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RESEARCH ARTICLE

OPTIMIZATION AND ENZYMATIC DEGRADATION OF COIR PITH AND SUGARCANE LEAVES BY PLEUROTUS PLATYPUS THROUGH UTILIZING NITROGEN, SULPHUR AND PHENOLIC COMPOUND

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ABSTRACT

Among white rot basidiomycetes *Pleurotus sp.,* is reported to be most efficient colonizers and degraders of lignocelluloses. The white rot edible mushroom *Pleurotus platypus* has considered as a moderate temperature loving fungus, having an optimum growth temperature ranges from 20-30°C. Alkaline pH (8) favored the fungal growth and extracellular lignin peroxidase and laccase production. *Pleurotus platypus* reported to produce extracellular lignin peroxidase under nitrogen and sulphur starvation. Among the nitrogen limiting condition tested, the organic rich yeast extract (1%) basal medium favored the production of lignin peroxidase (45.4 U/L) and in the absence of nitrogen favored the production of laccase (9300 U/L). The sulphur containing aminoacids cysteine and methionine comparatively reduced the ligninolytic enzymes production in all the basal media tested. The protein profile in SDS-PAGE gel showing band near 40 KDa and 60 KDa indicates the presence of lignin peroxidase and laccase in partially purified enzymes. *Pleurotus platypus* grown on sugarcane leaves and coir pith produced the extracellular lignin peroxidase and laccase.

INTRODUCTION

Apart from the classical biotechnological sciences which use microorganism capable of producing molecules of interest for industry, degradative or environmental biotechnologies have recently emerged. Bioconversion of the vast quantities of lignocellulosic materials of agriculture, house-hold and industrial origin into useful products has received considerable attention but has led to any commercially viable process (Coughlan 1985, Doelle 1984). Cultivation of mushroom around the world represents the only commercial successful large scale bioconversion of lignocellulosic residues into food. Economic and biotechnological significance of mushroom production as a successful large scale microbial technology has been appreciated (Wood 1989). Bacterial lignin degradation has been most extensively studied in actinomycetes, particularly Streptomyces spp. and Agropyron (Antai and Crawford 1981). Lignin is a recalcitrant heteropolymer of phenylpropanoid units present in woody plant tissues, that confers them rigidity and resistance to biological attack. In order to depolymerize and mineralize lignin, white-rot fungi have developed an oxidative and unspecific system including extracellular enzymes, low molecular weight metabolites and activated oxygen species. Due to the lack of specificity of the system involved in the lignin depolymerization, white-rot basidiomycetes and their enzymes are being studied for their application on the degradation of aromatic pollutants causing environmental problems (Mario carlos *et al.,* 2002). Laccases are copper-containing oxidase enzymes that are found in many plants, fungi, and microorganisms. Laccases act on phenols and similar molecules, performing a one-electron oxidations, which remain poorly defined. It is proposed that laccases play a role in the formation of lignin by promoting the oxidative coupling of lignols, a family of naturally occurring phenols. Among white rot basidiomycetes, *Pleurotus spp.* is reported to be most efficient colonizers and degraders of lignocelluloses (Rajaratham and Bano 1989, Zadrazil 1979). Utilization of *Pleurotus sp.* for decomposing coconut coir pith was reported by TheradiMani and Marimuthu 1992. Effect of nitrogen on production of extracellular degradative enzyme by *Pleurotus sajor caju* (Fr.) Sing. On wheat straw reported (Saxena and Rai 1992). *Pleurotus platypus* was one of the most efficient

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species in decomposing the substrates (Nallathambi and Marimuthu 1993). Thus *Pleurotus platypus* offers greater potential for mushroom production and recycling of substrates into useful animal feed and manure *Pleurotus platypus* degrades raw coconut and coir pith into valuable organic manure than other *pleurotus sp*. (TheradiMani and Marimuthu 1992). *Pleurotus platypus* a potent oyster mushroom considered for organic recycling of agricultural wastes (Nallathambi and Marimuthu 1993). Hitherto no work has been initiated on lignolytic enzymes such as lignin peroxidase, manganese dependent peroxidase and laccase of *Pleurotus platypus*. Since these enzymes are no intensive research, thus the aim was to optimize the growth conditions of *Pleurotus platypus* in liquid cultures. An attempt was made to study the extracellular enzymes such as lignin peroxides and laccase on different nitrogen, sulphur sources, phenolic substances such as gallic acid and tannic acid. The enzymatic degradation of coir pith and sugarcane leaves by *Pleurotus platypus* was also studied.

MATERIALS AND METHODS

Organism and Inoculum

The organism *Pleurotus platypus* (Cook and Massee) sacc. is a white rot fungus, belong to the class basidiomycetes.The organism was collected from the culture collection of Centre for Advance Studies in Botany, University of Madras, chennai and maintained at room temperature on potato dextrose agar slants. Mycelial disc of 4 mm diameter from seven day old culture of the fungus maintained in CDB medium were used as inoculums (Plate.1).

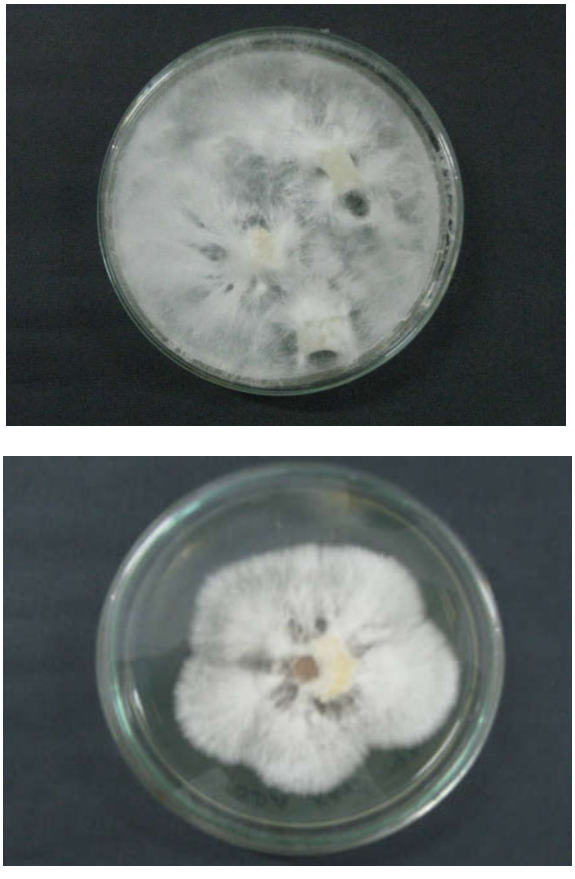


Plate 1. *Pleurotus platypus grown* in PDA

Assay for lignin peroxidase

The assay for the enzyme peroxidase was done by the modification of Kang *et al.,* 1993; Perumal and Kalaichelvan 1995 method in a Beckman spectronic DU-40 Spectrophotometer at an absorbance of 436nm. Pyrogallol was used as the substrate.

Assay mixture

Pipette	Volume	Concentration
Buffer pH 7	2.00 ml	0.1M
Pyrogallol	0.2 ml	0.1 M
Sample	1.0 ml	
H_2O_2	0.1 ml	0.1 M

The assay mixture was added in the order given above and the readings were recorded for 3 minutes each at 30 sec. intervals. The readings were recorded against a blank which was glass distilled water instead of H_2O_2. The enzyme activity was calculated and expressed in units / liter.

$$\text{Unit/liter} = \frac{V \times \Delta E \times 1000}{€ \times 1 \times t}$$

Where

V	=	Volume of assay mixture
ΔE	=	Extinction charge
€	=	Extinction co-efficient
l	=	Light path
t	=	Time

Assay for laccase

Laccase activity (or) production was measured polarographically using Yellos spring Model-5300 biological oxygen monitor by the method of Wood and Goodenough (1977). Values were calculated as enzyme Unit / l extract.

