African Journal of Microbiology Research Vol. 6(36), pp. 6545-6550, 20 September, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.476
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Identification and characterization of a fungal strain with lignin and cellulose hydrolysis activities

Ran Jin[#], Hongdong Liao[#], Xuanming Liu, Mang Zheng, Xianqiu Xiong, Xinwu Liu, Liyong Zhang and Yonghua Zhu*

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Biology, Hunan University, Changsha, China.

Accepted 3 September, 2012

A lignin and cellulose-degrading fungal strain Bio-1 was isolated from the soil of Yuelu Mountain in China. It was identified as a member of the genus *Cladosporium* by 18s rDNA, ITS sequences analysis and morphological characters. Bio-1 could hydrolyze at least 35% of the alkaline lignin in 4 days when incubated in screening liquid medium. Both laccase and cellulase activities could be detected in the fermentation liquid and cell homogenate. High extracellular and intracellular laccase activities of 3800 and 5352 U/L were obtained with copper induction, respectively. The extracellular cellulase production was reached (51 U/Ml). Based on these characteristics, Bio-1 could be well suited as a commercial fungus applied in degradation of lignocellulose biomass for environment protection.

Key words: *Cladosporium*, identification, laccase, cellulase, $CuSO_4$.

INTRODUCTION

Paper and pulp, textiles and petrochemical industries discharge highly colored effluents, which is not only aesthetically unacceptable but also leads to serious environment problems in soil, ground or surface water ecosystem. The color of effluent is mainly due to the presence of lignocellulose and its derivates. Application of biological methods based on fungi biodegradation is an environmentally friendly process to reduce pollution caused by lignocelluloses-containing material (Raghukumar et al., 2008).

As an efficient and typical lignin-degrading fungi (Rajeev et al., 2005), the white-rot basidiomycete attacks lignin mainly by secreting lignin-degrading isoenzymes, such as laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP) (Rajeev et al., 2005). Similarly, four laccase isoenzymes were synthesized by a *Pleurotus ostreatus* strain V-184 (Rajeev et al., 2005). *Daedalea quercina* was reported to produce the laccase and Mn-dependent peroxidase (Rajeev et al., 2005). Laccases are the most preferred enzyme and have attracted increasing scientific attention in recent years due to their ability to degrade a wide range of lignin related compounds, which make them very useful for their application in diverse industrial sectors, including decolourizing and degrading industrial dyes (Rodriguez-Couto and Toca-Herrera, 2006).

The cellulases have received tremendous attention from researchers over the last few decades due to their importance in several lignocellulose-based agricultural and waste treatment processes and could be widely used to improve environmental quality and a sustainable energy resource supply (Zhang et al., 2006). By far, *Trichoderma reesei*, *Humicola*, *Penicillium* and *Aspergillus* are generally studied fungi that could convert native or derived cellulose to glucose (Jahangeer et al., 2005). Interestingly, white-rot fungi are also a common fungi that could secrete cellulases such as *Phlebia gigantean* (Niranjane et al., 2007) and *Ischnoderma resinosum* (John, 1986), which also have the capacity to remove lignin.

Complex compounds such as cellulose, lignin and lignocelluloses are main components of colored effluent. As a result, microorganisms which could degrade both lignin and cellulose appear to be appropriate in the biological treatment of effluents (Ezeronye and

*Corresponding author. E-mail: zyh20@hotmail.com. Tel: +86 731 88821565. Fax: +86 731 88822606.

[#]Authors contributed equally to this work.

Table 1. Sequences of the primers.

Primer	Sequence
18sf	5'-AACCTGGTTGATCCTGCCAGT-3'
18sr	5'-CGACGGGCGGTGTGTAC-3'
ITS4	5'-TCCTCCGCTTATTGATATGC-3'
ITS5	5'-GGAAGTAAAAGTAACAAGG-3'

Okerentugba, 1999) and play an important role in the cycle of carbon (Sanche, 2009). Several fungal species were reported on the capacity to reduce chemical oxygen demand (COD) because of the capacity to degrade high-molecular weight polymers (Prasad and Gupta, 1997). However, in addition to white-rot fungi (Baldrian, 2004; Leonowicz et al., 1999), only a few fungi are reported to secrete both lignin and cellulose degrading enzymes (Sanche, 2009). In this study, one fungal strain Bio-1 isolated from the soil was found to produce both laccase and cellulase. $CuSO_4$ could highly improve its laccase activity. Considering the importance of the lignocelluloses-degrading process, Bio-1 has considerable application potential in the lignocellulose treating industry.

