

Isolation and partial purification of fungal ligninolytic enzymes from the forest soil fungi isolated from Bhadra Wildlife Sanctuary

SHIVAKUMAR P. BANAKAR (✉)¹, B. THIPPESWAMY²

¹ Department of Biosciences, Shri J.J.T. University, Vidyanaigari, Chudela-333 001 Jhunjhunu (Dist.), Rajasthan, India

² Department of Studies in Microbiology, Bioscience Complex, Kuvempu University, Jnanasahyadri, Shankaraghatta-577 451, Shivamogga (Dist.), Karnataka, India

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Abstract Screening was done for the isolation of effective lignin degraders from the forest soil samples, by providing lignin as a carbon source through the enrichment method, which leads to the isolation of 8 effective fungal isolates among 14 isolates. Submerged fermentation was done for the production of ligninolytic enzymes with the effective microorganisms by providing Guaiacol as a carbon source. The assay of laccase, lignin peroxidase activity and specific activity was done after the incubation intervals of 2, 4, 6, 7, 8, 10 and 12 days at $27 \pm 2^\circ\text{C}$ under shake culture condition. Partially purified protein content was estimated by using Lowry's method. *Pleurotus* sp. and *Phanerochaete chrysosporium* are more effective at the 2nd and 7th days of incubation for the production of laccase and lignin peroxidases among the effective isolates.

Keywords laccase, lignin peroxidase, extracellular enzyme, fungi, forest soil

Introduction

Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Wood is a complex of many types of chemicals whose concentrations vary among and within species. The chemical constituents of wood can be grouped into primary metabolites such as, soluble sugars, lipids, peptides, and the major storage compound, starch; cell wall components such as hemicellulose, cellulose and lignin; minerals and exclusive of extractable primary metabolites. One would therefore expect that the wood-inhabiting fungi would produce exoenzymes that solubilise these complex substrates to form breakdown products that can be taken up by the fungal cells and used for nutrition (Howard et al., 2003; Abdel-Raheem and Ali, 2004).

A diverse spectrum of lignocellulolytic microorganisms, mainly fungi (Falcon et al., 1995; Baldrian and Gabriel, 2003) and bacteria (Vicuna, 1988; Zimmermann, 1990) have been

isolated and identified over the years and this list still continues to grow rapidly. The main extracellular enzymes participating in lignin degradation are heme-containing lignin peroxidase, manganese peroxidase and Cu-containing laccase.

Only few organisms in nature belonging to the white-rot fungi are able to attack and destroy the lignin molecule. Both cellulose and lignin are rather rigid organic polymers which have been invented and optimized by nature during the evolution process for constructive and long-term preservation purposes. Harsh physicochemical conditions have to be applied to attack or modify these two compounds. Fungi, being ubiquitous in distribution, are highly successful in survival because of their great plasticity and physiologic versatility to secrete a wide array of enzymes involved in the breakdown of complex polymers (Carlsen and Nielsen, 2001).

A large number of microorganisms have been reported to produce ligninases, among which white rot fungi, belonging to the basidiomycetes are the most efficient and extensive lignin degraders (Gold and Alic, 1993; Akin et al., 1995; Wu et al., 2005). They have a powerful extracellular enzymatic complex, able to depolymerize this aromatic polymer into lower molecular weight compounds (Bajpai, 2004).

Several fungi are known to have the property of degradation PAHs like PCP, PCB, etc. The degradation of these compounds by ligninolytic fungi, including white-rot and litter-decomposing fungi has been intensively studied by various researchers. Wood and litter decay fungi are efficient degraders of these PAHs that were showed from the various investigations, they can mineralize PAHs with four and more condensed aromatic rings, in contrast to bacteria and soil fungi (Yu et al., 2011; Pozdnyakova, 2012).

Particularly *Phanerochaete chrysosporium* and *Pleurotus* sp. are considered to be the best having the desired qualities (Kerem et al., 1992), to produce copious amounts of a unique set of lignocellulytic enzymes (Howard et al., 2003). *Phanerochaete chrysosporium* is frequently investigated as a model organism for the study of ligninolytic enzymes since it was the first fungus found to produce lignin peroxidase and manganese peroxidase.

Phanerochaete chrysosporium has drawn a considerable attention as an appropriate host for the production of lignin-degrading enzymes or direct application in lignocellulose bioconversion processes (Bosco et al., 1999; Ruggeri and Sassi, 2003). Less known, white rot fungi such as *Daedalea flavida*, *Phlebia fascicularia*, *P. floridensis* and *P. radiata* have been found to selectively degrade lignin in wheat straw and hold out prospects for bioconversion biotechnology, where the aim is just to remove the lignin leaving the other components almost intact (Arora et al., 2002).