Estimation of protien

The protein estimation was determined according to the method of Bradford (1976), using Bovine Serum Albumin (BSA) as a standard protein. The amount of protein present in the sample was estimated from a standard graph prepared with Bovine Serum Albumin (Fr IV Sigma).

Comparision of growth on different temperature

Growth of *Pleurotus Platypus* at different temperature 0, 5, 10, 20, 30 and 40^{o}c were analyzed

Comparision of growth on different P^{h}

4mm disc of *Pleurotus platypus* were incubated at 30°C at static condition at different pH range such as 4, 5, 6, 7 and 8 pH. The mycelial growth was determined in terms of dry weight. The extracellular protein content was determined by the method of Bradford (1976). The H^+ ion concentration was

measure in expandable ion analyzer Ea 940 Orion Research (U.S.A).

Screening for lignolytic enzyme production

The production of lignolytic enzyme (Laccase and Peroxidase) of *Pleurotus platypus* was determined by the method of Adaskaveg and Gilbertson (1989).

Growth and ligninolytic enzyme production on diferent nitrogen and sulphur source

Different nitrogen sources such as glycine, asparigine, ammonium tartrate, peptone and yeast extract were selected.Glycine, asparagines, ammonium tartrate each at 0, 1, 5, 10 and 15mM concentration were amended in CDA medium. The sulphur sources such as cystine and methionine were amended at different concentration such as 0, 12.5, 25, 50 and 75mM in the CDB medium. A 4mm disc of was inoculated to 100ml Erlenmeyer flasks containing 30ml of respective media pH 6.5. The inoculated flaks were incubated at 30°C in still condition. The duplicate flasks were harvested at every 3 day intervals. The respective cultures were filtered through Whatman No.1 filter paper. The culture filtrate was used as a source of extracellular enzyme.

Partial purification of enzymes

Ammonium Sulphate precipitation

Ammonium sulphate is a particularly useful salt for the fractional precipitation of proteins. It is available in highly purified form, has great solubility allowing for significant changes in the ionic strength and is inexpensive. Changes in the ammonium sulphate concentration of a solution can be brought about either by adding solid substance or by adding a solution of known saturation, generally, a fully saturated (100%) solution. The enzymes separation from the exhausted medium was done by Ammonium sulphate precipitation technique. The Fungal enzymes extraction was made from culture filtrate using 83% w/v of Ammonium sulphate saturation (Fay.L.Myers and Northcote, 1958). The mixture was then stored in cold room for 24 hours to precipitate all the proteins. Then the precipitation was separated by centrifugation around 10000 rpm for 10 minutes. Then carefully the supernatant was discarded and the remaining precipitation was dissolved with 2 ml of 0.05M phosphate buffer (pH 7). Then the mixture was subjected in dialysis. The dialysed samples were lyophilized using the lyophiliser (Virtis). The enzyme powder that collected from the flask was transferred in to a sterile vial and stored at $4^{O}C$.

SDS PAGE ANALYSIS

The further protein profile and the presence of enzyme were confirmed by SDS PAGE analysis. The lyophilized protein sample we dissolved in 0.1M Tris (pH.9) and around 100µl of dissolved sample were heated at 90° c for ten minutes. Then around 10µl samples and a known molecular weight protein marker were loaded in separate wells into the polymerized gel (15% polyacrylamide). Tris glycine (pH 8.3) buffer was used as an electrode buffer. When the tracking dye reached the opposite end the run terminated and the gel was stained using Coomassie brilliant blue staining solution and destaining was done Alcohol – acetic acid mixture solution.

The enzymatic degration of coir pith and sugarcane leaves

The coconut coir pith and sugarcane leaves were collected from a farm near Vilupuram, Tamilnadu. The coir pith was chopped into small bits (1-2 cm) and dried in an oven at $80^{o}C$ for an overnight. 2 grams of coir pith was amended in 250 ml of Erlenmeyer flask containing 50 ml of distilled water and autoclaved. The discs of *Pleurotus platypus* (9 mm dia) were aseptically inoculated and incubated for 9 days at stationary condition. The duplicate cultures were harvested and filtered through Whatman No.1 filter paper. The filtrate served as the enzyme source for lignin peroxidase and laccase. Sugarcane leaves chopped into small bits and sun dried. The sun dried leaves were ground into fine powder. The fine powder sugarcane leaves 500 mg was amended into 250 ml Erlenmeyer flask containing 50 ml of water and autoclaved. The discs of *Pleurotus platypus* (9 mm dia) were aseptically inoculated and incubated for 9 days at 30ºC. The duplicate cultures were harvested and filtered through Whatman No.1 filter paper. The filtrate served as the enzymes source for lignin peroxidase and laccase.

RESULTS

Growth at different temperature

Pleurotus platypus was considerer moderate temperature fungus. *Pleurotus platypus* has an optimum temperature of 20-30°C with the maximum growth of 40.5 mm on the day 9 and the growth rate was 6 mm per day. At 20°C the maximum growth was 39 mm on the 9^{th} day (4 mm/day). There was no growth at 0, 5, 10 and 40°C. The optimum temperature for the growth of *Pleurotus platypus was* 30°C (Fig.1).

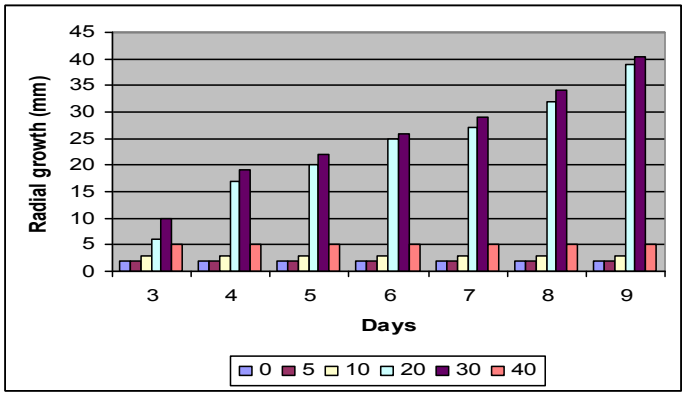
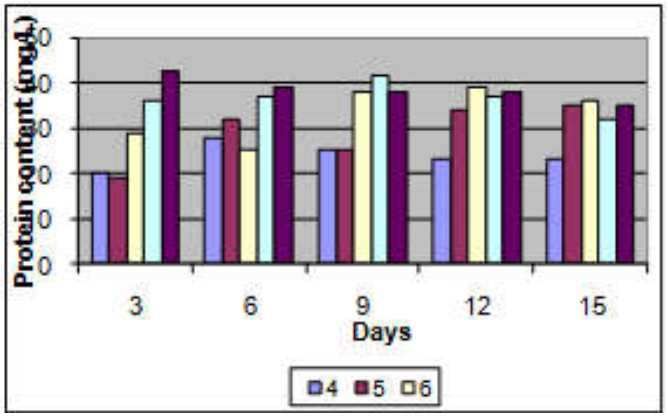


Fig.1. Mycelial growth of *Pleurotus platypus* at different temperature (°C)

Growth at different P^{h}

Of the different pH media tested the highest amount of the protein content was 43, 42, 39 and 28 mg/L in the pH 7, 6, 8, 5 and 4 observed on the day 9, 9, 12, 12 and 9 respectively (Fig.2.B). The highest amount of mycelial growth was 10.8, 4.9, 4.7 and 3.2 g/l in the pH 8, 7, 4, 6 and 5 observed on the day 12, 12, 12, 9 and 12 respectively (Fig.2.A). The optimum pH for the growth of *Pleurotus platypus* was pH 6-7.



