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Activity of Ligninolytic Enzymes during Growth and Fruiting Body Development of White Rot Fungi *Omphalina* sp. and *Pleurotus ostreatus*

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The activity of ligninolytic enzymes of white rot fungi (WRF) *Omphalina* sp. and *Pleurotus ostreatus* was observed during somatic and fruiting body development in solid substrate fermentation using empty fruit bunches of oil palm (EFB). The enzyme activity was dominated by laccase both of *Omphalina* sp. and *P. ostreatus*. The laccase activity on somatic phase (mycelium growth) was higher compared to that of fruiting body formation phase. The laccase activity of *Omphalina* sp. was slightly higher compared to that of *P. ostreatus*. The peak activity of manganese peroxidase (MnP) for *Omphalina* sp. was observed two weeks after inoculation, while *P. ostreatus* had two peaks i.e. two and four weeks after inoculation period. The MnP activity of *P. ostreatus* was higher compared to that of *Omphalina* sp. *Omphalina* sp. growth in EFB did not secrete lignin peroxidase (LiP) in contrast to *P. ostreatus*. The peak of LiP activity of *P. ostreatus* was reached two and four weeks after inoculation. The MnP and LiP activities declined during the development of fruiting bodies while laccase increased both in *Omphalina* sp. and *P. ostreatus*. It seems that the activities of ligninolytic enzyme profile were regulated in line with developmental phase of growth both in *Omphalina* sp. and *P. ostreatus*.

Key words: ligninolytic enzyme activity, *Omphalina* sp., *Pleurotus ostreatus*, empty fruit bunches of oil palm

INTRODUCTION

Empty fruit bunches of oil palm (EFB) is the lignocellulosic waste abundantly discharged by oil palm industry in line with the increasing oil palm acreage in Indonesia. In general EFB is a lignocellulosic materials consisting of a matrix of cellulose, hemicellulose, and lignin. The last compound was known as a recalsitrant material, though selected fungi especially white rot fungi (WRF) degrades efficiently the lignin to enable further digest of cellulose as sources of energy and carbon. Therefore, the highest lignin content of EFB is a potential to be utilized as a substrate in producing ligninolityc enzyme.

The main extracellular lignin degrading enzymes are laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP). Laccase is a copper-containing polyphenol oxidase. It catalyses the reduction of four-electron oxygen to water and this is typically accompanied by the oxidation of a phenolic substrate to fenoxil radical. Though the laccase produced by *Trametes vesicolor* is able to oxidize nonphenolic substrates provided with 2,2'azinobis(3-ethylbenzthiazoline-6-sulfonate). The molecular weight of laccase is between 60-80 kDa and its isoelectric point is between 3 and 4. Manganese peroxidase is an enzyme containing Fe which oxidize phenol to phenoxy radicals by oxidizing Mn^{1+} to Mn^{3+} using H_2O_2 as oxidant. Mn^{3+} was then chelated using oxalic acid or malic that spontaneous oxidize phenolic lignin. The molecular weight of MnP is 40-50 kDa and the isoelectric point is between 3 to 4 though neutral MnP is also found. Lignin peroxidase could also oxidize non phenolic aromatic compound. Sarkar *et al.* (1997) showed that beside those three enzymes, selected WRF also produced veratryl peroxidase combining LiP and MnP. This enzyme oxidizes Mn and phenolic compound and non phenolic aromatic compound. The veratryl peroxidase enzyme was isolated from *Pleurotus eryngii*, *P. ostreatus*, and *Bjerkandera* sp. BOS55.

Ligninolityc enzyme activities follow the pattern of lignin disappearance from the subtrate and directly correlated with the time of its disappearance. Durrant *et al.* (1991) showed that delignification increased from the spawning to the fruiting body formation and decrease during the maturation of the fruiting body. Degradation of lignin by fungi occured on the secondary metabolism and especially in the limiting N and carbon (Xiaoyan *et al*. 2007). But, Levin *et al.* (2002) showed that *Trametes trogii* produces the highest laccase, MnP, and GLOX in substrate containing high N and C simultaneously. The white rot fungi *Phanerochaetae chrysosporium* need N that is shown by expressing protease in the colonized wood (Sato *et al*. 2007). However, Levin *et al*. (2002) showed that high N could enhance pH and this affect the ability of ligninolytic capability. Cohen *et al.* (2002) showed that the regulation MnP was at the gene transcription together with N, Mn, heat shock and others factor. Ford *et al.* (2007) showed that the age of inoculant affected the ability of *Trametes* sp. in degrading pentachlorophenol (PCP). This research was aimed to examined ligninolytic enzyme activities under solid fermentation throughout the somatic and fruiting body development of *Omphalina* sp. and *P. ostreatus* using EFB as substrate.

