

Full Length Research Paper

Polycyclic aromatic hydrocarbons (PAHs) degradation by laccase from a tropical white rot fungus *Ganoderma lucidum*

Hunsa Punnapayak^{1*}, Sehanat Prasongsuk¹, Kurt Messner³, Khanchai Danmek^{1,2} and Pongtharin Lotrakul¹

¹Plant Biomass Utilization Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.

²Biotechnology Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.

³Abteilung Mykologie, Institut für Biochemische Technologie and Mikrobiologie, Technische Universität Wien, A-1060 Vienna, Austria.

Accepted 28 September, 2009

Laccase enzyme was produced from an isolate of the white rot fungus, *Ganoderma lucidum* Chaaim-001 BCU. The enzyme was subsequently evaluated for its degradative ability towards sixteen types of polycyclic aromatic hydrocarbons (PAHs). The *G. lucidum* laccase degraded anthracene completely with or without a redox mediator (2 mM 1-hydroxybenzotriazole) and also degraded benzo[a]pyrene, fluorine, acenaphthene, acenaphthylene and benzo[a]anthracene up to 100.0, 98.6, 95.4, 90.1 and 85.3 %, respectively, when the mediator was present. In the absence of the mediator, the ability to degrade these compounds dropped to 71.71, 62.9, 80.49, 85.85 and 9.14% respectively. Compared to the laccase enzyme from *Trametes vesicolor*, *G. lucidum* laccase appeared to retain more of its capability to degrade these PAHs when the mediator was absent.

Key words: Laccase, *Ganoderma lucidum*, polycyclic aromatic hydrocarbons.

INTRODUCTION

Lignin is the second most abundant renewable organic polymer on earth and is a major component of wood. Its degradation is an important and integral part of ecosystem. Moreover, because of the importance of lignocellulose based compounds and especially wood in the production of paper, feeds, chemicals and fuels there is much interest in lignin degradation (Boominathan and Reddy, 1992). The white rot basidiomycetous fungi are believed to be the most effective lignin-degrading microbes with the production of lignin-modifying enzymes (LMEs) including lignin peroxidase (LiP, E.C. 1.11.1.14), manganese peroxidase (MnP, E.C. 1.11.1.13) and laccase (Lac, E.C.1.10.3.2) (Pointing, 2001). Laccase is multi-copper containing oxidase (O'Malley et al., 1993)

which catalyzed the monoelectric oxidation of a range of inorganic and aromatic substances with relatively low redox potentials with the concomitant reduction of O₂ to water (Baldrian, 2006), including degradation of lignin and related compounds (Xu, 1996). The redox potential of laccase is rather low and less than those of non-phenolic substrates, which would be expected to limit their role in lignin degradation. However, in the presence of an appropriate effective redox mediator, or laccase-mediator system, laccases can oxidize the non-phenolic lignin structure (Bourbannais and Paice, 1996; Bourbannais et al., 1997).

Due to their abilities to degrade lignin and other phenolic compounds, laccase has been widely applied to different biotechnological processes, such as biopulping with a patented Lignozyme[®] process and the degradation of synthetic dyes as part of the waste treatment process in the textile industry (Howard et al., 2003). Moreover, the

*Corresponding author. E-mail: phunsa@chula.ac.th.

combined action of intracellular cytochrome P450 and extracellular laccase and peroxidase enzyme reactions enable white rot fungi to degrade a variety of toxic organopollutants, such as polychlorinated phenols, polychlorinated dibenzo-p-dioxins and polycyclic aromatic hydrocarbon (PAHs) and other xenobiotics (Boominathan and Reddy, 1992).

PAHs are recognized as a group of chemicals that are formed during the incomplete burning of coal, oil, gas, or other organic substances, contain several fused benzene rings and are biotoxic compounds with potentially carcinogenic and mutagenic properties (Cerniglia, 1997). Indeed, PAHs are currently regarded as ones of EPA-priority pollutants.