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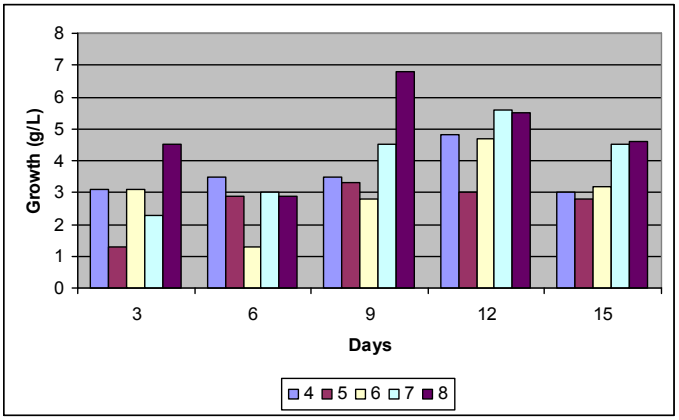


B

Fig. 2. Mycelial growth (A) and Protein content (B) of *Pleurotus platypus* at different pH (30° C).

which confirms the laccase production of *Pleurotus platypus* (Table.1 Plate 2 & 3).

Plate 2. Tannic acid plate

Peroxidase

The mycelial growth and oxidase reaction for lignin peroxidase were observed only on 0.05 and 0.10%. at 0.15 and 0.20% the growth was inhibited. The oxidase reaction was very strong (+++) on 0.05% and moderately strong (++) in 0.10%. The mycelial growth was more in 0.05% than in 10%, thus conforms the production of the lignin peroxidase by *Pleurotus platypus* (Table.1. Plate 4)

Table 1. Screening for lignin peroxidase and laccase on 2% Mea- Medium

Percentage %	Gallic Acid		Tannic acid		Pyrogallol and hydrogen peroxide	
	Mycelial growth (mm)	Oxidase reaction	Mycelial growth (mm)	Oxidase rection	Mycelial growth (mm)	Oxidase reaction
Control	20	-	20	-	20	-
0.05	6	+	17	+	5	++
0.10	11	++	9	++	2	+++
0.15	7	+++	9	+++	N.G	-
0.20	6	-	6	+++	N.G	-
0.25	N.G	-	N.G	-	N.G	-
0.50	N.G	-	N.G	-	N.G	-
0.75	N.G	-	N.G	-	N.G	-
1.0	N.G	-	N.G	-	N.G	-

- :Negative reaction + :Weak reaction
++ :Strong reaction +++ :Very strong reaction
N.G : No growth

Screening for lignolytic enzyme production

Laccase

The mycelial growth and oxidase reaction on the different concentration of gallic acid and tannic acid varied considerably between different concentrations. Mycelial growth and oxidase reaction were negatively correlated at 0.15 and 0.20% concentration of gallic acid and tannic acid the oxidase reaction was very strong, but did not grow well. At higher concentration above 0.20% of gallic acid and tannic acid the mycelial growth was greatly inhibited. The oxidase reaction rate was very strong (+++) in both 0.15 and 0.20%, moderately strong (++) in 0.10% and week (+) in 0.05%. Overall the mycelial growth was more in 0.5% (17 mm) in tannic acid and 0.10% (11 mm) in gallic acid. At 0.15 and 0.20% of tannic acid produced very strong oxidase reaction

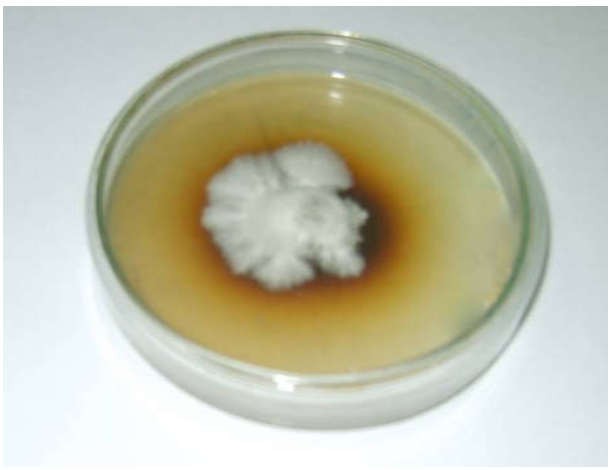


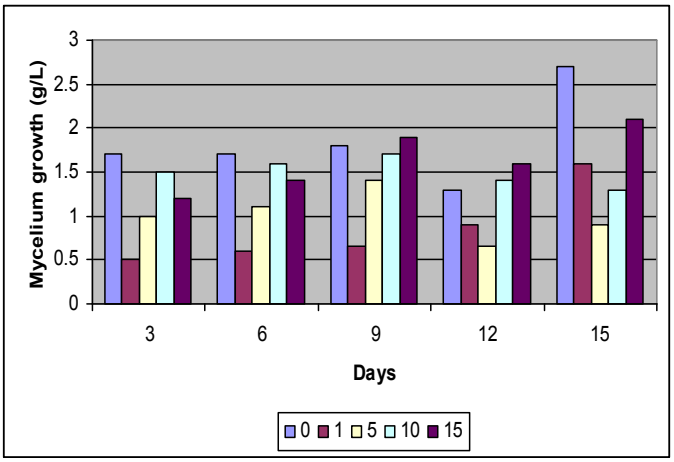
Plate 4. Pyrogallol plate

Nitrogen source

Production of lignin peroxidase and laccase under different organic and inorganic nitrogen sources was tested individually. The basal medium (CDB) was amended with different concentration of different nitrogen source such as glycine, aspargine, ammonium tartarate, peptone and yeast extract produced remarkable amount of lignolytic enzymes.

Glycine (0, 1, 5, 10 mM Conc.)

The basal medium amended with 0, 1, 5, 10 and 15 mM conc. of glycine produced the highest amount of mycelial growth was 2.7, 1.7, 1.3, 1.7 and 1.9 g/L recorded on the day 15, 15, 9, 9 and 9 respectively (Fig. 3.A). The extracellular protein content was 4, 5, 6, 8 and 7 mg/L recorded on the day 12, 15, 15, 12 and 12 respectively (Fig.3.B). T he maximum production of lignin peroxidase was 13.4, 7.2, 9.8, 12.3 and 10.3 U/L recorded on the day 9, 3, 9, 6 and 3 respectively in all the concentration of glycine tested (Fig.4).



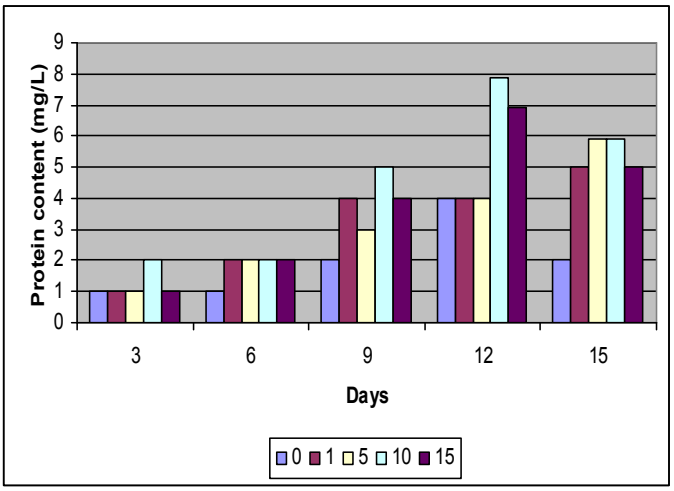


Fig. 3. Mycelial growth (A) and Protein content (B) of *Pleurotus platypus* on 0,1,5,10,15 mM concentraton of glycine in CDB media

Asparagine (0, 1, 5, 10 and 15 mM Conc.)

The basal medium amended with 0, 1, 5, 10 and 15 mM conc.of asparagine produced the highest amount of mycelial growth was 17.6, 6.9, 11.5, 6.5 and 13 g/L recorded on the day 15, 15, 12, 12 and 12 respectively (Fig. 5.A). The highest amount of extracellular protein was 4, 13, 15, 15 and 18 mg/L recorded on the day 9, 9, 12 and 12 respectively (Fig.5.B). The maximum production of lignin peroxidase was 6.7, 7.7, 8.2, 13.9 and 14.4 U/L recorded on the day 12, 12, 9, 9 and 9 respectively (Fig.6.A). The maximum production of laccase was 9370, 1040, 1250, 833 and 892 U/L recorded on the day 15, 6, 3, 12 and 3 respectively (Fig.6.B).