MATERIALS AND METHODS

Fungal Strain. *Omphalina* sp. used in this study is the collection of Indonesian Biotechnology Research Institute

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for Estate Crop (IBRIEC) isolated from oil palm factory in Kertajaya, PTPN VIII, Banten and *Pleurotus ostreatus* is the collection of Bogor Agricultural University.

Culture Condition. *Omphalina* sp. and *P. ostreatus* were cultured in potato dextrose agar (PDA) and incubated at room temperature for 5-7 days. Those agar plugs (10 mm diameter, 30 g) from the fungal colony grown on a PDA (14 d) were used as the inoculum of the sorghum media in a jam bottle (100 g in volume) and incubated for two weeks under room temperature (26-30 ^{o}C).

Preparation of EFB as WRF Production Medium. The EFB was obtained from PT Pinago, Palembang, South Sumatera. As much as 500 g of EFB was mixed with 30% of rice bran and submerged in 150 µM $CuSO_4$ and added with tap water up to 50% water content and filled to the bag log. The bag log is autoclaved under 1.2 kg/cm^2 pressure for 30 minutes. Sterilized EFB was inoculated with two species of WRF i.e. *Omphalina* sp. and *P. ostreatus* (from sorghum as media) and incubated in a dark room at 26-30 ^{o}C. To initiate the fruiting process, the fully colonized substrate was opened and the production room was flushed with fresh air (1-2 hours each day) to reduce both the temperatures and the level of carbon dioxide. The temperature of production room was about 28-30 ^{o}C with 70-80% relative humidity. The first fruiting body was produced 2-4 weeks after initiation (Figure 1).

Extraction of Enzyme. Phosphate buffer with pH 7.2 was used for extracting the enzyme from medium with substrate/buffer 1:3 (w/v), while being ground using mortar thoroughly, and centrifuged at 5,000 rpm for 10 min on 0-4 ^{o}C. The centrifugation was repeated untill clean filtrate was obtained.

Enzyme Activity Measurement. The measurement of ligninolytic activities was conducted every week during somatic phase until two weeks after the formation of fruiting body. The enzyme activity was analyzed from crude extract of the enzyme. The laccase activity was measured by method developed by Perez and Jeffries (1992). One unit laccase activity is defined as the amount of enzyme oxidizing 1 nmol ABTS (2,2-azino-bis-3-ethlybenzothiazoline-6-sulphonic acid) compound per min on 37 ^{o}C. LiP activity was measured by monitoring the oxidation of veratryl alcohol to veratraldehyde (Tien & Kirk 1984). One unit LiP is defined as 1 nmol of veratryl alcohol (guaiacol) oxidized to veratraldehyde per minute. MnP activity was determined by monitoring the oxidation of guaiacol spectrophotometrically at 465 nm (Hatakka 1994). One unit of activity was defined as 1 nmol/l of Mn^{2+} oxidized per minute.

The enzyme activity was analyzed from each of three replications and the sample was disturb sample. Datum points in all cases are means for three replicate cultures, with standard deviations indicated by error bars.

RESULTS

Ligninolytic Enzyme Activity of *Omphalina* sp. Mycelium growth of *Omphalina* sp. in EFB grew very fast that the somatic phase was only required four weeks for incubation. The fruiting body of *Omphalina* sp. was white in color with the glossy surface and 2-3 cm in diameter (Figure 1a).

Following initial colonization by *Omphalina* sp., the laccase activities were sharply increased in the compost collected on three weeks after inoculation (1.992 U/ml). During the development of fruiting bodies, the laccase activities gradually decreased, though it increased again two weeks after the maturation of fruiting bodies (Figure 2).

The MnP activity increased within two weeks after inoculation and then declined afterward. The activity was not detected during the fruiting body formation stages. Two weeks after the formation of fruiting bodies, the MnP activity was still not yet detected. In this study it was shown that *Omphalina* sp. grown in EFB did not excrete LiP.

