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Research Article

Preliminary Screening of fungal enzymes isolated from Marine ecosystem of East Coast of Tamil Nadu, India.

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Abstract

Screening and assay of fungal enzymes such as alpha amylase, alkaline protease, cellulase, pectate lyase, lipase, xylanase, phosphatase and laccase were studied on fungi isolated from mangrove habitat. Estimation of fungal protein, lipids, amino acids and rhamnolipids were studied. 31 species of fungi showed zone of clearance for amylase and protease followed by cellulase with 23 species, phosphatase (19 sp.), laccase (17sp.), xylanase (13sp.), lipase (11 sp.) and pectate lyase (12 sp.). Enzyme assays were also done. Protein, lipids, amino acids and rhamnolipids were maximum observed in *Rhizopus stolonifer, Mucor* sp*., Neurospora crassa, Aspegillus terreus, A. flavus*, *A. fumigatus, A. nidulans, A. niger, Pencicillium citrinum, P. rubrum* and *Fusarium semitectum.*

Keywords: fungal enzymes, Protein, lipids, amino acids and rhamnolipids.

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, 31 species of fungi (most dominant) were selected and screened for the production of 8 microbial enzymes (alpha amylase, alkaline protease, cellulase, pectate lyase, lipase, xylanase, phosphatase enzymes acid and alkaline and laccase).

Screening of Fungal Enzymes

Screening of enzymes were done by employing plating technique with specific media such as alpha amylase (Starch agar), alkaline protease (Skim milk agar), cellulase (Czapek - minimal salt agar), pectate lyase (Hankin's medium), lipase (Tributyrin agar), xylanase (Akiba and Horikoshi medium), phosphatase (Pikovskaya's agar) 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 Starch broth for alpha amylase, Modified Reese's medium for alkaline protease, Modified Czapek's – cellulose medium for cellulase, Hankin's medium for pectate lyase, Production medium C with coconut oil for lipase, Akiba and Horikoshi medium for xylanase, Pikovskaya's broth for phosphatase (acid and alkaline) 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 alpha amylase (Peter Bernfield, 1955), alkaline protease (Keay *et al*., 1970), cellulase (Denison and Koehn, 1977), pectate lyase (Collmer *et al*., 1988), lipase (Safarik, 1999), xylanase (Nanmori *et al*., 1990), phosphatase - acid and alkaline (Fiske and Subba Rao, 1925) and laccase (Ruttimann *et al.,* 1992).

Assay for alpha amylase enzyme

1 ml of starch solution was added to 1 ml diluted enzyme solution containing test tube. This solution was incubated at 27°C for 10min. 2 ml of dinitrosalicyclic acid reagent was added and heated in a boiling water bath for 5 min. 1 ml of potassium sodium tartrate was added and the tube was cooled in running tap water. This mixture was made up to 10 ml with sterile distilled water and absorbance was read at 560 nm. The enzyme activity was expressed as micromole of substrate utilized or product produced per min $^{-1}$ mg of protein $^{-1}$.

Assay for alkaline protease enzyme

0.2 ml of enzyme sample was taken and made up to 1 ml with sterile distilled water and 3ml of 0.4 M carbonate – bicarbonate buffer (pH.9) and 1 ml of casein were added. The mixture was incubated for 30 miuntes and 2 ml of TCA was added and again incubated at 37°C for 30minutes. The mixture was filtered and 0.1 ml of filtrate, 0.5 ml of 0.4 N sodium

carbonate and 1 ml of Folin- phenol were added. Then the solution was mixed well and incubated at 37°C for 20 minutes. The optical density was measured at 660 nm**.** One unit of protease activity was defined as the amount of enzyme that produced TCA soluble material equivalent to 1 μg of tyrosine from casein per minute under assay conditions.