MATERIALS AND METHODS

Screening of the lignocellulose-degrading strain

The lignocellulose-degrading fungus was screened using the screening medium at 28°C for 7 days from the strains which were isolated from Yuelu mountain soil in Hunan Province, China and preserved in our laboratory. The screening medium consists of (g/L): alkaline lignin 2, $(NH)_2SO_4$ 2, K_2HPO_4 1, $MgSO_4$ 0.2, $CaCl_2$ 0.1, $MnSO_4$ 0.02, KH_2PO_4 1, agar 15, and the pH of the medium was adjusted to 7.0. Then, the mycelia of survivor strains were incubated on the MEA medium (g/L): malt extract powder 20, glucose 20, peptone 1, agar 15, to be subcultured.

Identification of the strain

The isolated fungus was maintained on MEA medium plate at 28°C for 4 days and then transferred to liquid MEA medium. After incubation at 28°C for 7 days with shaking, the fruit bodies of the strains were collected. Genomic DNA was extracted using conventional methods and was then used as template in PCR with primers listed in Table 1. 18s rDNA gene was amplified with primers 18sf and 18sr (Daxboeck et al., 2004). ITS sequence was amplified with primers ITS4 and ITS5. The PCR reaction consisted of 30 cycles: denaturing for 40 s at 90°C, annealing at 56°C for 40 s, amplification at 72°C for 1 min. An initial denaturing step of 4 min at 94°C, and a unique final step of amplification at 72°C for 5 min were both included. The PCR products were analyzed on a 1.0% agarose gel and were purified using the "TIANgel Mini Purification" kit (Qiagen, Beijing) following the manufacturer's instructions. The products were sequenced in Sangong Biotech (Shanghai, China).

Analysis of alkaline lignin degradation capability

The fungus was maintained on MEA medium plate at 28°C for 4 days and then spores were collected. Conidial suspension at the concentration of 10^9 per milliliter was incubated at 28°C with screening liquid medium mentioned above with 7 g/L glucose in it to induce growth with shaking. To detect the lignin degradation rate, absorbance measurements at 280 nm were monitored every 2 days until the tenth day (Lundquist et al., 1977). According to the positive relationship between the concentration and absorbance, we could calculate the concentration of the alkaline lignin.

Analysis of the enzyme activity

The presence of laccase was rapidly detected by laccase detection medium (LDM) which consists of (g/L): glucose 10, KH_2PO_4 2, $MgSO_4.7H_2O$ 0.5, $CaCl_2$ 0.1, ammonium tartrate 0.5, 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) 0.1, agar 15 with the pH 5.0. The fungal mycelia were inoculated in LDM plate and incubated at 28°C. The strain that produced dark-green zone on the plate indicated the presence of laccase. MnP was detected by LDM containing 0.1 g/L $MnCl_2.4H_2O$ instead of ABTS. The formation of black and dark-brown flecks of manganese oxide (MnO_2) indicates the existence of MnP (Steffen et al., 2000). LiP was detected by LDM containing 0.1g/L Azure B instead of ABTS. The plate that has clearance of the blue coloured medium suggests the presence of LiP (Pointing, 1999).

Laccase activity was determined by measuring the absorbance of ABTS at 420 nm ($\varepsilon = 3.6 \times 10^4$ cm $^{-1}$ M^{-1}) at 28°C. Conidial suspension was incubated in Czapek's medium and the fermentation solution and intracellular solution were taken for extracellular and intracellular enzyme activities detection, respectively. The culture fluid was centrifuged at 8000 rpm and the supernatant was collected as extracellular fermentation solution. The fruit bodies were ground in a mortar and re-suspended with 0.1 nM Tris-HCl. Then, the suspension was centrifuged at 10000 rpm and the supernatant was collected as intracellular solution. The assay mixture in a total volume of 3 mL contains 30 µL of 100 mM ABTS and 100 µL aliquots of appropriately diluted culture fluid and tartaric acid buffer (pH 3.0). One unit of laccase activity is defined as the amount of enzyme required to oxidize 1 µmol ABTS per minute (Srinivasan et al., 1995). Activities of MnP and LiP were measured following the method of Gao et al. (2011). The activities were expressed in U/L.