Members of white rot basidiomycetous fungi have been reported as one group of organisms which extensively mineralize the recalcitrant PAHs due to their abilities to produce ligninolytic enzymes (Pointing, 2001). Certain white rot fungi have been well recognized for these capabilities including *Pluerotus* sp., *Phanerochaete chrysosporium*, *P. laevis*, *Trametes versicolor* (Pointing, 2001) and *Agrocybe* sp. (Chupungars et al., 2009). Recently an isolate of *Ganoderma lucidum* was identified as a dominant laccase producer (Murugesan et al., 2007). However, the ability of tropical white rot fungi, including *G. lucidum*, for PAHs degradation has yet to be described.

In this research, we investigated the ability of the laccase enzyme in the crude extracellular extract from a tropical isolate of white rot fungus, *G. lucidum* Chaaim-001 BCU, to degrade PAHs with or without the presence of 1-hydroxybenzotriazole (HOBT) as a redox mediator.

MATERIALS AND METHODS

The *G. lucidum* isolate Chaaim-001 BCU (isolated from tree stump in Pattani, Thailand) was maintained on potato dextrose agar (PDA) slants and deposited in the Fungi Section of Herbarium of Plant Biomass Utilization Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University. The culture was subsequently transferred to yeast malt agar (YMA, Difco) plates and incubated at 30°C for growth. The fungus was tested for thermotolerance by incubating at 25, 30, 35, 40 and 45°C and monitoring the growth by measuring the colony diameter. The crude extracellular extract containing laccase was prepared from the culture filtrates of the fungus grown in a semi-solid state cultivation on eucalyptus paper pulp (Punnapayak et al., 2005). The medium was filtered, assayed for laccase activity and the liquid filtrate was further concentrated by membrane filtration (10,000 Da MW cut off, Millipore). The assay for laccase activities was performed in triplicate using 2,6-dimethoxy phenol (2,6-DMP, Sigma) as the substrate (Bourbonnais and Paice, 1990). For the PAHs degradation test, sixteen of the EPA-priority pollutants including acenaphthene (ACE), acenaphthylene (ACY), anthracene (ANT), benzo[a]anthracene (BaA), dibenzo[ah]anthracene (BahA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[ghi]perylene (BghiPER), benzo[k]fluoranthene (BkF), chrysene (CHR), fluoranthene (FLA), fluorene (FLU), indole[123-cd]pyrene (IcdPYR), naphthalene (NAP), phenanthrene (PHE) and pyrene (PYR) were evaluated as substrates. Each PAH was dissolved to the final con-

centration of 16 ppm in 0.3% (w/w) tween 80. The reaction mixture (600 µl) consisted of 1.52 U/ml laccase, 50 mM sodium acetate buffer (pH 5.0), PAH solution and water with or without the presence of HOBT as the redox mediator. The reaction mixture was incubated in darkness for three days at 30°C with agitation (120 rpm) and then stopped by the addition of 600 µl acetonitrile. The degradation profile was detected by HPLC (Hewlett Packard series 1100) as previously described (Srebotnik and Hemmel, 2000).

RESULTS AND DISCUSSION

The *G. lucidum* species complex is one of the most broadly distributed white rot fungi, thriving in both temperate and tropical areas. It is of interest to investigate whether this isolate (Chaaim-001 BCU) of the fungus, obtained from a tropical country like Thailand, is thermotolerant. *G. lucidum* Chaaim-001 BCU grew well at 30°C, it grew poorly to not at all at temperatures above 30°C and thus appeared to not be thermotolerant. When cultivated on eucalyptus pulp, *G. lucidum* Chaaim-001 BCU yielded a laccase activity up to 0.507 U/ml. This crude enzyme preparation was further concentrated by membrane filtration (10,000 Da MW cut off) to 7.5 fold increased activity and was then used for the subsequent PAHs degradation test. Sixteen types of PAH were used as substrates to evaluate the degradation activity by this crude laccase preparation. A crude laccase from *Trametes versicolor*, a known PAH-degrading basidiomycetes fungus, was included for comparison as a reference.

