellulase enzyme (Gallo, 1978). Ojumu *et al*. (2003) reported that lignocellulose substrates used for the production of celluase enyzme using *A. niger* and cellobiose substrates for the production of cellulase enzyme using *Aspergillus* and *Trichoderma* (Romana *et al*., 1990).

Production of xylanses has been investigated using different fungi such as *Chrysosporium* (Eriksson and Rzedowski, 1969), *Sclerotium* (Sadana *et al*., 1980), *Aspergillus* (Stewart *et al*., 1983), *Aureobasidium* (Leathers *et al*., 1986), *A. niger* (Rodonova *et al*., 1983) and *A. fumigatus* (Kitpreechavanich *et al*., 1986). Safari Sinegani *et al*. (1999) reported that the lignolytic enzyme like laccase production using *A. terreus, T. ressei*, yeast and additional chemical compounds. In this, Manganse peroxidase and Lignin peroxidase activity were higher *A. terreus* and *T. ressei* than yeast and chemical compounds tested.

References

Collmer, A., Ried, J.L. and Mount, M.S. 1988. Assay methods for pectic enzymes. Methods Enzymol.161: 329-399.

Denison, D.A. and Koehn, R.D. 1977. Mycologia. LXIX: 592.

Eriksson, K.E. and Rzedowski, W. 1969. Extracellular enzyme system utilized by the fungus *Chrysosporium lignorum* for the breakdown of cellulose. Arch. Biochem. Biophys.129: 683-688.

Gallo, B.J., Andreotti, R., Roche, C., Rye, D. and Mandels, M. 1978. Cellulase production by a new mutant strain of *Trichoderma ressei* MCG 74. Biotechnol. Bioeng. Symp. 8: 89 –101.

Kitpreechavanich, V., Hayashi, N. and Nagai, S. 1986. Purification and characterization of extracellular beta-xylosidase and beta-glucosidase from *A. fumigatus*. Agr. Biol. Chem. 50: 1703 - 1711.

Leathers, T.D., Detroy, R.W. and Bothost, R.J. 1986. Induction and glucose respiration of xylanase from a color variant strain of *Aureobasidium pullulans*. Biotechnol. Lett. 8: 867-872.

Nanmori, T., Watanbe, T., Shike, R., Kohno. A. and Kawambera, Y. 1990. Purification and properties of thermostable xylanase and beta xylosidase produced by a newly isolated *Bacillus stearothermophilus* strain. J. Bacteriol. 172(12): 6669-6672.

Ojumu, T.V., Solomon, B.O., Betiku, E., Layikun, S.K. and Amigumn, B. 2003. Cellulase production by *Aspergillus flavus* Linn. Isolate NSPR 101 fermented in saw dust, baggase and corncob. African. J. Biotechmol. 2: 150 – 152.

Rodonova, N.A., Tavobilov, I.M. and Bezborodov, A.M. 1983. Beta-xylosidase from *A.niger* 15: Purification and properties. J. Appl. Biochem. 5: 300-312.

Rohrmann, S. and Molitoris, P. 1992. Screening of wood-degrading enzymes in marine fungi. Can. J. Bot. 70: 2116-2123**.**

Romana, T., Rajoka, M. I. and Malik, K. A. 1990. Production of cellulase and hemicellulase by an anaerobic mixed culture from lignocellulosic biomass. W. J. Microbiol. Biotechnol. 6: 39 – 49.

Ruttimann, C., Schwember, E., Salas, L., Cullen, D. and Vieuna, R. 1992. Lignolytic enzyme of the white rot Basidiomycetes *Phlebia bravispora* and *Ceriporiopsis subvermispora*. Biotechnol. Appl. Biochem.16: 64-76.

Sadana, J.C., Shewale, T.G. and Deshpande, M.V. 1980. High cellobiose and xylanase production by *Sclerotium rolfsii* UV–8 mutant in submerged culture. Appl. Environ. Microbiol. 39: 935-936.

Stewart, J.C., Lester, A., Milburen, B. and Parry, J.B. 1983. Xylanase and cellulase production by *Aspergillus fresenius*. Biotech. Lett. 6: 543-548.