Lignocellulosic wastes are majorly generated by forestry and agricultural practices, paper-pulp industries, timber industries and many agro-industries and they pose an environmental pollution problem (Howard et al., 2003). Metalloenzymes like, lignin peroxidases (Lip) and laccases are secreted by filamentous fungi from the class Basidiomycetes, are the only known enzymes being able to degrade the recalcitrant plant cell wall constituent lignin in the environment (Piontek et al., 2001).

The laccases are multicopper phenol oxidases, which oxidize phenolic compounds to phenoxy radicals with oxygen as final electron acceptor. In the presence of a mediator such as ABTS or 1-hydroxybenzotriazole, laccases are capable of oxidation of nonphenolic compounds (Eggert et al., 1996a). Lignin peroxidases (LiPs) are heme-containing peroxidases, they catalyzes C–C bond cleavage in the propyl side chains of two dimeric model compounds, as well as in spruce and birch lignins. This cleavage is prominent in the fungal degradation of lignin and is the first reaction in the metabolism of dimeric models in cultures. The studies here reveal that the enzyme is a heme-containing oxygenase, unique in that it requires H₂O₂ (Tien and Kirk, 1988).

Ligninolytic microbial systems and their enzymes were more applicable to improve the digestibility and nutritive value of animal feeds, degradation of toxic pollutants and bioconversion of lignin into useful organic compounds and to increase the fermentability of lignocellulosic residues and also for the commercial production of biofuels from feed-

stock, paper pulp (Arora et al., 2002).

Present study was taken up to isolate, characterize and screen the more efficient ligninolytic fungi from forest soils for the production of laccase and lignin peroxidase under submerged fermentation at different time of incubation and they were partially purified for the industrial applications.

Materials and methods

Study area and sample collection

Forest soil sample was collected from Bhadra Wildlife sanctuary, organic soil sample was collected in a depth of 5–10 cm by random mixed sampling method in forest by removing upper litter layer. Bhadra Wildlife Sanctuary (492.46 sq. km) is a hot spot biologic diversity in the Western Ghats, with a wide range of tree vegetation such as dry and moist deciduous, semi-evergreen and evergreen forests (Champion and Seth, 1968).

Enrichment technique

Isolation of extracellular ligninolytic enzyme producing fungi was done, by stepwise nutrient enrichment technique from a lower concentration of lignin sulphonic acid as sole carbon to a higher (0.5% and 1%), in a 250 mL Erlenmeyer's flask containing 100 mL of Czapek Dox broth. Culture media flasks were inoculated with 1 g of forest soil sample to the lower concentration of carbon source aseptically. The flask was incubated in a shake culture condition of 120–160 rpm at 27±2°C for 7 days, after the incubation, 1 mL of inoculum was transferred to the fresh sterile Czapek Dox broth supplemented with 1% w/v lignin sulphonic acid and incubated for further 7 days by maintaining similar conditions (Patil and Chaudhari, 2010). After the incubation it was used as inoculum for the screening and isolation of effective ligninolytic fungi.

Screening and isolation of effective ligninolytic fungi

Selection and isolation of effective ligninolytic fungi were done by using 0.1 mL of inoculum from the enriched culture medium Malt extract agar and Czapek Dox agar plates, supplemented with 0.02% Guaiacol and 10–25 µg/mL of Tetracycline at pH of 6.0 and incubated at 28±1°C for 5–7 days (Coll et al., 1993). After the incubation fungal colonies were observed for the colored zone around and below surface of the colony and purified for further use.

Secondary screening was done for the selection of more potent ligninolytic colonies for the production of extracellular ligninase by culturing on Czapek Dox agar plates supplemented with 0.02% Guaiacol, at pH 6.0. Discs of actively growing myceliums (3 days old) were removed from the growing edge of the fungal isolates cultured on malt extract

agar by using sterile cork borer of 6 mm dia. and they were inoculated to the pre-welled Guaiacol agar plates and incubated at $28 \pm 1^\circ\text{C}$ for 7–12 days, after the incubation plates were observed for the colored zone around and below the surface of colony. The cultures which are not produced zones within 7 days were further incubated up to 12 days and discarded. The rate of formation of colony with colored zone was an indication of the fungal species for their effectiveness for the production of lignolytic enzymes.

Identification of ligninolytic fungi

The fungal species were grown on the Czapek Dox agar plates, identified based on Cultural and morphological characteristics. Morphological characteristics of fungi like, mycelium, reproductive structures, spore shape, color, size and its arrangements were studied under microscope by using Lactophenol cotton blue staining method and they were identified using standard identification manuals (Ellis, 1971, 1976; Pitt, 1979; Domsch and Gams, 1972; Ellis M B and Ellis J P, 1997; Gilman, 2001; Nagamani et al., 2006).