When assayed for degradation, NAP could not be detected from the HPLC peaks, presumably because NAP was vaporized under the HPLC condition used in this study and thus was excluded from further analysis. Under the above fungal growth and extract assay conditions a higher level of degradation was observed when HOBT was present and appeared essential for degradation to any extent for seven of the 15 PAHs (BahA, BbF, BghiPER, BkF, CHR, FLA and PYR) for the extract from *T. versicolor* and were degradable for that from *G. lucidum* Chaaim-001 BCU (4/7; BahA, BkF, CHR and PYR) (Table 1). In the presence of HOBT, all 15 of the tested PAHs were degraded to some extent by the *T. versicolor* laccase, although only 11 of these were at levels above 40%. Only 13 and 6 PAHs were degraded at levels greater than 80 and 40%, respectively, by the *G. lucidum* Chaaim-001 BCU laccase. Complete degradation of FLU, ANT, BaA and BaP was observed under these conditions with the *T. versicolor* laccase, whereas only ANT and BaP were completely degraded by the *G. lucidum* Chaaim-001 BCU laccase. The lowest degradation level detected with the *T. versicolor* laccase was 1.59% for FLA whereas *G. lucidum* Chaaim-001 BCU laccase did not degrade BbF, IcdPYR and FLA. In the absence of HOBT, *T. versicolor* laccase dramatically lost its capability to degrade PAHs, especially BkF, BahA,

Table 1. Percent degradation of PAHs by laccases from *G. lucidum* Chaaim-001 BCU and *T. versicolor*.

PAHs	Degradation (%)*			
	Laccase from <i>T. versicolor</i>		Laccase from <i>G. lucidum</i>	
	With mediator	Without mediator	With mediator	Without mediator
ACE	92.00	48.35	95.36	80.49
ACY	73.64	40.18	90.09	85.85
ANT	100.00	79.55	100.00	99.41
BaA	100.00	1.30	85.30	9.14
BahA	93.68	0.00	36.53	0.00
BaP	100.00	31.10	100.00	71.71
BbF	38.69	0.00	0.00	0.00
BghiPER	93.16	0.00	20.33	0.00
BkF	79.65	0.00	21.71	0.00
CHR	37.34	0.00	7.93	0.00
FLA	1.59	0.00	0.00	0.00
FLU	100.00	15.71	98.63	62.86
IcdPYR	85.84	0.00	0.00	0.00
PHE	25.85	2.14	11.53	7.45
PYR	89.64	0.00	30.71	0.00

*Compared to the control reaction (without enzyme added).

BghiPER, IcdPYR and PYR, whilst only one PAH (ANT) was degraded more than 50%. In the absence of HOBT, expecting the seven common PAHs that neither the *T. versicolor* laccase nor the *G. lucidum* laccase could degrade (BahA, BbF, BghiPER, CHR, FLA and IcdPYR), the laccase from *G. lucidum* Chaaim-001 BCU appeared to retain a greater PAH-degrading capability than that of *T. versicolor*. Almost the same level of degradation was observed when ANT, ACY and ACE were exposed to *G. lucidum* Chaaim-001 BCU laccase with and without HOBT, whilst more than 50% degradation of FLU and BaP was observed. In most reports of PAHs degradation by microbial laccases, a redox mediator was found to be important for accelerating such degradation. For example, addition of the synthetic redox mediator ABTS was reported to increase the oxidation rate of phenanthrene by the *T. versicolor* laccase by about 40% (Han et al., 2004), whilst the degradation of PAHs by laccase from the white rot fungus, *Rigidoporous lignosus*, was reported to be insignificant without the addition of a mediator (Cambria et al., 2008). However, we found that the laccase from both *T. versicolor* and *G. lucidum* could degrade some PAHs, although less efficiently, without the addition of HOBT redox mediator.