Ligninolytic Enzyme Activity of *P. ostreatus*. The mycelium of *P. ostreatus* colonized the bag log within four weeks. The formation of fruiting bodies was observed two weeks after light induction. Figure 1b showed the fruiting body

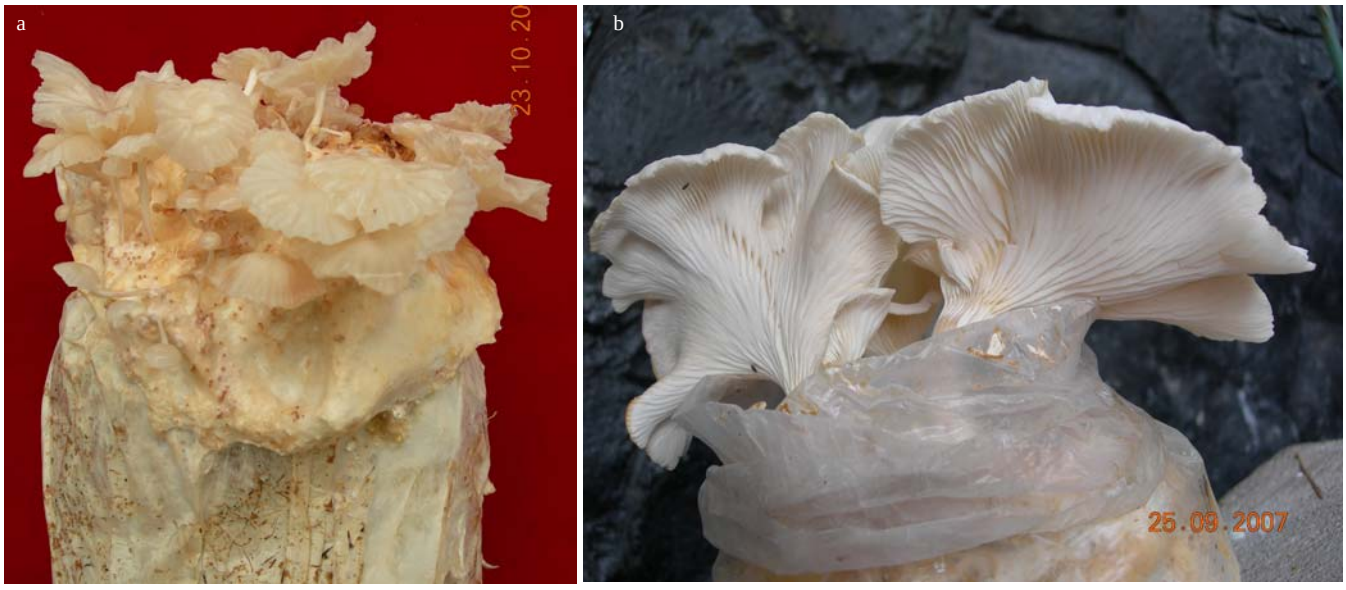

Figure 1. Fruiting body of *Omphalina* sp. (a) and *P. ostreatus* (b).

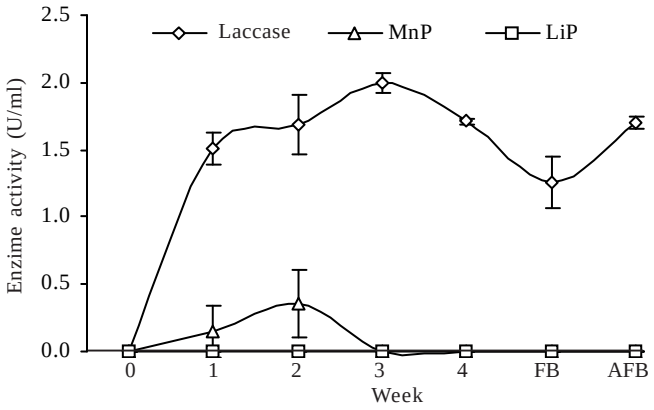

Figure 2. Activities pattern of ligninolytic of *Omphalina* sp. grown in EFB during somatic phase throughout two weeks after maturation the fruiting body (AFB). Vertical bars represent the standard error of the mean.