Cellulase detection medium (CDM) was used for cellulase determination, which composed of (g/L): $MgSO_4$ 0.5, $(NH_4)SO_4$ 4, KH_2PO_4 1, NaCl 1, CMC-Na 1, agar 15. After incubated at 28°C for 3 days, the plate was flooded with 0.1% Congo red for 15 min and then with 1 M NaCl twice (each for 5 min). Stain that produced clear zone on the plate indicated the presence of cellulase (Catcheside et al., 2003). The xylanase was detected by CDM containing 1.0% birchwood xylan instead of CMC-Na.

The fermentation solution and intracellular solution for cellulase activity detection was obtained using the method above and assayed in 2.5 mL reaction mixture containing 1% CMC-Na in citrate buffe, pH 5.0 and appropriately diluted enzyme solution. After incubation at 50°C for 30 min, the reaction was stopped by adding 2.5 mL Dinitorsalycilic acid solution and immersing the tube in boiling water for exactly 5 min. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 µmol of glucose from the appropriate substrate under the standard conditions (Ghose, 1987). The activity was expressed in U/mL. All values were the means of at least three replicates.

RESULTS AND DISCUSSION

Identification of the strain

As alkaline lignin is a by-product in the pulp and paper

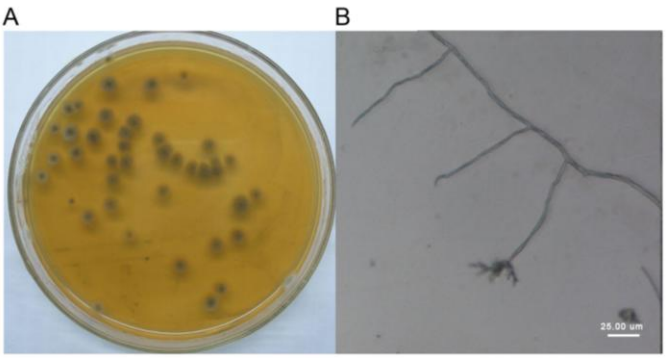

Figure 1. Morphological characteristics of Bio-1. (A) Colonies grown in screening medium at 27°C for three days. (B) Mycelia and fruit bodies under inverted microscope for magnifications of 40 times.

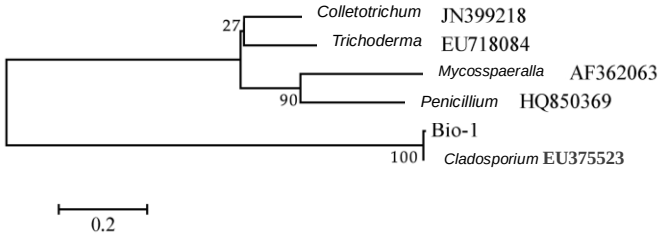

Figure 2. Phylogenetic tree of 18s rDNA of strain Bio-1. Branch lengths were drawn to scale, with the scale bar indicating the amount of divergence.

industry and contains significant similar structure to natural lignin, in this paper, the screening medium, containing alkaline lignin as the sole carbon source, was used for screening lignin-degrading fungi. One strain, named Bio-1, could grow well on the screening medium at 28°C. The basic shape of the Bio-1 colony is circular with entire edge and wrinkled surface, the colour of the fruit bodies is dark-green (Figure 1).

Total DNA was extracted from Bio-1 and amplified with the primers of 18 s and ITS, respectively. Two single bands of 1564 and 538 bp were obtained. Purified PCR products were sequenced and the results were compared with sequences in the GeneBank database by using BLAST (NCBI). The 18s rDNA sequence of Bio-1 has 99% similarity with *Cladosporium cladosporioides* EU375523.1. The phylogenetic tree of 18s rDNA was gotten by neighbor-joining using CLUSTAL X and the neighbor-joining tree is shown in Figure 2. The same result (99% similarity) was found from ITS sequence analysis. The morphological characteristics of Bio-1 were also similar with genus *Cladosporium* (Deacon, 1997). These results showed that Bio-1 belonged to the genus *Cladosporium*. The *Cladosporium* fungus has been found to have the capacity to biodegrade some aromatic compounds. Halaburgi et al. (2011) has purified a thermostable laccase from a strain of *C. cladosporioides*. According to Abrha and Gashe (1992), when grown in shaking-culture with medium containing carboxymethylcellulose, a *Cladosporium* species could produce cellulase components.