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 Lipase

250 mg of olive oil was taken in test tube containing 2 ml of phosphate buffer (pH.6.3). From this 1ml of enzyme sample was added and vortexed for 15 sec. The mixture was incubated at 37°C in a water bath under static conditions for 30 min. 1 ml of concentrated HCl was added and vortex for 10 min. 3 ml of benzene was added and vortexed for 90 sec. 2 ml of benzene layer was taken and added to 1 ml aqueous solution of 5% cupric acetate (pH.6.2). It was vortex for 90 sec. and centrifuged at 5000 rpm for 10 min. Clear orgainc phase of benzene layer was removed and used to estimate the liberated fatty acids by measuring the optical density at 715 nm. One unit of lipase activity is equivalent to μM of free fatty acid liberated min^{-1} ml^{-1}, under the assay conditions.

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.

Acid and Alkaline Phosphatase
Extraction of acid phosphatase

1ml of enzyme was taken and 10 ml of 50 mM Citrate buffer (pH.5.3) was added and filtered through Whatmann filter paper. The mixture was centrifuged at 10,000 rpm for 10 min. The collected supernatant was used as enzyme source.

Extraction of Alkaline phosphatase

1 ml of enzyme was taken in a test tube and 5 ml of 50 mM Glycine NaOH buffer (pH.10.4) was added and filtered through Whatmann filter paper. The mixture was centrifuged at 10,000 rpm for 10 min. The collected supernatant was used as enzyme source.

Assay method

0.5 ml of magnesium acetate solution was added to a test tube containing 2 ml of buffer solution. Then 2 ml of beta glycerol phosphate solution (substrate) was added and made up to 7 ml with sterile distilled water. 1 ml of enzyme extract of both acid and alkaline were added and incubated at 37°C for 1 h. Then 2 ml of Trichloro acetic acid was added to the mixture to stop the reaction. The content was centrifuged at 10000 rpm for 10 min. From this, 2 ml of sample was taken and made up to 5 ml with distilled water. 1 ml of ammonium molybdate solution was added and mixed well. 0.4 ml of ANSA reagent was added and allowed to stand for 10 min. The content was diluted to 10 ml with distilled water and the optical density was measured at 660 nm. The enzyme activity was expressed as amount of phosphorus released in unit time mg of protein.

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.

Estimation of Protein, Lipids, Amino acids and Rhamnolipids of fungi

All the 35 most dominant species of fungi were inoculated into Czapek dox broth containing 1% yeast extract. The broth was incubated for 3 days in rotary shaker at room temperature. After incubation period, the mycelium was collected and analyzed for protein (Lowry's *et al*., 1951), lipids (Birch *et al*., 1998), total free amino acids (Moore and Stein, 1948) and rhamnolipids (Koch *et al.,* 1991).

Estimaton of Protein (Lowry's et al., 1951)

0.2 ml of protein sample was added to test tube containing 0.8 ml-distilled water. Then 5 ml of alkaline copper solution was added and allowed to stand for 10 min. 0.5 ml of Folin reagent was added and optical density was read at 660 nm.

Calculation

Test OD / Std OD ×Conc. of Std ×1 / Volume of sample taken

Estimation of Lipids (Birch *et al*., 1998)

100 mg of fresh mycelium of fungi were weighed and 2 ml of methanol and chloroform (2:1) were added. This solution was allowed to stand for 4 hrs with occasional shaking. Then solvent was removed and re-extraction was done by repeating the steps. The solvent was collected and pooled with first extract. Again the lipid extract was redissolved in 1 ml choloform and washed with 1 ml of distilled water. The top layer was removed and bottom layer was collected. The collected bottom layer was dried and weighed.

Calculation

mg of lipid / volume of sample taken (mg) ×100

Estimation of total free amino acids (Moore and Stein, 1948)

0.2 ml of amino acid sample was taken in test tube containing 0.8 ml sterile distilled water. Then 1ml of ninhydrin reagent was added. The mixture was kept in boiling water bath for 20min. 5 ml of diluent was added and allowed to stand for 15 min. Then optical density was measured at 570 nm using spectrophotometer.