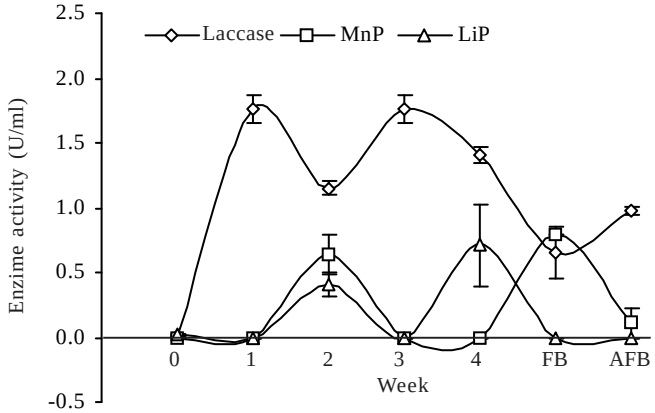

Figure 3. Activity pattern of ligninolytic of *P. ostreatus* grown in EFB during somatic phase throughout two weeks after maturation of the fruiting body (AFB). Vertical bars represent the standard error of the mean.

of *P. ostreatus* grown on EFB medium. The fruiting body was thick and white in colour with fresh biomass weight reached 170 g.

Following initial colonization of *P. ostreatus*, the laccase activities was very high i.e. 1.762 U/ml. The laccase activity declined after two weeks and increase in three weeks thereafter (Figure 3). But, in the fruit body formation the laccase activity reduced sharply. However, there was enhancement of laccase activity after the fruiting body maturation.

The peak activity of MnP from somatic till fruiting bodies development occurred two times. Firstly it accurred two weeks after inoculation and the second peak was observed during the fruiting body formation i.e. 0.634 and 0.799 U/ml respectively. But the MnP activity reduced after the maturation of fruiting body.

The LiP activity from the somatic till fruiting bodies development was fluctuated but the highest activity was reached in two and four weeks incubation i.e. 0.404 and 0.708 U/ml respectively. The LiP activity decreased during the fruiting body formation and was not found after the fruiting body maturation.

Comparation of Ligninolytic Enzyme Activity of *Omphalina* sp. and *P. ostreatus* and the Lignin Content on the Growth Media. The mycelium growth of *Omphalina* sp. in lignocellulosic EFB was faster than that of *P. ostreatus*. The maximum activity of laccase of *Omphalina* sp. was slightly higher though slower than that of *P. ostreatus.* In *P. ostreatus*, there was two peaks of laccase activity while on *Omphalina* sp. there was one peak only of laccase activity. The similar pattern was also found for MnP. However, *P. ostreatus* secretes LiP in EFB as growth media in contrast to *Omphalina* sp.

The lignin analyses of EFB during the somatic phase and fruiting body formation showed that the lignin content two weeks after fruiting body formation decrease on EFB inoculated with both *Omphalina* sp. and *P. ostreatus* i.e. 23.98 and 18.37% respectively. The highest reduction of lignin was observed in somatic phase (four weeks inculation) i.e. 17.52 and 7.04% in *Omphalina* sp. and *P. ostreatus* respectively.

Table 1. Lignin content of EFB inoculated with *Omphalina* sp. dan *P. ostreatus* throughout the somatic and two weeks after fruiting body maturation

Phase	Lignin content (%, b/b), (% tage of lignin decreasing)	
	Omphalina sp.	*P. ostreatus*
Before inoculation	29.40	29.40
Mycelium (1 week)	29.21 (0.65)	28.15 (4.25)
Mycelium (2 week)	27.00 (8.16)	27.82 (5.37)
Mycelium (3 week)	26.29 (10.58)	27.66 (5.92)
Mycelium (4 week)	24.25 (17.52)	27.33 (7.04)
Fruiting body (FB)	24.20 (17.69)	27.81 (5.41)
Two week after fruiting body (AFB)	22.35 (23.98)	24.00 (18.37)

DISCUSSION

Omphalina sp. synthesized more laccase compared to MnP in the medium incorporated with Cu. Levin *et al*. (2002) reported that Cu ion up to 1 mM strongly stimulated the laccase of *T. trogii* though the use of higher concentration of Cu ion could suppress its growth and decreased MnP. Laccase of *Omphalina* sp. was actively secrete at the mycelium phase and reached its peak activity after the incubation for three weeks, and then declined during the fruiting body formation. The laccase increased again two weeks after the maturation of fruiting body. This finding was resemble with Levin *et al*. (2002) report but the second peak produced by *T. trogii* was reached after the enzyme declined at stationary growth. Xie *et al*. (2001) reported that *P. ostreatus* still produced laccase when entering primordial phase of fruiting body and then sharply decreased when the fruiting body was harvested. Baldrian *et al*. (2000) showed that laccase of *Pleurotus* reached the optimum level of activity at two weeks after the medium was completely colonized with mycelium and then sharply dropped at further period of growth.