Degradation of the alkaline lignin

Based on the fact that lignin consists of large group of aromatic polymers with a characteristic absorption band at 280 nm, a simple lignin content detection method was used to measure the absorbance of medium at 280 nm. Proteins could contribute to the total amount of the absorbance value at 280 nm, which interfered with the results of the measurement. However, the content of the proteins in the fermentation broth in the first few days after inoculation was very low (data not shown), and the concentration of lignin was relatively high. Proteins contributed little to the absorbance values at 280 nm. Therefore, decrease of absorbance value at 280 nm reflected the degradation capacity of Bio-1 to a certain extent. A decreasing trend of the alkaline lignin was observed (Figure 3) and the minimum value of the alkaline lignin concentration was on the fourth day. This suggested that Bio-1 has secreted lignin-degrading enzyme after inoculation and 35% of the alkaline lignin could be hydrolyzed by Bio-1. With the process of lignin degrading, more aromatic units which were wrapped in the center of lignin structure were released, and some carbohydrates also have absorption at 280 nm. Meanwhile, with the increasing incubation time, proteins such as lignocelluloses degrading enzymes, which also had absorbance at 280 nm, were accumulated in the fermentation broth. These might be reasons why absorbance at 280 nm was slightly enhanced after the fourth day.

Rolz and colleagues (1987) showed that twelve tested white-rot fungi could decrease 38.64% of lignin after fermentation of sugarcane chips for 5 to 6 weeks at 20°C (Rolz et al., 1987). Considering that Bio-1 decreased 35% of the lignin only in the fourth day after inoculation, Bio-1 has great development potential in degrading lignin.

Assay of the lignin-degrading enzymes activity

To reflect the degradation capacity of Bio-1 more accurately, the detection of lignin-degrading enzyme activities was performed. The presence of laccase, LiP and MnP were detected to ensure which lignin-degrading enzyme was secreted by Bio-1. For visual demonstration of the presence of laccase, a simple rapid assay was used by incubating the mycelia on the LDM plate at 28°C. A dark-green zone was observed in the medium on the third day (Figure 4A), which suggested that Bio-1 showed

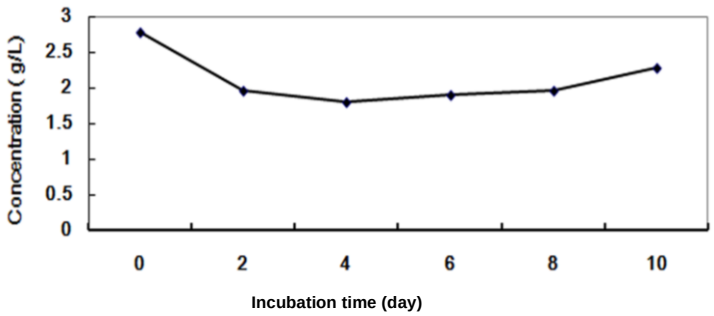

Figure 3. Lignin degradation curve. Conidial suspension was cultured at 28°C for 10 days and the concentrations of alkaline lignin were detected by measuring the absorption of medium at 280 nm. All values were the means of three replications.

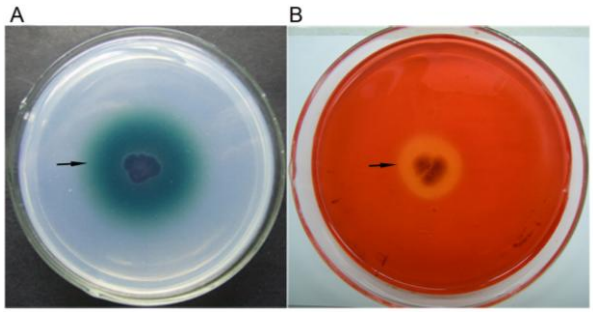

Figure 4. Detection of laccase (A) and cellulase (B) secretion of Bio-1 on the ager plates. (A) The strain cultured on the LDM at 28°C for 3 days; arrow indicates the dark-green ring. (B) The strain cultured on the CDM at 28°C for 3 days; arrow indicates the clear zone.