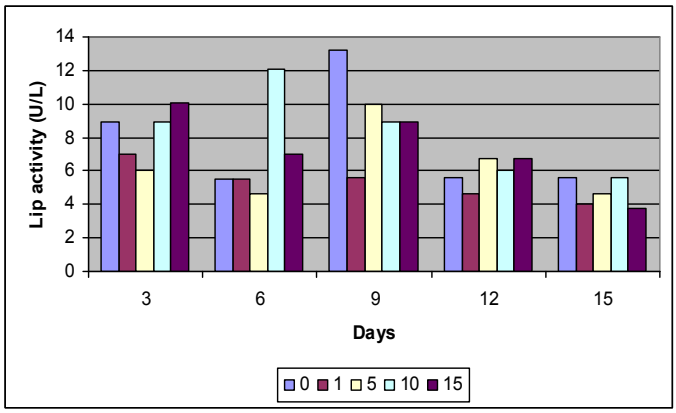
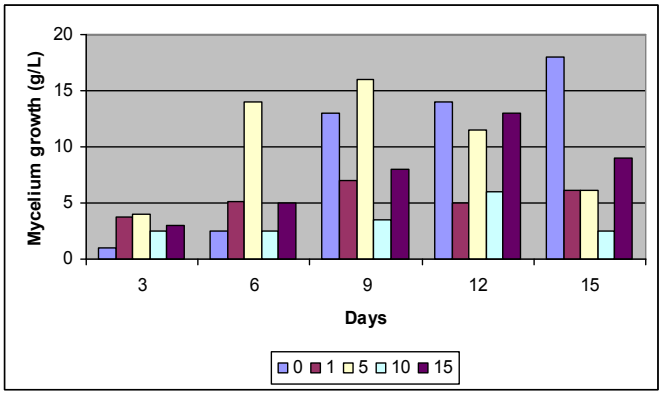


Fig. 4. Lignin peroxidase activity of Pleurotus platypus on 0,1,5,10,15 mM concentration of glycine in CDB media



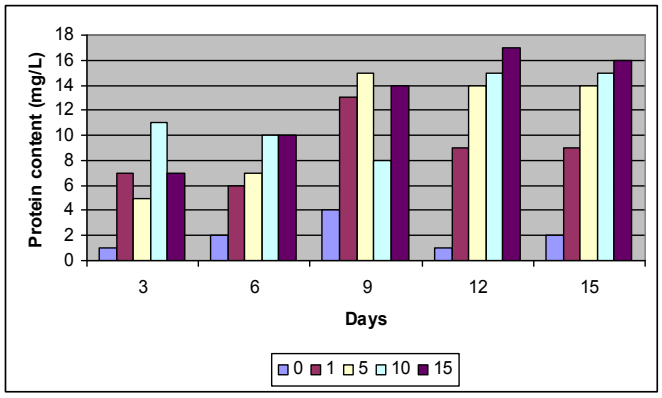


Fig. 5. Mycelial growth (A) and Protein content (B) of Pleurotus platypus on 0,1,5,10,15 mM concentration of asparagine in CDB media

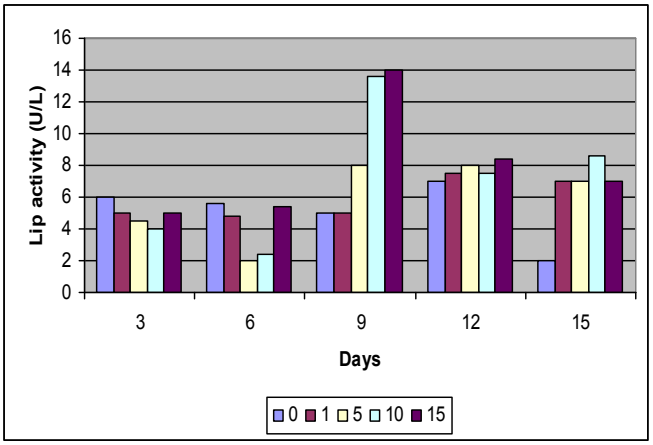
Ammonium Tartarate (0, 1, 5, 10 and 15mM Conc.)

The basal medium amended with different concentration of ammonium tartarate produced the highest amount of mycelial growth was 17.6, 10.1, 5.3, 5 and 9.1 g/L recorded on the 15th day for all the media (Fig.7.A). The highest amount of extracellular protein content was 4, 8, 9, 9 and 11 mg/L on the day 9, 15, 15, 15 and 15 respectively (Fig.7.B). The maximum production of lignin peroxidase activity was 6.7, 8.7, 8.2 and

9.2 U/L recorded on the day 12, 9, 6, 9 and 9 respectively (Fig.8.A). The maximum production of laccase was 9300, 6250, 3750 and 6250 U/L recorded on the day 15, 6, 6, 9 and 12 respectively (Fig.8.B).

Peptone (0, 0.5, 1.0, 1.5 and 2.0%)

The basal media amended with different percentage of peptone produced the highest amount of mycelial growth was 8.2, 11.7, 6.1, 3.4 and 8.9 g/L on the day 18, 18, 18, 9 and 18 respectively (Fig.9.A). The highest amount of extracellular protein content was 12, 107, 118, 224 and 176 mg/L recorded on the day12, 15, 12, 6 and 12 respectively (Fig. 9.B). The maximum production of lignin peroxidase was 9.2, 18, 28.4, 12.3 and 24.2 U/L recorded on the day 12, 15, 15, 15 and 15 respectively (Fig.10.A). The maximum production of laccase was 4100, 500, 533, 522 and 529 U/L recorded on the day 6, 18, 18, 15 and 15 respectively (Fig.10.B).



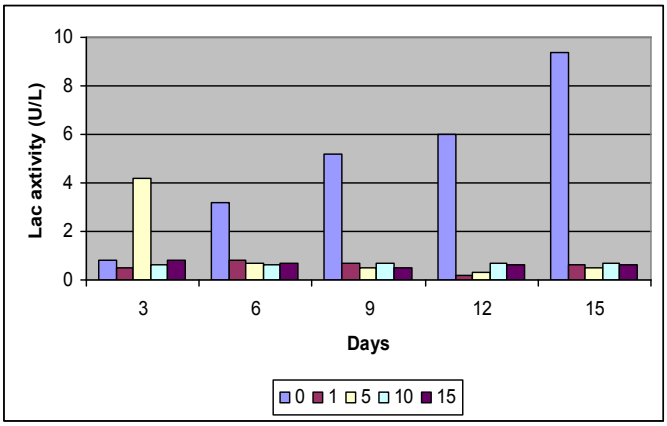
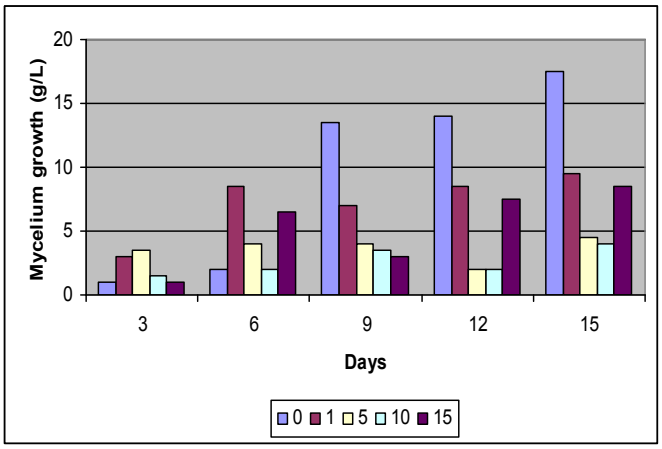


Fig. 6. Lignin peroxidase (A) and Laccase activity (B) of Pleurotus platypus on 0,1,5,10,15 mM concentration of asparagine in CDB media

Yeast Extract (0, 0.5, 1.0, 1.5 and 2.0%)

The basal medium amended with different percentage of yeast produced the higher amount of mycelial growth was 8.2,5.5,10.3,13.8 and 15.1g/L recorded on the day 18,15,12,15 and 18 respectively (Fig.11.A). The maximum production of lignin peroxidase was 9.2,37.6,45.4,40.7,and 35.6 U/L recorded on the day 12,15, 18,15 and 15 respectively (Fig.12.A).The highest amount of laccase activity was 4100,806,816,816 and 761 U/L recorded on the day 6,9,12,15 and 15 respectively (Fig.12.B).



