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Research Article

LIGNOLYTIC ENZYMES PRODUCTION FROM SELECTED MUSHROOMS

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Abstract

In this paper, ligninase enzymes produced by selected mushrooms have been reported. We collected mushrooms from Western Ghats, most of them were edible food. Thirty samples isolated were tested using a plate assay through direct agar plate assay by using ABTS, decolourisation containing the fifteen isolates were able to decolourise the dye, indicating a lignin-degrading ability. Spectrophotometric enzyme assays from all selected isolates were carried out to examine the production of Ligninolytic enzymes (Laccase, lignin peroxidase and manganese peroxidase). Ten selected isolates produced all three kinds of enzymes tested. Lignolytic enzymes are groups of enzymes these are actively involved in bioremediation.

Key words: Edible Mushrooms; Lignolytic Enzymes; ABTS.

Introduction

Mushroom which is a fleshy saprophyte fungus are found growing on damp rotten log of wood trunk of trees, decaying organic matter and in damp soil rich in organic substances. Edible mushroom are highly nutritious and can be compared with eggs, milk and meat (Oei, 2003).

The content of essential amino acids in mushroom is high and close to the need of the human body. Mushroom is easily digestible and it has no cholesterol content. However, the cultivation of mushroom is still very limited (Isikhuemhen and Okhuoye, 1996; Belewu, 2002, 2003).

Production of ligninolytic enzymes by solid-state cultivation of white-rot fungi on lignocellulosic substrates is essential in the emerging field of biotechnology. During the growth and the development of mushroom mycelia, secretions of these enzymes cause biochemical changes in the substrate. Such biochemical changes modify the insoluble and large components of lignocellulosic materials into the soluble and low-molecular-weight compounds which afterward are taken as source of nutrients and energy necessary for the fruiting bodies enlargement. Therefore, these enzymes have a significant role in mushroom growth and development. (Kuforiji and Fasidi, 2008). These enzymes are also important in various industrial processes in the production of chemicals, fuel, food, pulp and paper, textile, laundry, and animal feed (Elisashvili *et al.*, 2006).

Lignocellulose is the predominant component of woody plants and dead plant materials and the most abundant biomass on earth. White-rot fungi (Basidiomycetes) produce various extracellular enzymes, such as laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP), which are involved in the degradation of lignin and their natural lignocellulosic materials (Nagai *et al.*,2007).

This ligninolytic system of white-rot fungi is also directly involved in the degradation of various xenobiotic compounds and dyes (Hofrichter, 2002; Songulashvili *et al.*, 2007).

The potential application of ligninolytic enzymes in biotechnology has stimulated the investigation of their production with the purpose of selecting promising enzyme producers and increasing of their yield. The understanding of physiological mechanisms regulating enzyme synthesis in lognocellulose bioconversion could be useful for improving the technological process of edible and medicinal mushroom production (Songulashvili *et al.*, 2007).

To develop commercially significant and low cost technologies for ligninolytic enzyme production, two approaches can be applied. The first one involves search for powerful producers of enzymes and the second one is the utilization ability of lignocellulosic residues. Many studies indicate that the structure of lignocellulosic materials, the type of mushrooms species as well as mushroom cultivation techniques play a vital role in the enzyme production potential by white rot fungi during SSF (Reddy *et al.*, 2003; Elisashvili *et al.*, 2006; Elisashvili *et al.*, 2008; Kurt and Buyukalaca, 2010).

Moreover, most studies utilized cereal straw based-substrates to investigate ligninolytic activities trend in some

growth stages during solid state cultivation of *Pleurotus species* such as *Pleurotus ostreatus* (Elisashvili *et al.*, 2008; Rühl *et al.*, 2008; Isikhuemhen and Mikiashvilli, 2009; Sherief *et al.*, 2010).

Materials and Methods

Sampling and Extraction of Enzymes

In this study, we used mushroom species collected from Western Ghats North Canara and Shivamogga. . Samples were inoculated on to culture potato dextrose agar or Malt extract agar medium. Isolated fungal strains were maintained on 2% (w/v) MEA or PDA slants at 4ºC and the fungi were activated at 26ºC to determine their Ligninolytic activities.

Mycelium plug was transferred to screening medium plates for 21 days incubation at 27 ºC. Diameters of clearing zone formation around the mycelia plugs were measured for 21 days. Isolates, which formed clearing zones, were selected for enzyme assays.

Two plugs of inoculate taken from 7 day-old cultures of selected isolate, were transferred to a 100-ml flask containing 15 ml of liquid basal medium without addition of agar. After incubation at room temperature and 140 rpm (until the cultures reached their optimal growth rate), the culture was centrifuged (10000 gm, 4 ºC, 10min), and the supernatants were recovered for ligninolytic enzyme assays.