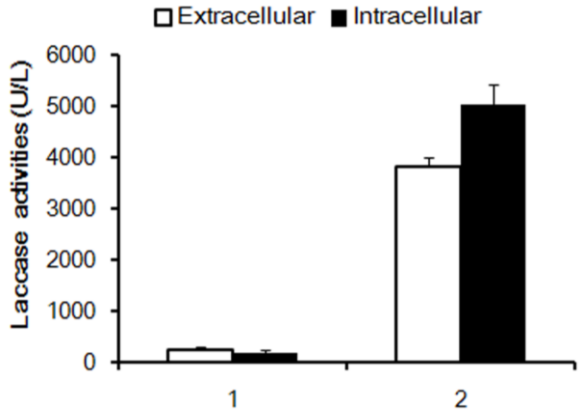

Figure 5. Activities of the extracellular and intracellular laccase on the eighth day. Without (1) and with (2) 0.5 mM $CuSO_4$ induction at 28°C in Czapek's medium.

laccase activity.

To be sure of the laccase existence, the extracellular and intracellular laccase activities were detected by ABTS method. The highest extracellular and intracellular laccase activities were detected on the eighth day of incubation, which were 241 and 187 U/L, respectively (Figure 5). This indicates that the degradation of the lignin should be continued after the fourth day, the above experimental results in which absorbance of medium at 280 nm were slightly increased after the fourth day may be disturbed by the impact of secreted proteins. Meanwhile, some intermediate products may enhance lignin degradation and act as the genes inducers, which promote the degradation of lignin after day 4.

Owing to the ever-increasing demand for laccase in biotechnological applications, its production process is required to be economical and further enhanced. Using inducers may be of benefit (Niladevi and Prema, 2008). Laccase is a multi-copper oxidase and copper could regulate the synthesis of several laccase isoforms at the level of gene transcription (Galhaup et al., 2002). According to Collons and Dobson (1997) results, 0.5 mM $CuSO_4$ was added to Czapek's medium on the fourth day. The extracellular and intracellular activities were enhanced to 3800 and 5352 U/L, respectively. Significant impact of copper on laccase synthesis in *Trametes versicolor* (Niladevi and Prema, 2008), *P. ostreatus* (Palmieri et al., 2000) and white rot fungus had been frequently reported. For the Bio-1, strain copper induced the extracellular laccase activity by 15 fold and the intracellular laccase activity by 28 fold (Figure 5). Before induction by $CuSO_4$, extracellular laccase activity was higher than the intracellular laccase, while after being induced, the intracellular laccase activity was much higher than the extracellular laccase.

Maybe intracellular laccase produced by Bio-1 was more sensitive to copper. Or, there was no enough time for the secretion of the enzyme. The effluents often contain copper component, so the sensitivity to copper of Bio-1 has large application potential in effluents treatment.

The highest extracellular laccase activity was 241 U/L, lower than some classical laccase-producing fungi. However, 0.5 mM $CuSO_4$ greatly enhanced laccase production as 3800 U/L, much higher than the same species, which has been reported earlier (Claus and Filip, 1998). According to Vijiaykumar et al. (2006), a fungus *C. cladosporioides* isolated from a coal sample showed laccase activity with 1413 U/L. Interestingly, only a few articles have reported that genus *Cladosporium* strain could produce intracellular laccase (Tetsch et al., 2006; Froehner and Eriksson, 1974).

In our case, the intracellular laccase of Bio-1 was detected and more importantly, it could be greatly enhanced by $CuSO_4$, even much stronger than extracellular laccase. So, the strain Bio-1 could be an attractive source of laccase producer.