Submerged fermentation (SmF)

Cultures were grown in 250 ml Erlenmeyer flask containing 100 ml of production medium of pH 6.0, contains 0.2%, NaNO_3 ; 0.1%, K_2HPO_4 ; 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.05%, KCl ; 0.01%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 3%, Sucrose; 0.001%, ZnSO_4 ; 0.001%, CuSO_4 and 0.01 mL of guaiacol were used for laccase and lignin peroxidase enzyme production. After the sterilization of the Erlenmeyer flasks containing fermentation medium, cooled and discs of actively growing fungal myceliums of 3 day old culture on malt extract agar at growing edge were used to inoculate aseptically. Cultures were incubated in the incubator shaker operating 120–160 rpm at $28 \pm 1^\circ\text{C}$ for 7–12 days. Aseptically transferred 10 mL of incubated broth from the culture flasks at different days intervals of incubation (2, 4, 6, 7, 8, 10 and 12 days), mycelia and spores were harvested by centrifugation under 4°C at 10000 rpm for 10 min., the supernatants obtained from the centrifugations were carried to filtration through sterile whatman filter paper they were used as crude enzyme sources for assaying purpose.

Protein estimation

The protein concentration was determined by the Lowry's method (Lowry et al., 1951), by using bovine serum albumin (BSA) as a standard (0.2 mg/mL), absorbance was read at 660 nm using JENWAY-6305, UV-VIS Spectrophotometer and plotted the standard protein calibration curve. The culture filtrates from the culture flasks at different days of incubation were used as crude proteins and ammonium sulfate precipitated and dialyzed proteins were used as partially purified proteins and they were quantified.

Determination of laccase activity

Culture filtrates from the incubated shake culture flasks at different days intervals (2, 4, 6, 7, 8, 10 and 12 days), they were used as crude laccases enzyme sources, and activity was assayed using a method described by Coll et al. (1993) with slight modification. Preparation of 46 mM guaiacol solution was done in buffer (50 mM, pH 4.6) solution. Laccase activity was measured by taking the optical density of reaction mixture, prepared by mixing 0.5 mL of distilled water, 2 mL of sodium acetate buffer (50 mM, pH 4.6), 0.5 mL of substrate solution (46 mM guaiacol) and 0.5 mL of crude enzyme extract was added, by maintaining a blank solution containing above reaction mixture, added buffer instead of enzyme source. Absorbance was read at 440 nm up to 5 min. with 30 s of time interval using JENWAY-6305, UV-VIS Spectrophotometer. The laccase enzyme activities were calculated using an extinction coefficient of $25.5 \cdot \text{M}^{-1} \cdot \text{cm}^{-2}$ and expressed as one activity unit was defined as the amount of enzyme necessary to oxidize 1 μmol of substrate per minute or Katal (Bergmeyer, 1974; Das et al., 1997).

Determination of lignin peroxidase activity

Culture filtrates from the incubated shake culture flasks at different days intervals (2, 4, 6, 7, 8, 10 and 12 days), they were used as crude lignin peroxidase enzyme sources, and activity was assayed (Bergmeyer, 1974; Sarkanen et al., 1991). Lignin peroxidase activity was measured by taking the optical density of reaction mixture, prepared by mixing 2 mL of sodium acetate buffer (50 mM, pH 4.6), 0.5 mL of guaiacol solution (46 mM), 0.5 mL of crude enzyme extract and 0.5 mL of substrate solution of H_2O_2 (12.3 mM) was added, by maintaining a blank solution containing above reaction mixture, added buffer instead of enzyme source. Absorbance was read at 440 nm up to 5 min. with 30 s of time interval using JENWAY-6305 UV-VIS Spectrophotometer. The lignin peroxidase enzyme activities were calculated using an extinction coefficient of $25.5 \cdot \text{M}^{-1} \cdot \text{cm}^{-2}$ and expressed as one activity unit, it was defined as the amount of enzyme necessary to oxidize 1 μmol of substrate (H_2O_2) per minute or Katal.

Partial purification of enzymes

Culture filtrates after the incubation of 12th day, the crude enzyme was precipitated with different concentrations of ammonium sulfate up to the saturation level from 20%–100%, kept under magnetic stirrer at 4°C for 24 h, the protein precipitate was collected by refrigerated centrifugation at 10000 r/min for 10 min. The pellet was suspended in sodium acetate buffer of pH 4.6 and dialyzed against 2–3 changes of buffer during the process under magnetic stirrer at 4°C for 24 h. An aliquot of partially purified proteins were quantified (U/mL) (Hara et al., 1984).