Eggert (1997) showed that WRF laccase besides functioning as the lignin degradating enzyme, it was also important in pigment production, polyphenol detoxification, fruiting body formation, sporulation, and antimicrobial agent.

The difference pattern of laccase activity in white-rot fungi might be due to the genetic variability of the fungi, the difference of culture condition and age of culture (Levin *et al*. 2002). Mansur *et al*. (1998) reported that the expression of laccase gene was influenced by culture condition and its production was regulated by differential pattern. Levin *et al*. (2002) says that design production of isozim laccase was not influenced by medium composition but more influenced by culture age. Xie *et al*. (2001) found the different result since laccase activity and MnP of *Pleurotus* sp. 2 reached the highest activity at tenth days in broth medium with lower value of N, without high amount of carbon and inorganic salt. The different result of activities of laccase between this research and Xie *et al*. (2001) may be caused by the difference of growth media. It is suspected that the nutrient in broth medium, was more available than that in the solid medium. More over, according to Xiaoyan *et al*. (2007) the lower value of N used in Xie *et al*. (2001) was considered stimulating laccase activity. The fluctuations of ligninolytic enzyme activity showed that the culture age affect the mushroom activity in degrading. Ford *et al*. (2007) also said that inoculum age affects the ability of WRF *Trametes* sp. to pentachlorophenol biodegradation (PCP).

Bonnen *et al*. (1994) reported that the highest MnP activity in *Agaricus bisporus* was detected in the first phase of fruiting body formation and decreased in parallel to the ripening of fruiting body. The pattern seems has the same way with laccase activity reported before. Baldrian *et al*. (2000) said MnP activity in *P. ostreatus* increased two weeks after increasing of the laccase activity. This finding is the same as this research result where MnP activity reaches the first peak at one week after secretes of laccase. In condition Galliano *et al*. (1991) assumed that in *Rigidoporus linosus*, while degrading the lignin, the MnP and laccase become synergic. Schlosser and Hoefer (2002) also found synergetic between MnP and laccase. Since *Omphalina* sp. grown in EFB did not secrete LiP, it supposed that LiP is not involved in degrading of lignin.

The patterns of ligninolytic enzyme activity in *Omphalina* sp. apparently was not different with other WRF reported. The *Omphalina* sp. secrete actively the laccase enzyme both during somatic and fruiting body development while the MnP activity was detected only in mycelium stage. In generale, ligninolytic enzyme-increased at somatic phase and decreased at the fruiting body phase. However, it is assumed that when the fruiting body was reformed at next incubation, the fluctuation of ligninolytic enzyme activity occurred. This result showed that there was fluctuations in the laccase and MnP peroxidase activity of *Omphalina* sp. and maximum enzymes activity coincided with the mycelium stages of *Omphalina* sp. development.

On the EFB, the *P. ostreatus* produced ligninolytic enzymes such as laccase, MnP, and LiP. As in *Omphalina* sp., the laccase activity of *P. ostreatus* was higher than the other enzymes. It is assumed that the high laccase production due to the addition of Cu in substrate. Palmieri *et al*. (2000) said that the addition of Cu increase the total laccase activity and the induction of laccase gene transcription. The role of metallothionein, a metal gene, in protecting metal toxicity which has correlated with the ability of many metal ion such as Zn, Cu, Cd, and the other to activated *mt* gene transcription through metal organizing protein which is function as metal receptor and transcription factor (Faraco *et al*. 2003). The drastic escalation of laccase activity was recurred at one weeks incubation i.e. 1.762 U/ml. At the third week, the laccase activity increased back after decreasing at the second week. The laccase activity decreased again when the fruiting body was formed. The fluctuated laccase activities of *P. ostreatus* during the experiment have the same pattern as reported by Schlosser and Hoefer (2002).