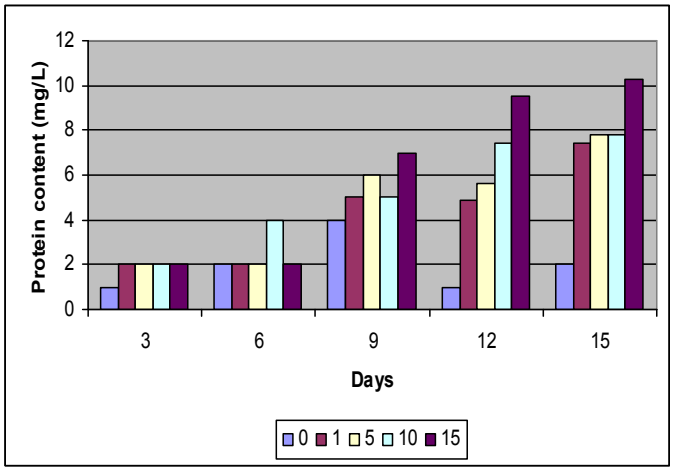
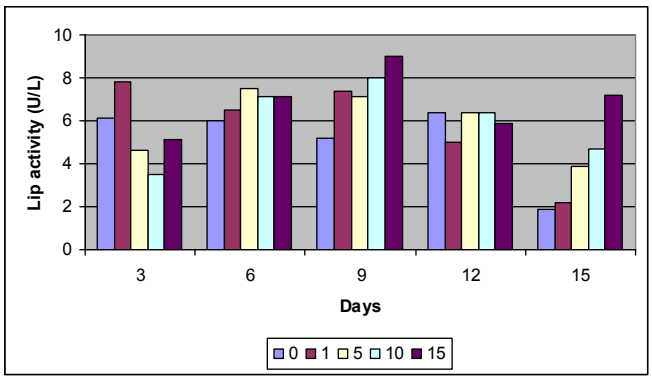


Fig. 7. Mycelial growth (A) and Protein content (B) of Pleurotus platypus on 0,1,5,10,15 Mm concentration of ammonium tartarate in CDB media



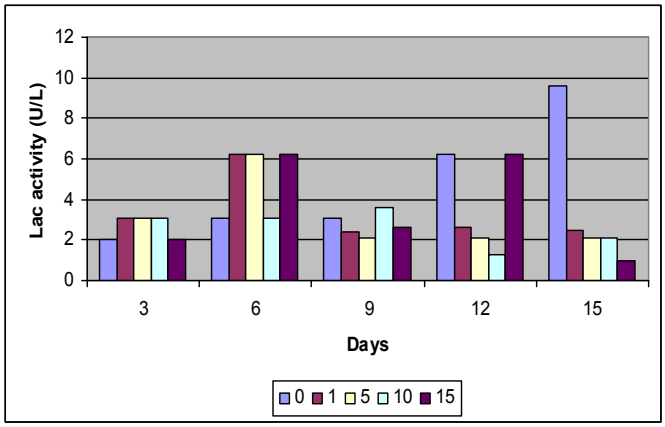
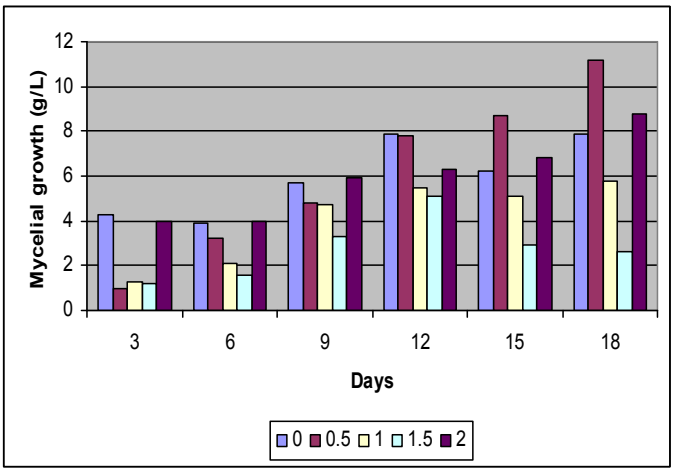


Fig. 8. Lignin peroxidase (A) and Laccase activity (B) of Pleurotus platypus on 0,1,5,10,15 mM concentration of ammonium tartarate in CDB media



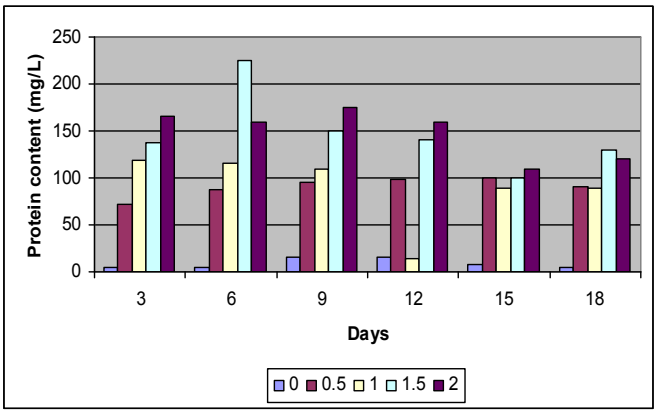
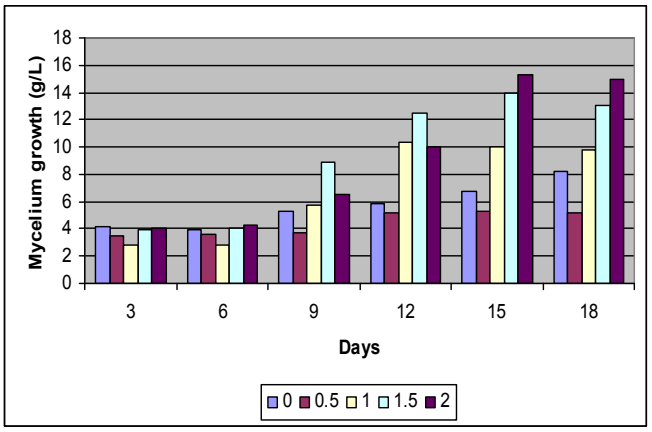


Fig. 9. Mycelial growth (a) and Protein content (B) of Pleurotus platypus on 0,0.5,1,1.5,2% of peptone in CDB media