Results

Isolation and identification of effective ligninolytic fungi

Screening and Isolation of ligninolytic fungi was done from the inoculums of forest soil samples inoculated with nutrient enriched culture on the CDA and Malt extract agar plates and cultural and morphological characteristics of the isolates were examined under microscope. The morphological characters were studied under microscope by using lactophenol cotton blue and identified as *Chaetomium globosum*, *Trichoderma* sp., *Theliophora terristrus*, *Humicola* sp., *Talaromyces* sp., *Pleurotus* sp., *Trametes versicolor*, *Trametes hirsute*, *Phenerochaetae sordida*, *Lenzitis betulina*, *Pleurotus ostreatus*,

Stereum ostrea, *Phenerochaetae chrysosporium* 1. and *Phenerochaetae chrysosporium* 2 with the help of standard fungal identification manuals. The isolated strains were further screened for qualitative ligninolytic activity, based on the rate of zone of red or brown red coloration around the fungal colony (Fig. 1). The more efficient eight ligninolytic fungal strains were selected among the others like, *Chaetomium globosum*, *Humicola* sp., *Talaromyces* sp., *Pleurotus* sp., *Trametes versicolor*, *Trametes hirsuta*, *Pleurotus ostreatus* and *Phenerochaetae chrysosporium* 1. (Table 1), and they were used for extracellular laccase and lignin peroxidase enzyme production studies under submerged fermentation.

Culture filtrates of fungal extracellular crude enzyme preparations were assayed for laccase and lignin peroxidase

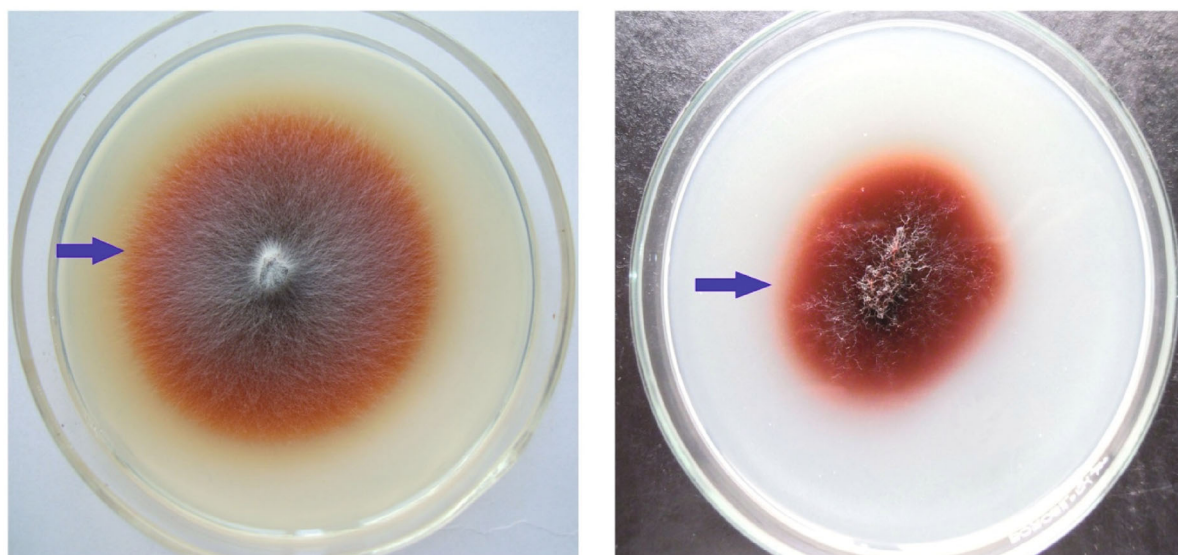


Figure 1 Ligninolytic activity of fungi on CDA plates supplemented with guaiacol as substrate.

Table 1 Screening of extracellular ligninolytic fungi from forest soil

Sl. No.	Fungal isolates	Lignolytic activity (dia. in mm)	
		Colony	Clear zone
1	<i>Chaetomium globosum</i>	55	45
2	<i>Trichoderma</i> sp.	33	33
3	<i>Theliophora terristrus</i>	60	60
4	<i>Humicola</i> sp.	25	25
5	<i>Talaromyces</i> sp.	90	70
6	<i>Pleurotus</i> sp.	62	60
7	<i>Trametes versicolor</i>	70	65
8	<i>Trametes hirsuta</i>	80	70
9	<i>Phenerochaetae sordida</i>	20	6
10	<i>Lenzitis betulina</i>	22	8
11	<i>Pleurotus ostreatus</i>	45	10
12	<i>Stereum ostrea</i>	46	12
13	<i>Phenerochaetae chrysosporium</i> 1.	45	45
14	<i>Phenerochaetae chrysosporium</i> 2.	43	43

enzyme activity at different days of incubation (2, 4, 6, 7, 8, 10, and 12 days) and they were showed varied levels of enzyme activities among the species investigated. All eight investigated species were showed very high laccase and lignin peroxidase activities at the 2nd and 4th day of incubation, then gradually decreases at the 6th day of incubation and further showed slighter variations up to the 10th day of incubation, that showed by a linear curve.