Calculation

Test OD / Std OD ×Conc. of Std ×1 / Volume of sample taken

Estimation of Rhamnolipids (Koch *et al.,* 1991)

100 µl of sample was taken in test tube containing 900 µl of orcinol reagent (53% v/v N_2SO_4 and 0.19% w/v Orcinol). Then the mixture was heated at 100°C for 20 min. The solution was cooled and the optical density was read at 421 nm.

Calculation

Test OD / Std OD ×Conc. of Std ×1 / Volume of sample taken

Results and Discussion

Screening and activity of fungal enzyme

Among the fungal isolates, 31 species were most dominant and selected for enzyme studies. In this study, all the 31 species showed zone of clearance in alpha amylase and alkaline protease on selective screening media followed by cellulase with 23 sp., pectate lyase with 12 sp., lipase with 11 sp., xylanse with 13 sp., phosphatase activity was observed in 19 sp. and laccase in 17 sp. were showed zone of clearance around the colony on selective screening media. *Aspergillus niger* showed maximum zone of clearance in all the media employed for enzyme screening assay (Table 1).

a). Alpha amylase activity

Maximum alpha amylase activity was observed in *A. niger* with 4.7752 U/ml folloed by *R. stolonifer* with 4.3569U/ml, *A. sulphureus* with 4.153 U/ml, *R. nigricans* with 3.7605, U/ml *N. crassa* with 3.077 U/ml, *A. sacchari* with 3.037 U/ml, *A.erythrocephalus* with 3.264 U/ml.The minimum enzyme activity was observed in *A. funiculosis.* (Table 2).

b). Alkaline protease activity

Maximum alkaline protease activity was observed in *A. funiculosis* with 6.8520U/ml followed by *A. sulphureus* with 6.4649 U/ml, *A. wentii* with 6.419U/ml, *A. conicus* with 6.104 U/ml. The minimum enzyme activity was observed in *A. carbonarius* with 0.0606 U/ml, *R. stolonifer* with 1.1906 U/ml and *A. luchensis* with 1.5025 U/ml (Table 2).

c).Cellulase activity

Cellulase activity was maximum in *F. semitectum* with 11.460 U/ml followed by *A. versicolor* with 3.991 U/ml, *R. nigricans* with 3.635 U/ml,*A. sydowi* with 3.313 U/ml *A. fumigatus* with 3.2077 U/ml, *A. wentii* with 3.1070 U/ml. Cellulase activity was minimum in *A. sacchari* (0.7428U/ml) (Table 2).

d)Pectate lyase activity

Maximum pectate lyase activity was observed in *R. oryzae* (11.529U/ml), *A. niger* (3.3557 U/ml), *A. conicus* (3.0193 U/ml) and minimum in *A. flavus* (0.6956 U/ml) (Table 2).

e). Lipase enzyme activity

Lipase activity was maximum in *Mucor* sp. (2.982 U/ml) followed by *R. nigricans* (2.44U/ml) and *R. oryzae* (1.67 U/ml) and minimum in *A.nidulans*(0.6643 U/ml) (Table 2).

f). Xylanaseactivity

Maximum xylanase activity was observed in *A. flavus* (5.805 U/ml) followed by *A. versicolor* (5.424 U/ml), *A. clavatus* (5.232 U/ml), *A. funiculosis* and *A. terreus* (5.125 U/ml). The minimum enzyme activity was observed in *A. niger* with 3.688 U/ml (Table 2).

g). Phosphatase activity
(Acid phosphatase and alkaline phosphatase)

Maximum acitivity of acid phosphatase was observed in *R. oryzae* (2.549 U/ml) and minimum activity in *A. sacchari* (0.5329 U/ml). Maximum acitivity of alkaline phosphatase was observed in *A. funiculosis* (1.854 U/ml) and minimum activity in *A. ochraceus* with 0.7282U/ml (Table 2).