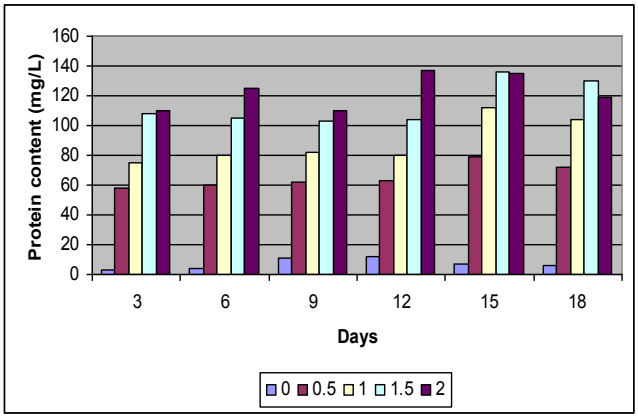
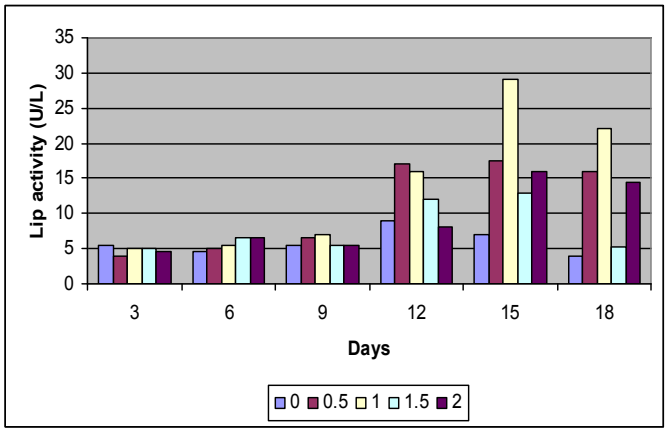


Fig. 11. Mycelial growth (A) and Protein content (B) of Pleurotus platypus on 0,0.5,1.0,1.5,2.0% of yeast extract in CDB media



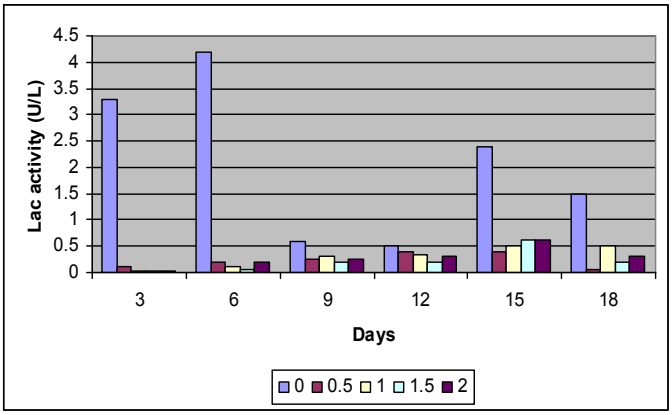
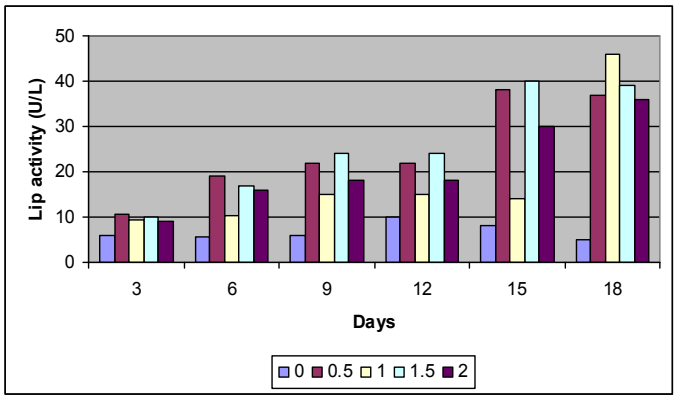


Fig.10. Lignin peroxidase (A) and Laccase (B) of Pleurotus platypus on 0,0.5,1,1.5,2% of peptone in CDB media.



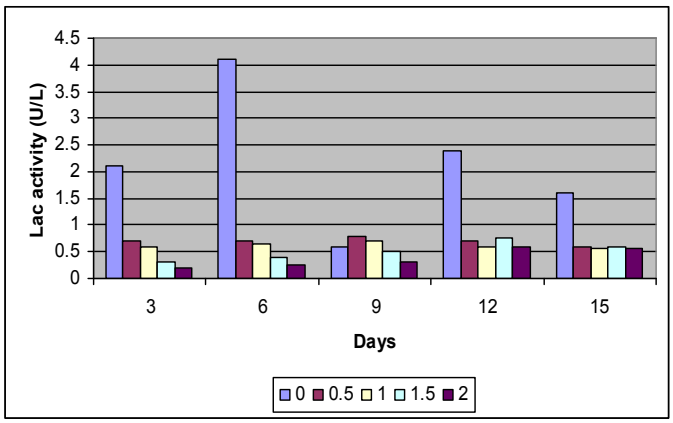
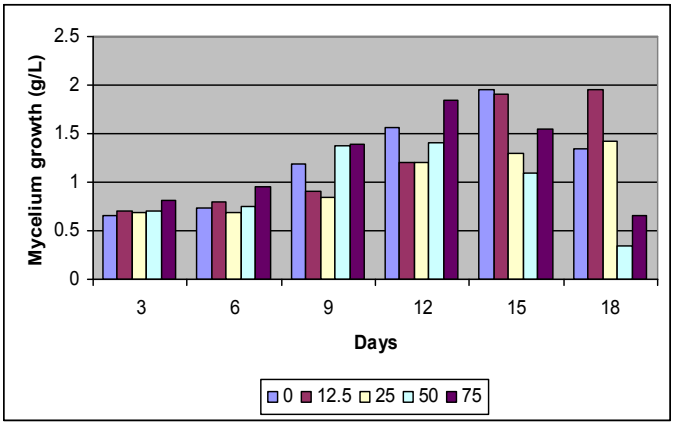


Fig.12. Lignin peroxidase (A) and Laccase activity (B) of Pleurotus platypus on 0,0.5,1.0,1.5,2% of yeast extract in CDB media

Sulphur Sources

Cystine (0, 12.5, 25, 50 and 75µM)

The basal medium amended with 0, 12.5, 25, 50, and 75µM concentration of cystine produced the highest amount of mycelial growth was 1.998, 1.998, 1.165, 1.465 and 1.864 g/L recorded on the day 15,18,12,12 and 12 respectively (Fig.13.A).The highest amount of extracellular protein content was 28, 32, 37, 38 and 40 mg/L recorded on the day 3 for all the concentration respectively (Fig.13.B).The maximum production of lignin peroxidase activity was 6.1, 6.7, 7.2, 5.6 and 6.7 U/L recorded on the day 18, 15, 15, 18 and 18 respectively (Fig.14.A).The maxium production of laccase activity was 446, 382, 500, 565 and 284U/L recorded on the day 3, 6, 9 and 9 respectively (Fig.14.B).



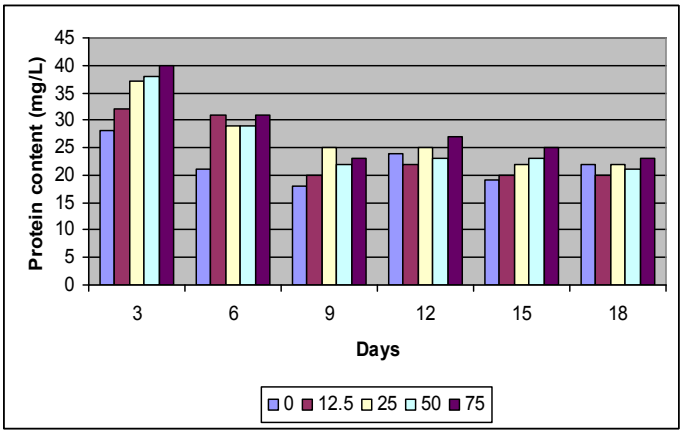
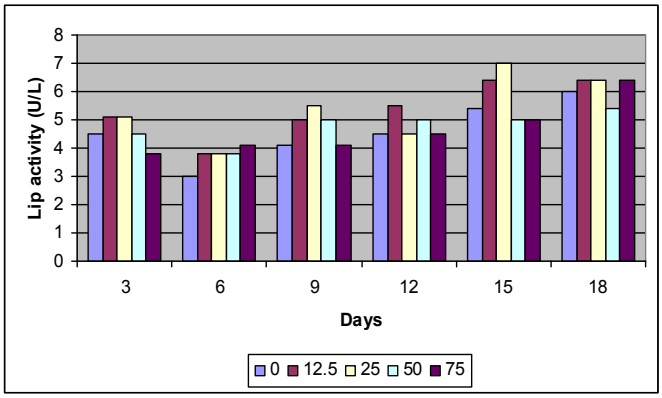


Fig. 13. Mycelial growth (A) and Protein content (B) of Pleurotus platypus on 0,12.5,25,50,75µM concentration of cystine in CDB media

Methionine

The basal medium amended with 12.5, 25, 50 and 75µM concentration of methionine. These basal medium produced the highest amount of mycelia growth was 1.5, 1.4, 2.0, 1.91.5 g/L recorded on the day 15 for all the respective concentration (Fig.15.A).The highest amount of extracellular protein content was 25,27,28 and 30mg/L recorded on the day 15 for all the respective concentration (Fig.15.B). The maximum production of lignin peroxidase activity was 8.7, 7.7, 10.3, and 8.7 U/L recorded on the day 12, 12, 18 and 12 respectively (Fig.16.A). The maximum production of laccase activity was 625, 370, 328 and 328 U/L recorded on the day 18,18,3 and 3 respectively (Fig.16.B).