Laccase activity

Culture filtrates were assayed for laccase activity, the following fungal species showed different rate of activities under assay conditions. Maximum laccase activity was showed by *Pleurotus* sp. (0.159 IU/mL) at the 2nd day of incubation followed by *P. chrysosporium* 1. (0.141 IU/mL), *C. globosum* (0.057 IU/mL), *P. ostreatus* (0.029 IU/mL), *Trametes hirsute* (0.027 IU/mL), *T. versicolor*, (0.025 IU/mL), *Talaromyces* sp. (0.021 IU/mL) and *Humicola* sp. (0.011 IU/mL) respectively and at the 4th day of incubation was by *Pleurotus* sp. (0.117 IU/mL), *P. chrysosporium* 1. (0.074 IU/mL), *T. versicolor* (0.049 IU/mL), *C. globosum* (0.04 IU/mL), *Talaromyces* sp. (0.03 IU/mL), *Trametes hirsute* (0.029 IU/mL), *P. ostreatus* (0.02 IU/mL), and *Humicola* sp. (0.01 IU/mL) respectively and at the 7th day of incubation was by *P. chrysosporium* 1. (0.042 IU/mL) followed by *T. versicolor* (0.041 IU/mL), *Pleurotus* sp. (0.032 IU/mL), *P. ostreatus* (0.030 IU/mL), *C. globosum* (0.029 IU/mL), *Talaromyces* sp. (0.027 IU/mL), *T. hirsute* (0.015 IU/mL) and *Humicola* sp. (0.014 IU/mL) respectively.

Specific activity of laccase was maximum by *P. chrysosporium* 1. (0.673 IU/mg) followed by *Pleurotus* sp. (0.419 IU/mg), *T. versicolor* (0.075 IU/mg), *C. globosum* (0.033 IU/mg), *Talaromyces* sp. (0.028 IU/mL), *P. ostreatus* (0.023 IU/mg), *T. hirsute* (0.017 IU/mL) and *Humicola* sp. (0.012 IU/mL), respectively at the 7th day of incubation (Figs. 2A and 3), (Table 2).

Lignin peroxidase activity

Culture filtrates were assayed for lignin peroxidase activities, the following fungal species showed different rate of activities under assay conditions. Maximum laccase activity was showed by *Pleurotus* sp. (0.164 IU/mL) at the 2nd day of incubation followed by *P. chrysosporium* 1. (0.119 IU/mL), *T. versicolor* (0.026 IU/mL), *Trametes hirsute* (0.022 IU/mL), *C. globosum* (0.02 IU/mL), *P. ostreatus* (0.018 IU/mL), *Talaromyces* sp. (0.015 IU/mL) and *Humicola* sp. (0.014 IU/mL) respectively and at 4th day of incubation was by *Pleurotus* sp. (0.109 IU/mL) followed by *P. chrysosporium* 1. (0.054 IU/mL), *T. versicolor* (0.046 IU/mL), *C. globosum* (0.037 IU/mL), *Trametes hirsute* (0.035 IU/mL), *Talaromyces* sp. (0.023 IU/mL), *P. ostreatus* (0.011 IU/mL) and *Humicola* sp. (0.007 IU/mL), respectively and at 7th day of incubation was by *T. versicolor* (0.042 IU/mL) followed by *P.*