Ligninolytic enzymes Activity

1. Laccase Activity

Laccase activity was determined by reaction assay mixture 1.5 ml contained 0.5 mM of 2,2'-azino-bis (3-ethylbenzthiazoline)-6-sulfonate (ABTS) in 100 mM sodium acetate buffer pH 5.0 and 50 µl of the enzyme filtrate. Oxidation of ABTS was observed by the formation of intense blue-green colour which was monitored by measuring the increase in absorbance at 420 nm, with using UV-Visible spectrophotometer (Silva *et al.*, 2005).

2. Manganese Peroxidase Activity

Manganese peroxidase activity was assayed as described by with some changes, using a reaction mixture containing 5 mM guaiacol and 2mM MnSO4 in 50 mM sodium succinate buffer pH 4.5, 10 mM H2O2 and 100 µl enzyme filtrate in a total volume of 1.5 ml. Hydrogen peroxide was added lastly to initiate the reaction after incubating the mixture 5 minutes. The increase in absorbance was monitored by the oxidation of guaiacol (2-methoxyphenol) as the substrate at 465nm using UV-Visible spectrophotometer. One unit of MnP activity was defined as the amount of enzyme that oxidized 1.0 µmole of 2- methoxyphenol per minute (Leonowicz *et al.*, 1999).

3. Lignin Peroxidase Activity

Lignin peroxidase activity was assay mixture 1.5 ml contained 10 mM veratryl alcohol in 20 mM citrate buffer pH 3.5, 10 mM H_2O_2 and 100 µl enzyme filtrate. H_2O_2 (300µl, 50 mM) was added last to initiate the reaction after incubating the substrate and enzyme for 5 minutes. The increase in absorbance was followed spectrophotometrically at 310nm where the oxidation of veratryl alcohol to veratraldehyde (3, 4-dimethoxybenzaldehyde) took place. One unit of LiP was defined as the amount of enzyme that oxidizes 1.0µmole of veratryl alcohol per minute at pH3.5 and 30°C (Kuwahara *et al.*, 1984).

Table: 1: Screening for ligninolytic enzyme in fresh mushroom extracts (In mm).

SL No	Organisms	Green zone formation (ABTS)	Reddish-brown zone formation (Guaiacol)	Controle
1	*Agaricus sp 1*	12±1	81±1	19± 1
2	*Agrocybe aegerita*	18±4	32 ±3	20 ±1
3	*Agrocybe sp.*	20±3	7±2	45±1
4	*Coprinus comatus*	26±3	23± 3	42±5
5	*Gloeophyllum trabeum (GT)*	28±2	30± 4	38± 3
6	*Meripilus giganteus (MG*	19±3	12± 4	35± 2
7	*P. djamor*	62± 2	4±0 2	55± 2
8	*Pleurotus ostreatus*	47±1	39± 2	60± 3
9	*Rigidoporus ulmarius (RU)*	ND	ND	62± 4
10	*Tricholoma caligatum (TC)*	ND	ND	70±5

Note: All values are media of three replications ± standard error. ND: Not detected.

Results and Discussion

Screening of lignin degrading enzymes in Mushrooms

In the fresh mushroom extracts, laccase, manganese peroxidase and lignin peroxidase activities were screened. All substrate formulations were fully colonized by mycelia after 14 days of incubation (Table 1). As laccases oxidize various types of substrates, several different compounds have been used as indicators for laccase production. ABTS is a very sensitive substrate that allows a rapid screening of fungal strains production. Extracellular ABTS-oxidizing enzymes by means of a color reaction. Colonies that showed green halo on GAE medium containing ABTS that exceeded the colony diameter were considered as ABTS oxidizing activity producers. Enzymatic activities of 15 fungal strains were detected to screen their ligninolytic enzymes by using primary screening method on solid medium. The solid cultures inoculated by the fungal strains on GAE medium containing ABTS have yielded 10 positive strains which exhibited green halo formation after incubation.

The diameter of the halo and the color intensity indicating a positive extracellular oxidoreductases secretion from mycelium was used to screen the level of ligninolytic enzyme production of each strain. *R. ulmarius and T. caligatum* exhibited no green halo during the incubation period.