We could not detect the MnP and LiP activities, which

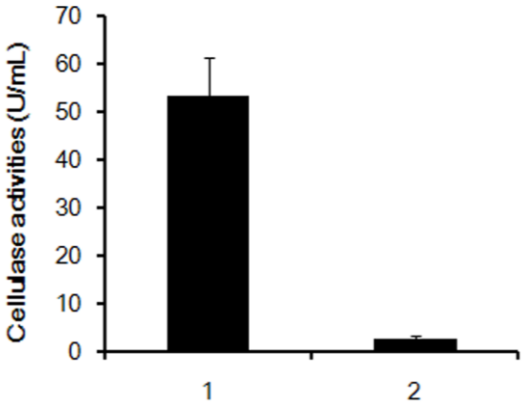

Figure 6. Extracellular (1) and intracellular (2) cellulase activities on the eighth day. Conidial suspension was cultured in Czapek's medium at 28°C.

was similar to the previous reports (Vijiaykumar et al., 2006) (data not show).

Assay of the cellulase activity

Qualitative measurement of cellulase was operated using CDM plate. A clear zone was formed around the colony and was observed in the CDM plate at 28°C on the third day after flooded with Congo red and NaCl (Figure 4B), which indicated that the strain could also secrete cellulases.

Similarly, the extracellular and intracellular cellulase activities were detected by DNS method. On the eighth day, the highest extracellular cellulase activity, 51 U/mL was obtained. At the same time, the intracellular cellulase was comparatively low, only 2.9 U/mL (Figure 6). Although *C. cladosporioides* had been reported as laccase-producer, there were few articles that mentioned that *C. cladosporioides* had capacity to degrade cellulosic materials (Eveleigh, 1970). According to Abrha, a *Cladosporium* species produced cellulase activity of 81 U/mL (Abrha and Gashe, 1992), and the lignin-degrading activity was not mentioned. Based on the characteristics that Bio-1 could secrete both laccase and cellulase, the strain has huge potential in commercial applications in lignocellulose treatment.

ACKNOWLEDGEMENTS

This research was supported by Doctoral Fund of Ministry of Education of China (20110161120020), Science and Technology Planning Project of Hunan Province, China (2010FJ4105), the Natural Science Foundation of Hunan Province, China (12JJ6023) and Fundamental Research Funds for the Central Universities.

REFERENCES

Abrha B, Gashe BA (1992). Cellulase production and activity in species of Cladosporium. World J. Microbiol. Biotechnol. 8(2):164-166.

Baldrian P (2004). Purification and characterization of laccase from the white-rot fungus *Daedalea quercina* and decolorization of synthetic dyes by the enzyme. Appl. Microbiol. Biotechnol. 63(5):560-563.

Catcheside DEA, Rasmussen JP, Yeadon PJ, Browing FJ, Cambareri EB, Kato E, Gabe J, Stuart WD (2003). Diversification of exogenous genes in vivo in Neurospora. Appl. Microbiol. Biotechnol. 62(5-6):544-549.

Claus H, Filip Z (1998). Degradation and transformation of aquatic humic substances by laccase-producing fungi *Cladosporium cladosporioides* and *Polyporus versicolor*. Acta. Hydrochim. 26(3):180-185.

Collons PJ, Dobson ADW (1997). Regulation of laccase gene transcription in *Trametes versicolor*. Apply. Environ. Microbiol. 63(9):3444-3450.

Daxboeck F, Dornbusch HJ, Krause R, Assadian O, Wenisch C (2004). Verification of false-positive blood culture results generated by the BACTEC 9000 series by eubacterial 16S rDNA and panfungal 18S rDNA directed polymerase chain reaction (PCR). Diagn. Micr. Infec. Dis. 48(1):1-3.

Deacon JW (1997). Modern Mycology. In: Fungal growth, 3rd edn. Wiley New York, pp. 50-59.

Eveleigh DE (1970). Fungal disfigurement of paper and soft rot of cedar shingles. Appl. Microbiol. 19(5):872-874.

Ezeronye OU, Okerentugba PO (1999). Performance and efficiency of a yeast biofilter for the treatment of a Nigerian fertilizer plant effluent. World. J. Microbiol. Biotechnol. 15(4):515-516.

Froehner SC, Eriksson KE (1974). Purification and properties of Neurospora crassa Laccase. J. Bacteriol. 120(1):458-465.

Galhaup C, Goller S, Peterbauer CK, Strauss J, Dietmar H (2002). Characterization of the major laccase isoenzyme from *Trametes pubescens* and regulation of its synthesis by metal ions. Microbiology 148(7):2159-2169.