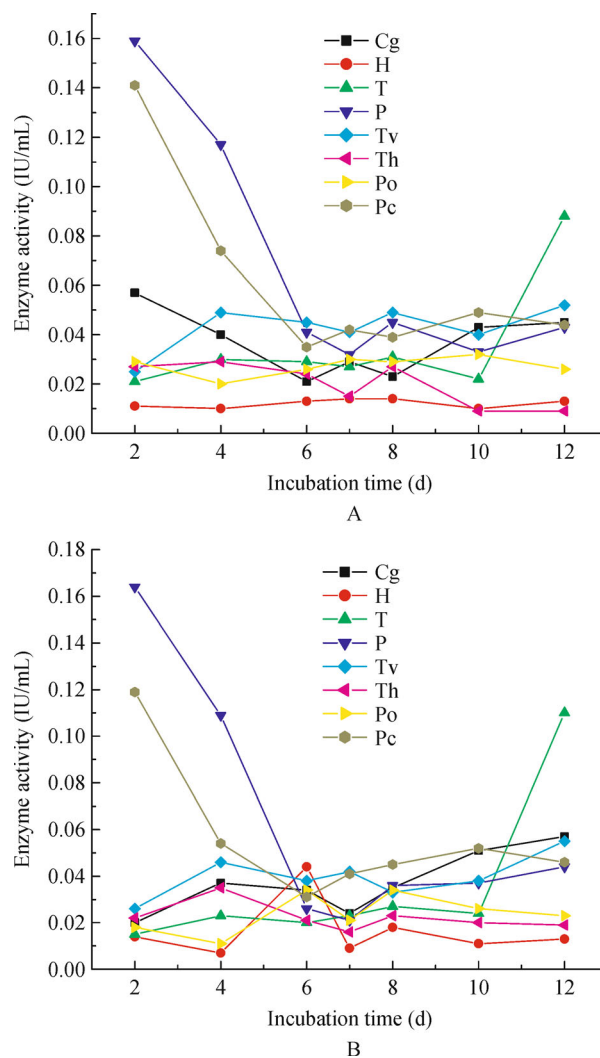


Figure 2 Fungal laccase (A) and lignin peroxidase (B) activity at different days of incubation. Cg–*Chaetomium globosum*, H–*Humicola* sp., T–*Talaromyces* sp., P–*Pleurotus* sp., Tv–*Trametes versicolor*, Th–*Trametes hirsute*, Po–*Pleurotus ostreatus* and Pc–*Phenerochaetae Chrysosporium* 1.

chrysosporium 1. (0.041 IU/mL), *C. globosum* (0.024 IU/mL), *Talaromyces* sp. (0.023 IU/mL), *Pleurotus* sp. and *P. ostreatus* (0.021 IU/mL), *T. hirsute* (0.016 IU/mL) and *Humicola* sp. (0.009 IU/mL), respectively.

Specific activity of laccase was maximum by *P. chrysosporium* 1. (0.655 IU/mg), followed by *Pleurotus* sp. (0.275 IU/mg), *T. versicolor* (0.077 IU/mg) and *C. globosum* (0.028 IU/mg), *Talaromyces* sp. (0.023 IU/mL), *T. hirsute* (0.019 IU/mL), *P. ostreatus* (0.016 IU/mL) and *Humicola* sp. (0.007 IU/mL), respectively at the 7th day of incubation (Figs. 2B and 3), (Table 2).

Protein content

Soluble crude proteins were more in *Pleurotus ostreatus* (1308 µg/mL) followed by *Humicola* sp. (1192 µg/mL),

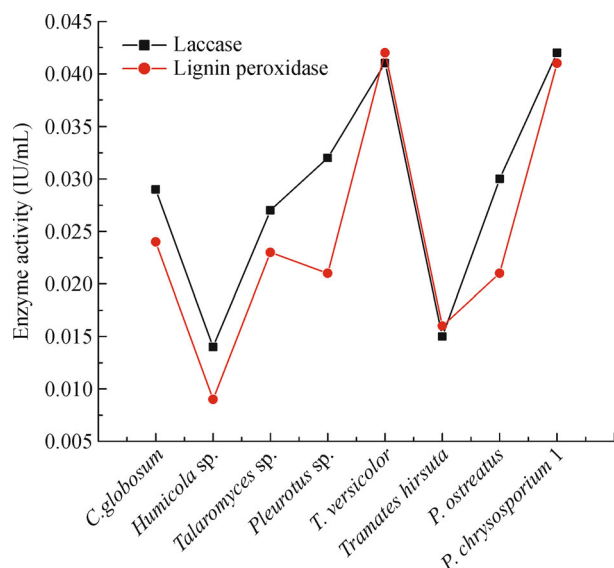


Figure 3 Fungal laccase and lignin peroxidase activity at the 7th day of incubation.

Talaromyces sp. (992 µg/mL), *Tramates hirsute* (890 µg/mL), *Chaetomium globosum* (868 µg/mL), *Tramates versicolor* (540 µg/mL), *Pleurotus* sp. (76 µg/mL) and *Phenerochaetae chrysosporium* 1. (62 µg/mL), they were obtained from the culture filtrates of fungal species grown at the 7th day of incubation (Fig. 4) (Table 2).

Partially purified proteins were obtained from the fungal culture filtrates at the 7th day of incubation was maximum by *Talaromyces* sp. (300 µg/mL), *Humicola* sp. (250 µg/mL), *Pleurotus ostreatus* (240 µg/mL), *Tramates hirsute* (200 µg/mL), *Tramates versicolor* (178 µg/mL), *Chaetomium globosum* (28 µg/mL), *Phenerochaetae chrysosporium* 1. (26 µg/mL) and *Pleurotus* sp. (24 µg/mL), respectively (Table 2).