The lower activities of MnP may be increased by addition of Mn and other minerals. Cohen *et al*. (2002) said that the addition of Mn^{2+} at cotton stalk inoculated with *P. ostreatus* increased peroxides expression, and the effect can be seen directly at two hours after the addition. Furthermore Giardina *et al*. (2000) showed that the addition increased the MnP and LiP production from *P. ostreatus*. Ma *et al*. (2004) said that Mn^{2+} mineral and the low amount of N were needed to activate the MnP gene code. The increase of peroxidase activity in this study may due to the addition of Mn^{2+} and the controlled amount of N and C.

MnP activity of *P. ostreatus* from the somatic phase to the fruiting body grown in EFB has two peak. It is seemingly that the decrease of laccase activity at the fruiting body followed by increasing MnP activity. These results are apparently different with those of Vladimir *et al*. (2003). Vladimir *et al*. (2003) reported that other WRF species, like *A. bisporus*, show higher MnP activity during colonization and become lower at early primordial and fruiting body phase. Profile of ligninolytic enzymes especially MnP and LiP, was influenced by medium composition and this was shown by isoenzyme analyzes conducted by Collin and Dobson (1997). Moreover, Giardina *et al*. (2000) reported that *P. ostreatus* grew on sawdust produce different isoenzymes compared to the basic medium.

Degradation of lignin both of *Omphalina* sp. and *P. ostreatus* were lower in fruiting body formation phase compared to somatic phase. It was shown that the pattern of lignin degradation was similar with Durrant *et al*. (1991). They showed that button mushroom (*A. bisporus*) grown on straw compost, the lignin concentration in the compost decreased from the nursery time to the first fruiting body formation and the lowest lignin degradation occurred at the fruiting body phase.

The earlier result showed that the laccase activity of *P. ostreatus* (0.6 U/ml) was higher than *Omphalina* sp. (0.2 U/ml) especially in the medium supplemented with Cu and a mixture of rice and bran (Widiastuti *et al*. 2007). In this research, *P. ostreatus* reach 1.762 U/ml while that of *Omphalina* sp. reach 1.992 U/ml. This result might be caused by the different level of culture maturity. Levin *et al*. (2002) said that culture age influence to ligninolytic activity. Widiastuti *et al*. (2007) observed the enzyme activity after three months incubation (although it is still in somatic phase). While in this research, *Omphalina* sp. activity was observed from 0 until 10 weeks of incubation, and for *P. ostreatus* it was observed from 0 until 8 weeks covering somatic and the

fruiting body phase. Schloserr and Hoefer (2002) said that the laccase and MnP titer were affected by condition and age culture. Ford *et al*. (2007) observed that biodegradation of pentachlorophenol (PCP) was affected by the culture age, and co-substrate and substrate composition. However, compared to *Trametes gallica*, the decrease of lignin concentration in this research is lower 3-4 times (Xie *et al*. 2001). This difference may be caused by the different substrate form, the high lignin concentration and the type of lignin structure.

In general, the pattern of ligninolytic activity of WRF enzymes were probably affected by the growth, stage and the development of mushroom. The pattern most likely becomes the problem in developing ligninolytic enzyme production since will affect to low production and enzyme stability.

REFERENCES

Baldrian P, der Wiesche C, Gabriel J, Nerud F, Zadrazil F. 2000. Influence of cadmium and mercury and activities of ligninolytic enxymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotas ostreatus* in soil. *Appl Environ Microbiol* 66:2471-2478.

Bonnen AM, Antón LH, Orth AB. 1994. Lignin-degrading enzymes of the comercial button mushroom, *Agaricus bisporus*. *Appl Environ Microbiol* 60:960-965.

Cohen R, Yarden O, Hadar Y. 2002. Lignocellulose affects Mn^{2+} regulation of peroxidase transcript levels in solid–state cultures of *Pleurotus ostreatus*. *Appl Environ Microbiol* 68:3156-3158.

Collins PJ, Dobson ADW. 1997. Regulation of laccase gene transcription in trametes versicolor. *Appl Environ Microbiol* 63:3444-3450.

Durrant AJ, Wood DA, Cain R. 1991. Lignocellulose biodegradation by *Agaricus bisporus* during solid substrate fermentation. *J Gen Microbiol* 137:751-755.

Eggert. 1997. Laccase is responsible for antimicrobial activity of *Picnoporus cinnabarinus*. *Microbiol Res* 152:315-318.