Conclusion

The laccase produced from *G. lucidum* Chaaim-001 BCU isolate was able to degrade some of 12/15 tested types

of PAH and could degrade anthracene completely with or without a redox mediator. The enzyme could also degrade benzo[a]pyrene, fluorine, acenaphthene, acenaphthylene and benzo[a]anthracene at high (> 80%) and medium (> 60%) degradation levels when the redox mediator was present or not, respectively. It is of interest that *G. lucidum* Chaaim-001 BCU laccase was still able to degrade such compounds without a redox mediator given its expected lower redox potential and this paradox awaits further investigation to solve.

ACKNOWLEDGMENTS

The authors wish to thank Oesterreichischer Austauschdienst (Austria), the Royal Golden Jubilee (RGJ) Ph.D. grant 2. B. CU /47/ F. 1 contract number PHD/0168/2547 and the Ministry of Education, Thailand for financial supports. Valuable suggestions from Prof. Dr. E. Srebotnik are highly appreciated. Technical assistance from P. Wulz, M. Weisgram and T. Kuncinger are acknowledged.

REFERENCES

- Boominathan K, Reddy CA (1992). Fungal degradation of lignin: biotechnological applications, Arora DK, Elander RP, Mukerji KG (ed)., In handbook of applied mycology, Vol. 4. Marcel Dekker Inc., New York, pp. 763-822.
- Pointing SB (2001). Feasibility of bioremediation by white-rot fungi. Appl. Microbiol. Biotechnol. 57: 20-33.
- O'Malley DM, Whetten R, Bao W, Chen CL, Sederoff R (1993). The role

- of laccase in lignification. *Plant J.* 4: 751-757.
- Baldrian P (2006). Fungal laccases-occurrence and properties. *FEMS. Microbiol. Rev.* 30: 215-242.
- Xu F (1996). Oxidation of phenols, anilines, and benzenethiols by fungal laccases: correlation between activity and redox potentials as well as halide inhibition. *Biochemistry*, 35: 7607-7614.
- Bourbannais R, Paice MG (1996). Enzymatic delignification of Kraft pulp using laccase and a mediator. *TAPPI. J.* 79: 199-204.
- Bourbannais R, Paice MG (1990). Oxidation of nonphenolic substrates-an expanded role for laccase in lignin degradation. *FEBS. Lett.* 267: 99-102.
- Bourbannais R, Paice MG, Freiermuth B, Brodie E, Borneman S (1997). Reactivities of various mediators and laccases with Kraft pulp and lignin model compounds. *Appl. Environ. Microbiol.* 63: 4627-4623.
- Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S (2003). Lignocellulose biotechnology: issues of bioconversion and enzyme production. *Afr. J. Biotechnol.* 2: 602-619.
- Cerniglia CE (1997). Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. *J. Ind. Microbiol. Biotechnol.* 19: 324-333.
- Chupungars K, Rerngsamran, and Thaniyavarn S (2009). Polycyclic aromatic hydrocarbons degradation by *Agrocybe* sp. CU-43 and its fluorene transformation. *Int. Biodeterior. Biodegrad.* 63: 93-99.
- Murugesan K, Nam IH, Kim YM, Chang YS (2007). Decolorization of reactive dyes by a thermostable laccase produced by *Ganoderma lucidum* in solid state culture. *Enzyme Microbiol. Technol.* 40: 1662-1672.
- Srebotnik E, Hemmel KE (2000). Degradation of non-phenolic lignin by the laccase- /1-hydroxybenzotriazole system. *J. Biotechnol.* 81: 179-188.
- Punnapayak H, Kuhirun M, Watanabe T (2005). Liquid-state prebleaching of paper pulp with white rot fungi. *J. Natl. Res. Council, Thailand.* 37: 25-35.
- Han MJ, Choi HT, Song HG (2004). Degradation of phenanthrene by *Trametes versicolor* and its laccase. *J. Microbiol.* 42: 94-98.
- Cambria MT, Minniti Z, Librando V, Crambria A (2008). Degradation of polycyclic aromatic hydrocarbons by *Rigidoporus lignosus* and its laccase in the presence of redox mediators. *Appl. Microbiol. Biotechnol.* 149: 1-8.