Discussion

The present study showed that forest soil is rich source of

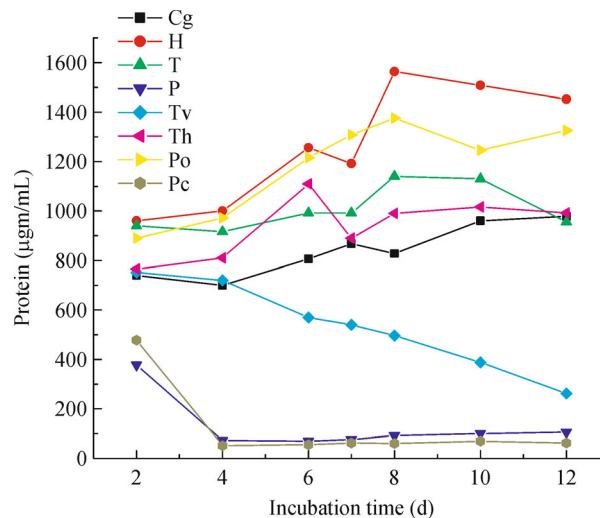


Figure 4 Fungal soluble crude ligninase protein content at different time of incubation. Cg–*Chaetomium globosum*, H–*Humicola* sp., T–*Talaromyces* sp., P–*Pleurotus* sp., Tv–*Tramates versicolor*, Th–*Tramates hirsute*, Po–*Pleurotus ostreatus* and Pc–*Phenerochaetae Chrysosporium* 1.

fungal diversity, especially ligninolytic fungal species, those were played an important role in the biogeochemical cycles in the environment. The indigenous fungi from the forest soils were more potent for the production of industrially important extracellular laccase and lignin peroxidase enzymes than the others.

Ligninases are the extracellular enzymes, they were produced by fungus during secondary metabolism, brought about by nutrient starvation (Tien and Kirk, 1988). High ligninase activities were initiated only at third–fourth day of incubation but not during exponential growth phase (Xavier et al., 2007).

Fungi, belonging to ascomycetes, deuteromycetes, and basidiomycetes, are known to produce laccases of ecological as well as biotechnological importance, such as biodegradation and bioremediation. In addition, laccases are also responsible for various physiologic functions in fungi. Due to their broad specificity toward substrate, they can oxidize a

Table 2 Laccase and Lignin peroxidase activity, specific activity and protein content at the 7th day of incubation

Sl. No.	Fungal species	Laccase*		Lignin peroxidase*		Protein content (µg)*	
		Activity (IU/mL)	Specific activity (IU/mg)	Activity (IU/mL)	Specific activity (IU/mg)	Crude (µg/mL)	Partially purified (µg/mL)
1	<i>Chaetomium globosum</i>	0.029±0.003	0.033±0.003	0.024±0.004	0.028±0.002	868±2	28±2
2	<i>Humicola</i> sp.	0.014±0.002	0.012±0.002	0.009±0.001	0.007±0.001	1192±5	250±3
3	<i>Talaromyces</i> sp.	0.027±0.003	0.028±0.003	0.023±0.003	0.023±0.003	992±4	300±6
4	<i>Pleurotus</i> sp.	0.032±0.002	0.419±0.006	0.021±0.001	0.275±0.005	76±3	24±2
5	<i>Tramates versicolor</i>	0.041±0.003	0.075±0.005	0.042±0.002	0.077±0.004	540±5	178±3
6	<i>Tramates hirsute</i>	0.015±0.002	0.017±0.002	0.016±0.003	0.019±0.002	890±4	200±4
7	<i>Pleurotus ostreatus</i>	0.030±0.001	0.023±0.003	0.021±0.002	0.016±0.003	1308±5	240±2
8	<i>Phenerochaetae chrysosporium</i> 1.	0.042±0.003	0.673±0.008	0.041±0.004	0.655±0.005	62±2	26±1

* Mean of three replicates±standard deviation (SD)

range of chemical compounds leading to various industrial applications (Dhakar and Pandey, 2013).

In the present study was conducted and reported about the laccase and lignin peroxidase enzymes from the indigenous fungal species like, *Phanerochaete chrysosporium* 1., *Trametes versicolor*, *Pleurotus* sp., *Pleurotus ostreatus* and *Chaetomium globosum* are more effective when compared to the other species. The major enzymes associated with lignin-degrading ability are lignin peroxidase, manganese peroxidase and laccase. Some fungi produce all these enzymes while others produce only one or two of them (Xavier et al., 2007). *Trametes versicolor* is a basidiomycete that produces three ligninolytic enzymes and it has an efficient degradation capacity of lignin, polycyclic aromatic hydrocarbons, a polychlorinated biphenyl mixture and a number of synthetic dyes (Tanaka et al., 1999; Novotny et al., 2004).