h). Laccaseenzyme activity

Laccase enzyme activity was maximum in *R. stolonifer* (0.1738 U/ml) and minimum enzyme activity was observed in *A. oryzae* (0.0103 U/ml) (Table 2).The enzyme screening and their acitivity was well correlated with previous reports by Goto *et al*. (1998) who studied that the amylase enzyme production from the fungi *A. oryae* and *A. niger*. Alpha amylase enzyme production was carried out under the influence of nitrogen sources using *A. oryzae* by Pederson and Neilson (2000). Under solid substrate fermentation potato waste was fermented by *R. oryae* and yielded 3.2 g crude alpha enzyme (Arora *et al*., 2000). Alkaline protease enzyme production from *A. niger* was also reported by Singh *et al*., (1973). Bathomeuf *et al*. (1992) and Monod *et al*. (1991) also studied the enzyme production in *A. fumigatus,A. sojae* by Nausho and Ohara (1971), *A.nidulans* by Stevens (1985), Cohen (1973) and *A. melleus* (Luisetti *et al*., 1991). Alkaline protease enzyme production by solid substrate fermentation using *A. flavus* (Malathi and Chakraborthy, 1991). Fungi like *A. niger, A. flavus, A. fumigatus* and *P. glacum* are reported as potential lipase producers (Bhaduria, 1989). Kamini *et al*. (1997) screened for the production of lipase enzyme on tributyrin agar plates using *A. niger* and maximum halozone observed on agar plates which indicates the production of lipase.

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),

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

Name of the fungi	Alpha amylase	Alkaline protease	Cellulase	Pectate lyase	Lipase	Xylanse	Phos-phatase	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. luchensis	+	+	+	-	-	+	-	-
A. nidulans	+	+	-	+	+	-	-	+
A. niger	+	+	+	+	+	+	+	+
A. ochraceus	+	+	+	-	-	-	+	-
A. oryzae	+	+	+	+	+	-	-	+
A. sacchari	+	+	+	-	-	-	+	+
A. sulpuhreus	+	+	-	-	-	-	-	-
A. sydowi	+	+	+	-	-	+	+	+
A. terreus	+	+	-	-	-	-	-	+
A. terricola	+	+	-	-	-	+	-	+
A. ustus	+	+	-	-	-	-	+	+
A. versicolor	+	+	+	+	-	+	-	+
A. wentii	+	+	+	-	-	-	+	+
Pencillium citrinum	+	+	+	-	+	-	+	-
P. janthinellum	+	+	+	+	+	-	-	-
P. rubrum	+	+	+	-	-	-	-	+
Cladosporium britannicum	+	+	+	+	-	-	-	+
Fusarium semitectum	+	+	+	+	-	-	+	+
Total No.of Fungi	**31**	**31**	**23**	**12**	**11**	**13**	**19**	**17**