Screening of ligninolytic enzyme activities in liquid media

The highest level of Lac activity in strains was found in *Agaricus sp 1* (61.37 ± 0.22 U/l) at 27°C for 14 days, while lowest level of this enzyme was determined in *P. djamor* (2.40 ± 0.07U/l) at the same incubation period . The highest level of MnP activity in strains was found in *Pleurotus ostreatus* (174.63 ± 0.34 U/l) at 27°C for 14 days, while lowest level of this enzyme was determined in *Meripilus giganteus* (MG) (17.03 ± 0.13 U /l) at the same incubation period. The highest level of Lip activity in strains was found in *Agrocybe aegerita* (19.74 ± 0.13 U/l) at 27°C for 14 days, while lowest level of this enzyme was determined in *Pleurotus ostreatus* (0.71 ± 0.07 U /l) at the same incubation period (Table 2).

Conclusion

This study emphasizes the need to explore more organisms to evaluate the real potential of fungi producing ligninolytic enzymes. Target enzyme synthesis may play an important role in the development of an efficient technology. The results of the present study allow us to conclude that wild *Agrocybe aegerita* and *P. ostreatus* are good candidates for scale-up ligninolytic enzyme production.

Table 2. Maximum ligninolytic enzyme production in liquid culture.

Organisms	Laccase Activity	MnP Activity	Lip Activity
Agaricus sp 1	61.37 ± 0.22 (6)	166.69 ± 0.24 (11)	0.90 ± 0.07 (13)
Agrocybe aegerita	30.80 ± 0.51 (5)	70.38 ± 0.17 (16)	19.74 ± 0.13 (15)
Agrocybe sp.	41.70 ± 0.23(3)	52.29±0.11(13)	18.63±0.05(12)
Coprinus comatus	3.16 ± 0.07 (14)	27.65 ± 0.06 (15)	ND
Gloeophyllum trabeum (GT)	18.00 ± 0.15 (11)	24.43 ± 0.17(13)	3.53 ± 0.07 (15)
Meripilus giganteus (MG)	6.30 ± 0.05 (9)	17.03 ± 0.13 (11)	ND
P. djamor	2.40 ± 0.07 (7)	26.05 ± 0.15 (13)	ND
Pleurotus ostreatus	55.27 □□0.39 (9)	174.63 ± 0.34 (16)	0.71 ± 0.07 (13)
Rigidoporus ulmarius (RU)	ND	ND	ND
Tricholoma caligatum (TC)	ND	ND	ND

Note: All values are media of three replications ± standard error ND: not determined, Lac: Laccase, MnP: Manganase peroxidase, Lip: Lignin peroxidase