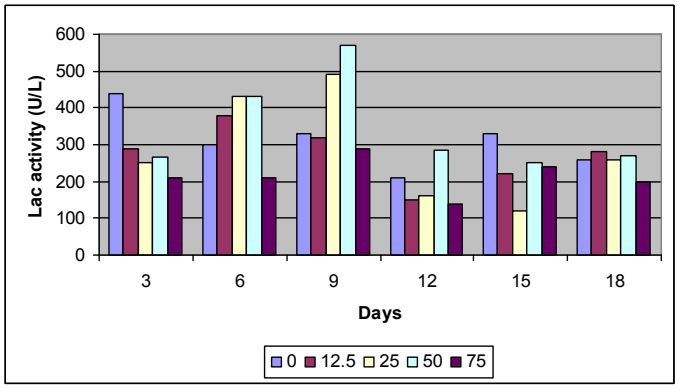
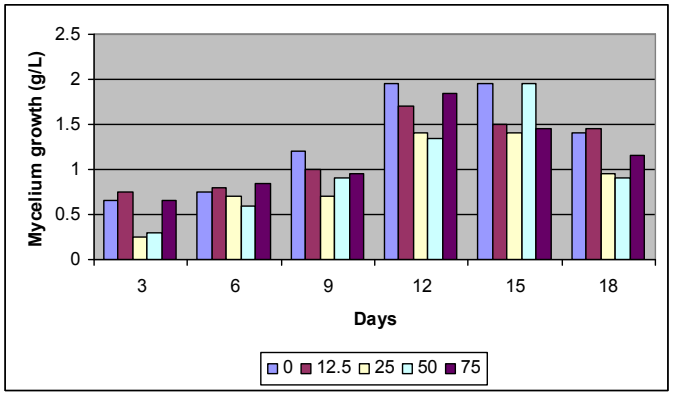


Fig.14. Mycelial growth (A) and Laccase activity (B) of Pleurotus platypus on 0,12.5,25,50,75µM concentration of cystine in CDB media



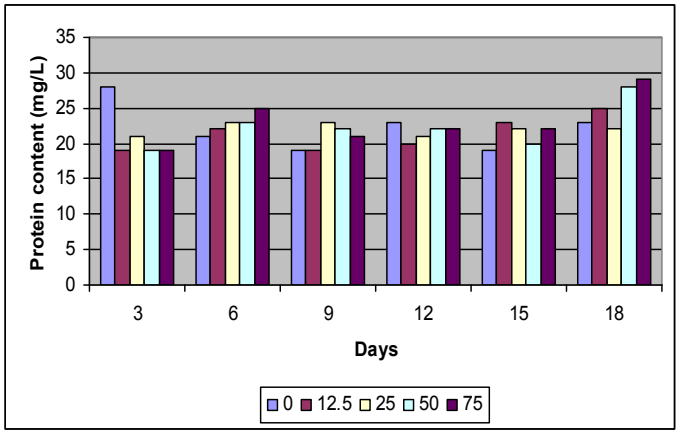
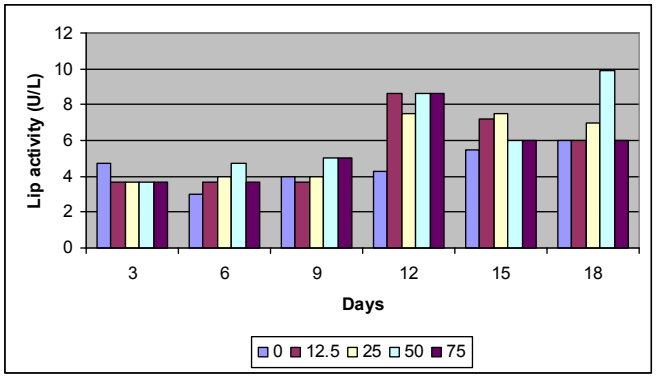


Fig. 15. Mycelial growth (A) and Protein content (B) of Pleurotus platypus on 0,12.5,25,50,75 µM concentration of methionine in CDB media



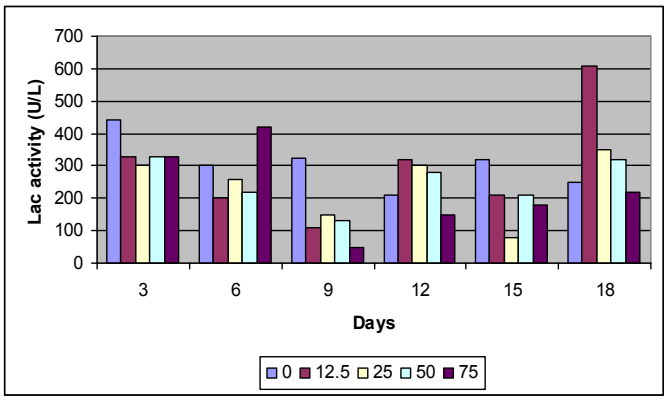


Fig. 16. Lignin peroxidase (A) and Laccase activity (B) of Pleurotus platypus on 0,12.5,25,50,75 µM concentration of methionine in CDB media

The enzymatic degradation of coirpith and sugarcane leaves

Pleurotus platypus grown on natural lignocellulosic such as coir pith sugarcane leaves and indulin, produced extracellular lignoinolytic enzymes in the culture media tested. The coir pith amended media produced 60.93 U/L of lignin peroxidase and 892 U/L of laccase. The sugarcane leaves amended media produced 6.2 U/L of lignin peroxidase and 155 U/L of laccase. The lignin model compound produced 12.3 U/L of lignin peroxidase and 288U/L of laccase. (Table 2 Plate. 5, 6).