Enzyme production patterns differ between the species. *Phanerochaete chrysosporium* has not been reported to produce laccase, although other selective degraders of wood produces a combination of MnP and laccase like, *Pleurotus ostreatus* (Giardina et al., 1996), *Pleurotus eryngii* (Muñoz et al., 1997) and *Dichomitus squalens* (Périeré et al., 1998) and a combination of MnP, LiP and laccase by *Phlebia radiata* (Lundell and Hatakka, 1994) or only laccase by *Pycnoporus cinnabarinus* (Eggert et al., 1996b). *P. radiata* produces at least 3 LiPs, 3 MnPs and one laccase (Moilanen et al., 1996).

Laccases are interesting proteins for industrial applications because extensive studies have shown the potential of fungal phenol oxidases as a biological alternative for chemical oxidative processes, e.g. phenol removal from waste water, pulp bleaching and ethanol production (Xavier et al., 2007). The extracellular lignin peroxidase from the white rot basidiomycete *Phanerochaete chrysosporium* is thought to play an important role in lignin biodegradation. However, the majority of lignin-derived preparations actually experience overall polymerization at the hands of the enzyme in vitro (Sarkanen et al., 1991).

The white rot fungi in the forests and reported 20 three isolates of white-rot fungi and they were tested for their efficiency of wood degradation based on guaiacol-oxidation, lignin peroxidase, laccase and manganese peroxidase activities (Metuku et al., 2011). Their main purpose of testing was for the selection of the best isolates that show high efficiency of producing ligninolytic enzymes. From their overall observations, they identified KU1009 (*Pycnoporus* sp. DIS 343f) strain was as the most superior for lignin degradation among the other isolates.

Enhancement of laccase production, by modifying the nutritional and physiologic conditions during cultivation of promising fungi, is a prerequisite for their optimum utilization at industrial scale (Dhakar and Pandey, 2013). Laboratory studies have found that increased concentrations of inorganic N can suppress the transcription of fungal genes required for

the metabolism of lignin and lignocellulose (Eisenlord et al., 2013).

Some studies reported about the biodegradation of PCP in soil, water and some bioreactors by *P. chrysosporium* and the application of inoculation with *P. chrysosporium* to treat hazardous wastes has showed to be effective in PCP degradation and remediation of PSW at laboratory-scales but less information is available about the effect of inoculation, due to lack of further research (Yu et al., 2011).

There is not much information published on the influence of pH on laccase production, but most reports indicated that initial pH levels has to be set between pH 4.5 and pH 6.0 prior to inoculation, the levels were not controlled during most cultivation (Thurston, 1994; Gochev and Krastanov, 2007). The optimal temperature for laccase production is between 25°C and 30°C, when cultivated fungi at temperatures higher than 30°C the activity of ligninolytic enzymes was reduced (Pointing et al., 2000; Zadrazil et al., 1999).

Ligninases are most active at pH below 3, they are not very stable, and thus reaction rates are linear only for about 2 min. The ligninase is also inactivated by H₂O₂ in the absence of a reducing substrate, such as veratryl alcohol (Kerem et al., 1992). Lignin peroxidases and laccases have great potential for the application in various industrial processes, due to because of their high redox potentials and enlarged substrate range in the presence of specific mediators (Piontek et al., 2001).

One of the principal problems with many of the fungi, which perform well under laboratory conditions, is that they fail to compete well in natural soil environments. This provides an impetus to isolate new strains directly from polluted sites that may have greater potential for industrial application (Dritsa et al., 2007).

Peroxidase and laccase catalyzes the substrate oxidation by a mechanism involving free radicals and showing low substrate specificity. These ligninolytic enzymes are directly involved in the degradation of various xenobiotic compounds, including industrial dyes, polycyclic aromatic hydrocarbons, pesticides, dioxins, chlorophenols, explosives and kraft pulp bleaching (Metuku et al., 2011).

Conclusions

Different studies were reported about the ligninase enzyme activity, very less reports are available about the isolation of potential ligninase producers from the forest soils. The present study was followed for the isolation and screening of potential fungi for the production of industrially important ligninases from the forest soil samples. From the above study researcher came to know the white rot fungi like, *Phanerochaete chrysogenum* 1. and *Talaromyces versicolor* are having more potential than the other type of fungi for the production of laccases and lignin peroxidases.

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Compliance with ethics guidelines

SHIVAKUMAR P. BANAKAR and B. THIPPESWAMY declare that they have no conflict of interest in the publication. This article does not contain any studies with human or animal subjects performed by any of the authors.

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