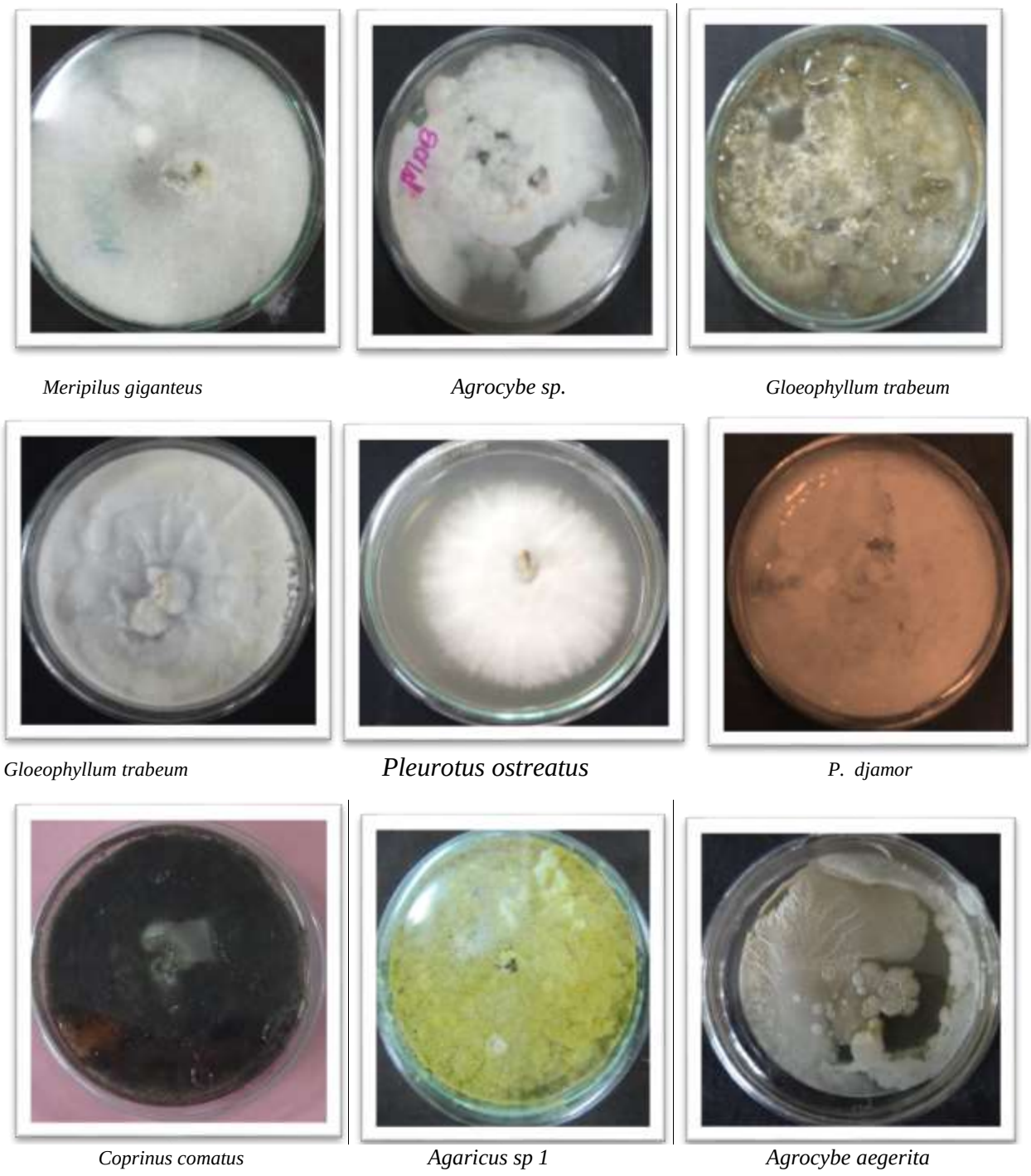

Fig. 2: Various enzyme producing fungal species showing clearing zone.

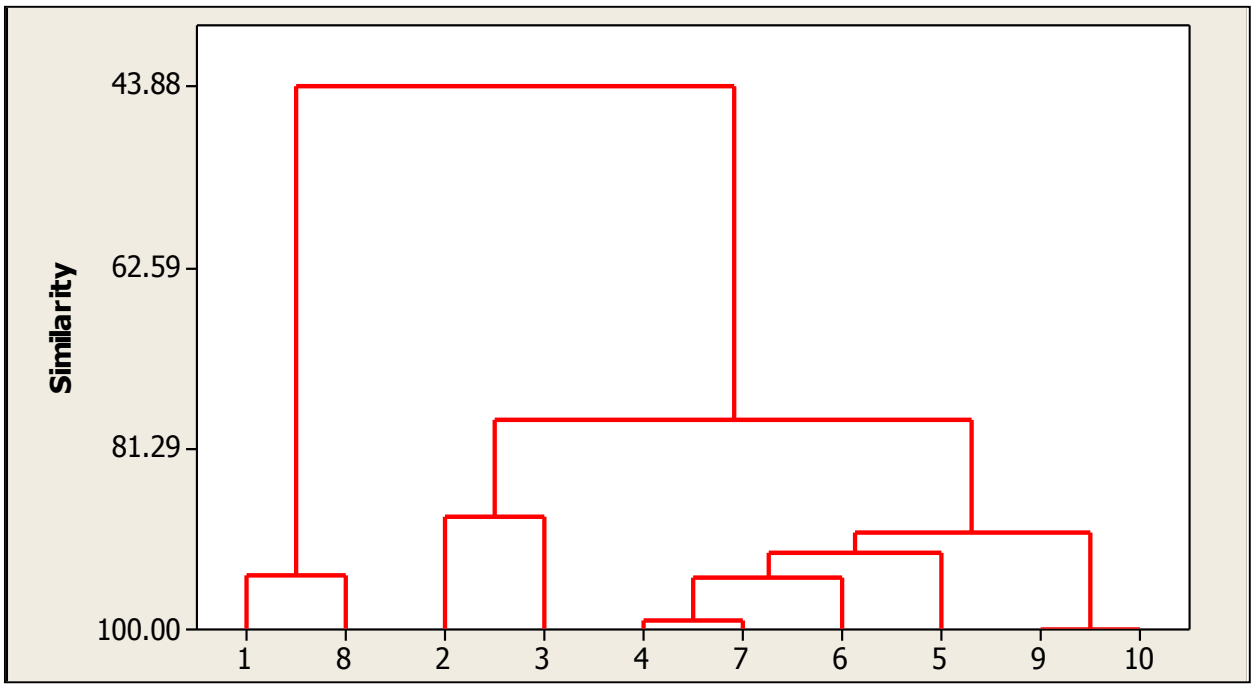

Fig. 3: Similarity of Laccase, Manganese peroxidase and Lignin peroxidase Activity SL no 1-10 is the organism name mentioned in Table no 1

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