Faraco V, Giardina P, Sannia G. 2003. Metal-responsive elements in *Pleurotus ostreatus* laccase gene promoters. *Microbiology* 149:2156-2162.

Ford CI *et al*. 2007. Fungal inoculum properties: extracellular enzyme expression and pentachlorophenol removal by New Zealand *Trametes* species in contaminated field soils. *J Int Qual* 36:1749-1759.

Galliano H, Gas G, Seris JI, Bondet AM. 1991. Lignin degradation by *Rigidiporus lignosus* involves synergistic action of two oxidizing enzymes: Mn peroxidase and laccase. *Enzyme Microb Technol* 13:478-482.

Giardina P, Palmieri G, Fontanella B, Rivieccio V, Sannia G. 2000. Manganese peroxidase isoenzymes produced by *Pleurotus ostreatus* grown on wood sawdust. *Arch Biochem Biophys* 376:171-179.

Hatakka A. 1994. Lignin modifying enzyme from selected white-rot fungi: Production and role in lignin degradation. *FEMS Microbiol Rev* 13:125-135.

Levin I, Forchiassin F, Ramos AM. 2002. Copper induction of lignin-modifying enzymes in the white-rot fungus *Trametes trogii*. *Mycologia* 94:377-383.

Ma B, Mayfield MB, Godfrey BJ, Gold MH. 2004. Novel promoter sequence for manganese regulation of manganese peroxidase isozyme 1 gene expression in *Phanerochaete chrysosporium*. *Eucaryotic Cell* 3:579-588.

Mansur M, Suarez T, Gonzalez AE. 1998. Differential gene expression in the laccase gene family from basidiomycete 1-62 (CECT 20197). *Appl Environ Microbiol* 64:771-774.

Palmieri G, Giardina P, Bianco C, Fontanella B, Sannia C. 2000. Copper induction of laccase isoenzymes in the lignolytic fungus *Pleurotus ostreatus*. *Appl Environ Microbiol* 66:920-924.

Perez J, Jeffries TN. 1992. Mineralization of $^{14}CO_2$ ring labeled synthetic lignin correlates with the production of lignin peroxidase, not manganese or laccase. *Appl Environ Microbiol* 58:1806-1812.

Sarkar S, Martinez AT, Martinez AJ. 1997. Biochemical and molecular characterization of a manganese peroxidase isoenzyme from *Pleurotus ostreatus*. *Biochim Biophys Acta* 1339:23-30.

Sato S, Liu F, Koc H, Tien M. 2007. Expresión análisis of extracellular proteins from *Phanerochaete chrysosporium* grown on different liquid and solid substrates. *Microbiol* 153:3023-3033.

Schlosser D, Hoefer C. 2002. Laccase-catalyzed oxidation of Mn^{2+} in the precence of natural Mn^{3+} chelators as a novel source extracellular H_2O_2 production and its impact on manganese peroxidase. *Appl Environ Microbiol* 68:3514-3521.

Tien M, Kirk TK. 1984. Lignin-degrading enzyme from *Phanerochaete chrysosporium*: Purification, characterization, and catalytic properties of a unique H_2O_2-requiring oxygenase. *Proc Natl Acad Sci USA* 81:2280-2284.

Vladimir, David, Eva, Nino, Tamar. 2003. Lignocellulolytic enzyme activity during growth and fruiting of the edible and medicinal mushroom *Pleurotus ostreatus* (Jacq. Fr) Kumm. (Agaricomycetideae). *Int J Medicinal Mushroom* 5:193-198.

Widiastuti H, Siswanto, Suharyanto. 2007. Optimasi pertumbuhan dan aktivitas enzim ligninolitik *Omphalina* sp. dan *Pleurotus ostreatus* pada fermentasi padat. *Menara Perkebunan* 75:93-105.

Xiaoyan Z, Xianghua W, Yan F. 2007. Influence of glucose feeding on the ligninolytic enzyme production of the white rot fungus *Phanerochaete chrysosporium*. *Front Environ Sci Engin China* 1:89-94.

Xie J, Sun X, Ren L, Zhang YZ. 2001. Studies on lignocellulolytic enzymes production and biomass degradation of *Pleurotus* sp. 2 and *Trametes gallica* in wheat straw cultures. *Sheng Wu Gong Cheng Xue Bao* 80:575-578.