(+) - 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	Alpha amylase	Alkaline protease	Cellulase	Pectate lyase	Lipase	Xylanse	Phosphatase		Laccase
							Acid	Alkaline	
Rhizopus nigricans	3.7605	3.0474	3.635	-	2.44	4.804	-	-	-
R .oryzae	1.7680	2.036	-	11.529	1.67	-	2.549	0.8147	-
R. stolonifer	4.3569	1.1906	2.241		1.318	-	1.708	1.1960	0.1738
Mucor sp.	2.5957	4.0633	0.8397	1.2153	2.982	3.712	1.109	1.122	-
Neurospora crassa	3.077	3.7352	1.812	-	0.4820	4.0178	0.9618	0.9537	-
Aspergillus candidus	1.9613	3.4550	0.8552	-	-	3.732	1.101	1.3786	-
A. carbonarius	2.1814	0.0606	0.7698	1.6470	-	-	0.8822	0.8606	0.0416
A. clavatus	2.422	5.0594	1.3407	1.3513	0.690	5.232	1.316	1.5723	0.0169
A. conicus	2.7975	6.4649	2.3700	3.0193	-	-	0.7725	1.0266	-
A. erythrocephalus	3.264	5.791	-	-	-	-	1.121	1.0495	-
A. flavus	1.6290	3.360	1.3906	0.6956	-	5.805	0.856	0.8875	0.0366
A. fumigatus	2.570	4.3794	3.2077	-	-	4.162	-	-	-
A. funiculosis	0.5940	6.8520	-	-	-	5.125	1.000	1.854	-
A. luchuensis	2.8507	1.5025	2.700	-	-	4.85	-	-	-
A. nidulans	2.602	2.8198	-	2.2857	0.6643	-	-	-	0.0189
A. niger	4.7752	2.1341	2.486	3.3557	1.341	3.688	1.366	1.850	0.0258
A. ochraceus	1.5614	2.6445	2.200	-	-	-	0.8612	0.7282	-
A. oryzae	1.0656	3.8408	2.477	-	1.021	-	-	-	0.0103
A. sacchari	3.037	2.689	0.7428	-	-	-	0.5329	1.2170	0.0608
A. sulpuhreus	4.153	6.104	-	-	-	-	-	-	-
A. sydowi	1.9776	3.3116	3.313	-	-	4.123	1.380	1.4375	0.0212
A. terreus	1.8522	4.9392	-	-	-	-	-	-	0.0207
A. terricola	2.863	5.7555	-	-	-	3.980	-	-	0.0504
A. ustus	1.9356	4.8613	-	-	-	-	0.8134	1.3045	0.0264
A. versicolor	2.0460	4.7346	3.991	1.999	-	5.424	-	-	0.0113
A. wentii	1.0427	6.419	3.1070	-	-	-	0.9214	1.2835	0.0151
Penicillium citrinum	2.1117	5.304	1.437	-	0.7638	-	1.115	1.4718	-
P. janthinellum	2.1061	5.482	2.951	1.8151	1.1933	-	-	-	-
P. rubrum	1.8506	3.3894	2.707	-	-	-	-	-	0.0861
Cladosporium britannicum	1.6480	2.4854	1.5983	-	-	-	-	-	0.0366
Fusarium semitectum	2.016	5.1991	11.460	1.085	-	-	1.6207	1.809	0.0149

Table 3. Estimation of protein, lipids, amino acids and rhamnolipids content of selected fungi

S.No	Name of the fungi	Protein (mg/g)	Lipids (%)	Amino acids (mg/g)	Rhamno Lipids (mg/g)
1	*Rhizopus nigricans*	1.2325	0.92	1.2587	0.1445
2	*R. oryzae*	0.9525	0.28	1.232	0.1445
3	*R. stolonifer*	1.5075	1.15	1.65	0.3545
4	*Mucor* sp.	1.6975	1.04	1.6062	0.4690
5	*Neurospora crassa*	1.5225	1.06	1.8275	0.3909
6	*Aspergillus candidus*	1.0525	0.95	1.4012	0.1854
7	*A. carbonarius*	0.945	0.47	1.16	0.1381
8	*A. clavatus*	1.0175	0.53	1.4187	0.1381
9	*A. conicus*	1.02	0.08	1.21	0.1636
10	*A. erythrocephalus*	1.2575	0.20	1.4112	0.1072
11	*A. flavus*	1.725	1.14	1.9862	0.5090
12	*A. fumigatus*	1.6325	1.07	1.866	0.52
13	*A. funiculosis*	1.13	0.36	1.2525	0.1254
14	*A. luchensis*	1.2275	0.55	1.5012	0.0836
15	*A. nidulans*	1.675	1.02	1.7362	0.52
16	*A. niger*	1.73	1.73	2.0025	0.2163
17	*A. ochraceus*	0.74	0.53	1.27	0.1563
18	*A. oryzae*	0.99	0.72	1.2412	0.1509
19	*A. sacchari*	1.2025	0.73	1.2362	0.1545
20	*A. sulphureus*	0.95	0.27	1.4862	0.1145
21	*A. sydowi*	0.725	0.27	1.2612	0.1381
22	*A. terreus*	1.625	1.22	1.9775	0.50
23	*A. terricola*	0.9625	0.24	1.24	0.1581
24	*A. ustus*	1.08	0.63	1.1262	0.1275
25	*A. versicolor*	0.7325	0.82	1.2262	0.1445
26	*A. wentii*	0.92	0.18	1.135	0.0963
27	*Penicillium citrinum*	1.5425	1.04	1.7362	0.3090
28	*P. janthinellum*	1.14	0.49	1.2475	0.1763
29	*P. rubrum*	1.5225	0.24	1.76	0.1445
30	*C. britannicum*	0.7925	0.50	1.3612	0.1872
31	*Fusarium semitectum*	1.5	1.13	1.7325	0.5327