Plate 5. Degradation of coir pith

SDS PAGE

Finally, the protein profile was analysed in SDS-PAGE; it showed the presence of multiple bands. Obviously, because the medium contain protein source, so unutilized protein also may be present in the exhausted medium. Moreover, along with laccase and lignin peroxidase can also be produced by the organism. But the presence of protein band nearing the molecular weight 60Kda in lane 2 and near 25-30 kda in lane 3 confirms the presence laccase of and lignin peroxidase. (Saranyu khammuang *et al.,* 2004; Mariana Mansur *et al.,* 2003). (Plate.7)

Plate 6. Degradation of sugarcane leaves

Table 2. Production of lignin peroxidase and laccase by pleurotus platypus on natural lignocellulosic substrate

Substrates	Lignin peroxidase (U/L)	Laccase (U/L)	Protein (mg/L)
Coir pith	60.93	892	7
Sugarcane leaves	6.2	155	7
Indulin + Minimal Media	12.3	288	115
Minimal media	18.0	187	50

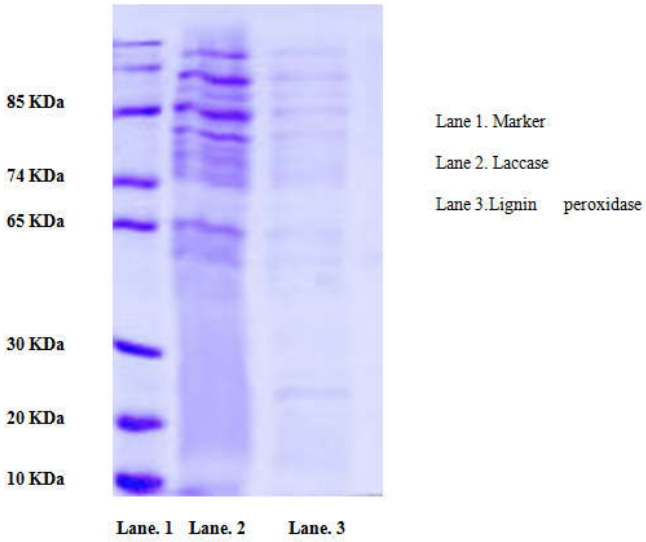


Plate 7. Protein profile in SDS – PAGE

DISCUSSION

Pleurotus Spp. are white rot fungi having a wider growth temperature ranges from 15 C to 40°C of which P.ostreatus grew at 28°C (Youn *et al.,* 1995) and *P.sajor-caju* (Saxena and Rai 1992). *P.erynii* (Guillen and Evans 1994) grew well in the optimum temperature at 25 & 28°C. The test fungus *Pleurotus platypus* had a good growth at optimum temperature ranges from 20 to 30°C and had a growth rate of 6 mm/day. The

Pleurotus platypus can be considered as moderate temperature loving fungus. The mycelial growth and the extracellular enzyme production of many white rot fungi were pH dependent (Jefferies *et al.,* 1981; Kall *et al.,* 1993). In many *Pleurotus Spp*. the enzyme production appears as constitutive nature and produced at different phases under different culture conditions (Guillen *et al.,* 1990; Valmaseda *et al.,* 1991). The results on pH indicated that the alkaline pH favored the mycelial growth. The extra cellular protein content was maximum at pH 7 and the fungal growth was maximum at pH 8. Thus the alkaline pH influenced the enzymatic degradation of lignolytic substrates by *Pleurotus platypus.* However these fungi capable of growing under alkaline conditions and extracellular industrially useful enzymes have been little investigated (Bansod *et al.,* 1993). Most of these white rot fungi produces lignolytic enzyme such as lignin peroxidase, manganese dependent peroxidase and laccase. The lignin peroxidase can directly oxidize non-phenolic lignin models, whereas manganese dependent peroxidase and laccase are supposed to degrade mainly phenolic units (Camarero *et al.,* 1994). Some of the white rot fungi produced lignin peroxidase but not laccase. In *Phanerocheate chrysorium* no detectable amount of laccase was recorded (Krik and Farrell 1987). Among the white rot fungi the *Pleurotus sp.,* were not produced lignin peroxidase but produced laccase (Gutierrez *et al.,* 1994; Martinz *et al.,.,* 1994.).

In three species of the genus Pleurotus in which neither lignin peroxidase nor manganese dependent peroxidase has been found (Guillen *et al.,* 1990; Sannia *et al.,* 1991; Guillen *et al.,* 1992), but Kang *et al.,.,* 1993 reported that *Pleurotus ostreatus* produced peroxidases and characterized this enzyme from culture filtrate of *Pleurotus ostreatus.* The experimental results suggested that *Pleurotus platypus* produced extracellular lignin peroxidase under experimental conditions. Thus it may be concluded that *Pleurotus platypus* produced both lignin peroxidase and laccase which may be involved in degradation of phenolic and non-phenolic lignin models. According to present day paradigm most of the white rot fungi produce ligninolytic enzymes in response to carbon, nitrogen and sulphur limitations (Kaal *et al.,* 1993). The most studied ligninolytic system was in *Phanerochaete chrysosporium*. The ligninolytic activity is an expression of secondary metabolism triggered not only by nitrogen limitation but also by carbon and sulphur limitation (Jefferies *et al.,* 1981). However Kimura *et al,.,* 1990 found that some wild type white rot produced lignin peroxidase only in organic rich medium. In accordance with results the *Pleurotus platypus* in organic rich yeast extract 1% media induced lignin peroxidase (45.4U/L in 1%) production. In *Pleurotus platypus* lignin peroxidase (28.4 U/L) could also be induced by 1% peptone amended basal medium. The organic rich yeast extract media repressed the laccase production. The aminoacids glycine and asparagines comparatively induced the lignin peroxidase production in *Pleurotus platypus,* but reduced the laccase production under the nitrogen limited condition. These studies indicated that a distinct regulation of lignin peroxidase production in *Pleurotus platypus* exists compared with *Phanerochate chrysosporium.* Ligninolytic activity of *Pleurotus platypus* is controlled by nitrogen and carbon limitation (Keyser *et al.,* 1978) but also by carbohydrate and sulphur limitation (Jefferies *et al.,* 1981).The experiments on different carbon sources by *Pleurotus platypus* indicated that increasing concentration of carbon sources increased the fungal growth and induced the enzyme production. At 25mM concentration of sources in basal medium induced the maximum production (35.1 U/L) of lignin peroxidase. In *Phanerochaete chrysosporium* ligninolytic activity begins when the physiological equilibrium is reached. Decreasing mycelium growth induced the onset of ligninolytic enzyme (Jefferies *et al.,* 1981) but the results remarkably indicated the increasing the mycelial growth increased the production of lignin peroxidase. Thus the result evidently indicated that the ligninolytic enzymes production is expressed during the primary and secondary mycelial growth of *Pleurotus platypus*. The relationship between ligninolytic enzymes production and growth substrate is unclear (Fenn and Krik 1980).Evidently the carbon and nitrogen source remarkably induced the lignin peroxidase and decreased the laccase, where as in absence of nitrogen source significantly increased the production of laccase but not lignin peroxidase. *Pleurotus platypus* was reported to be a better degrader of raw coconut coir pith with higher bioefficiency (Nallathambi and Marimuthu, 1993). The experimental result of *Pleurotus platypus* on coir pith and sugarcane leaves remarkably produced both laccase and lignin peroxidase. The production of lignin peroxidase and laccase is involved in the degradation of phenolic and non phenolic subunits (Camararo *et al.,* 1994). *Pleurotus platypus* was reported to degrade lignin 78.07% in coir pith (Theradimani and Marimuthu 1992). The production of lignin peroxidase and laccase of *Pleurotus platypus* on the coir pith and sugarcane leaves might be in response to the lignin degradation. Four laccase isozymes synthesized by *Pleurotus ostreatus* have molecular masses of about 60 and 65kDa (Mariana Mansur 2003), were lignin peroxidase having the molecular weight of about 35- 40 Kda. Where laccase and lignin peroxidase of our isolates showed bands nearing 60KDA and 25-30 Kda.

Summary

The white rot edible mushroom *Pleurotus platypus* has considered as a moderate temperature loving fungus, having an optimum growth temperature ranges from 20-30°C. Alkaline pH (8) favored the fungal growth and extracellular lignin peroxidase and laccase production. *Pleurotus platypus* reported to produce extracellular lignin peroxidase under carbon nitrogen and sulphur starvation. Among the nitrogen limiting condition tested, the organic rich yeast extract (1%) basal medium favored the production of lignin peroxidase (45.4 U/L) and in the absence of nitrogen favored the production of laccase (9300 U/L). The sulphur containing aminoacids cysteine and methionine comparatively reduced the ligninolytic enzymes production in all the basal media tested. The protein profile in SDS-PAGE gel showing band near 40 KDa and 60 KDa indicates the presence of lignin peroxidase and laccase in partially purified enzymes. *Pleurotus platypus grown* on sugarcane leaves and coir pith produced the extracellular lignin peroxidase and laccase.

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