Gao HJ, Wang YW, Zhang WT, Wang WL, Mu ZM (2011). Isolation, identification and application in lignin degradation of an ascomycete GHJ-4. Afr. J. Biotechnol. 10(20):4166-4171

Ghose TK (1987) Measurement of cellulase activities. Pure. Appl. Chem. 59(2):257-268.

Halaburgi VM, Sharma S, Sinha M, Singh TP, Karegoudar TB (2011). Purification and characterization of a thermostable laccase from the ascomycetes *Cladosporium cladosporioides* and its applications. Process Biochem. 46(5):1146-1152.

Jahangeer S, Khan N, Jahangeer S, Sohail M, Shahzad S, Ahmad A, Khan AS (2005). Screening and characterization of fungal cellulases isolated from the native environmental source. Pak. J. Bot. 37(3):739-748.

John B (1986). Regulation of Cellulolytic Activity in the White-Rot Fungus *Ischnoderma redinosum*. Mycologia 78(1):52-55.

Leonowicz A, Matuszewska A, Luterek J, Ziegenhagen D, Wojtas-Wasilewska M, Cho.NS, Hofrichter M, Rogalski J (1999). Biodegradation of Lignin by White Rot Fungi. Fungal Genet. Biol. 27(2-3):175-185.

Lundquist K, Kirk TK, Connors WJ (1977). Fungal Degradation of Kraft Lignin and Lignin Sulfonates Prepared from Synthetic 14C-Lignins. Arch. Microbiol. 112(3):291-296.

Niladevi KN, Prema P (2008). Effect of inducers and process parameters on laccase production by *Streptomyces psammoticus* and its application in dye decolourization. Bioresour. Technol. 99(11):4583-4589.

Niranjane AP, Madhou P, Stevenson TW (2007). The effect of carbohydrate carbon sources on the production of cellulase by *Phlebia gigantean*. Enzyme. Microb. Technol. 40(6):1464-1468.

Palmieri G, Giardina P, Bianco C, Fontanella B, Sannia G (2000). Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. Apply. Environ. Microbiol. 66(3):20-924.

Pointing SB (1999). Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. Fungal Diversity 2:17-33.

Prasad GK, Gupta RK (1997). Decolourization of pulp and paper mill effluent by two white-rot fungi. Inidan J. Environ. Health 39(2):89-96.

Raghukumar C, Ticlo DD, Verma AK (2008). Treatment of colored effluents with lignin-degrading enzymes: An emerging role of marine-derived fungi. Crit. Rev. Microbiol. 34(3-4):189-206.

Rajeev KS, Reeta RS, Ashok P (2005). Microbial cellulases production application and challenges. J. Sci. Ind. Res. 64(11):832-844.

Rodriguez-Couto S, Toca-Herrera JL (2006). Industrial and biotechnological applications of laccases: A review. Biotechnol. Adv. 24(5):500-513.

Rolz C, Leon R, Arriola MC, Cabrera S (1987). White-rot fungal growth on sugarcane lignocellulosic residue. Appl. Microbiol. Biotechnol. 25(6):535-541.

Sanche C (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnol. Adv. 27(2):185-194.

Srinivasan C, Dsouza TM, Boominathan K, Reddy CA (1995). Demonstration of Laccase in the White Rot Basidiomycete *Phanerochaete chrysosporium* BKM-F1767. Appl. Environ. Microbiol. 61(12):4274-4277.

Steffen KT, Hofrichter M, Hatakka A (2000). Mineralisation of 14C-labelled synthetic lignin and ligninolytic enzyme activities of litter-decomposing basidiomycetous fungi. Appl. Microbiol. Biotechnol. 54(6): 819-825.

Tetsch L, Bend J, Holker U (2006). Molecular and enzymatic characterization of extra- and intracellular laccases from the acidophilic ascomycete *Hortaea acidophila*. Antonie van Leeuwenhoek 90(2):183-194.

Zhang YHP, Himmel ME, Mielenz JR (2006). Outlook for cellulase improvement: Screening and selection strategies. Biotechnol. Adv. 24(5): 452-481.

Vijiaykumar MH, Veeranagouda Y, Neelakanteshwar K, Karegoudar TB (2006). Decolorization of 1:2 metal complex dye Acid blue 193 by a newly isolated fungus *Cladosporium cladosporioides*. World J. Microbiol. Biotechnol. 22(2):157-162.