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.

Estimation of protein, lipids, amino acids and rhamnolipids

Maximum of protein content was observed in *R. stolonifer* (1.507 mg/g) followed by *Mucor* sp. (1.697 mg/g), *N. crassa* (1.522 mg/g), *A. terreus* (1.625 mg/g), *A. flavus* (1.725 mg/g), *A.fumigatus* (1.632 mg/g), *A. nidulans* (1.675 mg/g), *A. niger* (1.73 mg/g), *P. citrinum* (1.542 mg/g), *P. rubrum* (1.522 mg/g) and *F. semitectum* (1.5 mg/g)than other fungi employed for proteinestimation (Table 3).

Rhizopus stolonifer (1.15%), *Mucor* sp. (1.04%), *N. crassa* (1.06%), *A. terreus* (1.22%), *A. flavus* (1.14%), *A. fumigatus* (1.07%), *A. nidulans* (1.02%), *A. niger* (1.73%), *P. citrinum* (1.04%), *P. rubrum* (1.24%) and *F. semitectum* (1.13 %) showed maximum lipid content than other fungi (Table 3).

Maximum contents of amino acid content was observed in *R. stolonifer* (1.65 mg/g) followed by *Mucor* sp.(1.606 mg/g), *N. crassa* (1.827 mg/g), *A. terreus* (1.977 mg/g), *A. flavus* (1.986 mg/g), *A. fumigatus* (1.866 mg/g), *A. nidulans* (1.736 mg/g), *A. niger* (2.002 mg/g), *P. citrinum* (1.736 mg/g), *P. rubrum* (1.76 mg/g) and *F. semitectum* (1.732 mg/g) than other fungi employed for amino acid estimation (Table 3). *Rhizopus stolonifer* (0.354 mg/g), *Mucor* sp. (0.469 mg/g), *N. crassa* (0.390 mg/g), *A. terreus* (0.50 mg/g), *A. flavus* (0.509 mg/g), *A. fumigatus* (0.52 mg/g), *A. nidulans* (0.52 mg/g), *A. niger* (0.216 mg/g), *P. citrinum* (0.309 mg/g), *P. rubrum* (0.176 mg/g) and *F. semitectum* (0.532 mg/g) showed maximum amount of rhamnolipids than other fungi (Table 3).

Protein from fungi has been studied by several authors (Plesofsky-vig and Brambl, 1985; Silver *et al*., 1983; Freemann *et al*., 1989). Lipids content of spore of fungi ranges from 5 to 17% dry weight but spores of some species such as rusts contain up to 35% lipid (Shen, 1966). In fungi lipids are important for germination, in addition to other functions (Cochrane *et al*., 1963; Owens, 1955; Turain and Bianchi, 1972). Low concentration of lipids observed in *Rhizopus stolonifer* (Weete *et al*., 1973).

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