J. Microbiol. Biotechnol. (2007), **17**(7), 1147–1151

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Biodegradation of Endocrine-disrupting Bisphenol A by White Rot Fungus *Irpex lacteus*

SHIN, EUN-HYE, HYOUNG TAE CHOI, AND HONG-GYU SONG*

Division of Life Sciences and Research Institute of Life Sciences, Kangwon National University, Chuncheon 200-701, Korea

Received: January 19, 2007 Accepted: March 10, 2007

Abstract Biodegradation of endocrine-disrupting bisphenol A was investigated with several white rot fungi (*Irpex lacteus*, *Trametes versicolor*, *Ganoderma lucidum*, *Polyporellus brumalis*, *Pleurotus eryngii*, *Schizophyllum commune*) isolated in Korea and two transformants of *T. versicolor* (strains MrP 1 and MrP 13). *I. lacteus* degraded 99.4% of 50 mg/l bisphenol A in 3 h incubation and 100% in 12 h incubation. which was the highest degradation rate among the fungal strains tested. *T. versicolor* degraded 98.2% of 50 mg/l bisphenol A in 12 h incubation. Unexpectedly, the transformant of the Mn-repressed peroxidase gene of *T. versicolor*, strain MrP 1, degraded 76.5% of 50 mg/l bisphenol A in 12 h incubation, which was a lower degradation rate than wild-type *T. versicolor*. The removal of bisphenol A by *I. lacteus* occurred mainly by biodegradation rather than adsorption. Optimum carbon sources for biodegradation of bisphenol A by *I. lacteus* were glucose and starch, and optimum nitrogen sources were yeast extract and tryptone in a minimal salts medium; however, bisphenol A degradation was higher in nutrient-rich YMG medium than that in a minimal salts medium. The initial degradation of endocrine disruptors was accompanied by the activities of manganese peroxidase and laccase in the culture of *I. lacteus*.

Keywords: Biodegradation, bisphenol A, endocrine disruptor, white rot fungi

Since the late 20th century, certain chemicals have been known to affect the reproductive systems of various animals including human beings, and the compounds mimicking or interfering with the action of endogenous gonadal steroid hormones have been named endocrine-disrupting chemicals (EDCs) [3]. Their harmful effects are reported to expand to chromosomal abnormalities [23] and earlier onset of diseases such as mammary and prostate cancers [16]. Among 67 chemicals registered as EDCs by the Ministry of Environment, Korea, bisphenol A [2,2-bis(4-hydroxyphenyl)propane] is widely used as a material for the production of epoxy and phenol resins, polycarbonates, polyester, and lacquer coatings on food cans, and released to environments from bisphenol-A-producing factories, leaching of plastic wastes, and landfill sites [22].

Because of the potential adverse health and ecological effects, the removal of EDCs including bisphenol A is very important and has been extensively studied [3, 9]. There are increasing concerns about the degradation of EDCs by white rot fungi. There are several reasons for the attractiveness of white rot fungi in the degradation of environmental pollutants, such as the mineralizing capability of a wide variety of toxic xenobiotics, potential to oxidize substrates with low water solubility, the constitutive nature of the key enzymes (ligninolytic enzymes) involved in degradation, and the inexpensive growth substrates [18]. Recently, the degradation of EDCs by lignin-degrading enzymes has attracted the attention of many researchers [6, 14, 17, 20]. However, fungi with high bisphenol A degradability are also limited [1], and the fungal strains having a high degradability are critical to the application of white rot fungi or their ligninolytic enzymes to elimination of EDCs including bisphenol A. In this study, several white rot fungi isolated in Korea, which were active in degrading polycyclic hydrocarbons and trinitrotoluene [5, 11], were tested for their removal of bisphenol A.

MATERIALS AND METHODS

Chemicals and Fungal Strains

Bisphenol A and HPLC-grade organic solvents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A.) and Fisher Scientific Co. (Pittsburg, PA, U.S.A.), respectively. The fungal strains tested were *Irpex lacteus*, *Trametes versicolor*, *Fomitella fraxinea*, *Merulis tremellosus*, *Ganoderma lucidum*, *Polyporellus brumalis*, *Pleurotus ostreatus*, and *Schizophyllum commune*, which

*Corresponding author
Phone: 82-33-250-8545; Fax: 82-33-251-3990;
E-mail: hgsong@kangwon.ac.kr

were all isolated in Korea, and *T. versicolor* MrP 1 and MrP 13 (transformants of the Mn-repressed peroxidase gene). Fungi were cultivated on YMG medium (yeast extract 4 g, malt extract 10 g, and glucose 4 g in 1 l H_2O) solidified with 2% agar at 30°C with different incubation periods.

Fungal Degradation of Bisphenol A

Five agar plugs (5×5 mm) of active mycelium of each fungal strain were grown in YMG broth on a rotary shaker (130 rpm) for 5 days at 30°C, and the fungal culture was blended with a homogenizer. Culture flasks containing 20 ml of YMG medium or Kirk's basal salts medium [24] were inoculated with 0.5 ml of the blended fungal inoculum. Bisphenol A dissolved in methanol (5,000 mg/l) was added at the beginning or after 5 days of incubation. During the incubation of the fungi at 30°C (130 rpm), the residual bisphenol A was analyzed at certain time intervals. The whole fungal culture was mixed with an equal volume of HPLC-grade dichloromethane in a separatory funnel, shaken for 10 min at 250 rpm on a reciprocal extraction shaker (Jeio Tech., Korea), and the dichloromethane phase containing residual bisphenol A was separated. This process was repeated three times and the dichloromethane was combined. After removal of water in extracts with anhydrous sodium sulfate, dichloromethane was concentrated with a rotary vacuum evaporator and the residual bisphenol A was measured.

For investigation of the removal mechanism of bisphenol A, the fungal culture was separated into supernatant and mycelium by centrifugation (6,140 ×g, 30 min), and the mycelium was homogenized and extracted with dichloromethane. The residual bisphenol A in whole fungal culture and associated mycelium were quantified and compared.

Effects of the addition of different carbon sources (5 g/l each of glucose, sucrose, fructose, glycerol, lactose, and starch) and nitrogen sources (0.22 g/l each of peptone, tryptone, yeast extract, asparagine, ammonium tartrate, ammonium nitrate, ammonium sulfate, and sodium nitrate) in Kirk's basal salts medium on bisphenol A degradation were investigated. The effect of preincubation on bisphenol A degradation was also examined. All the procedures utilized were the same as described above.

Analytical Methods

Bisphenol A was analyzed by HPLC (Waters, U.S.A.) using a Gemini C6-phenyl column (5 μm, 150×4.6 mm, Phenomenex, U.S.A.). The sample was eluted by a linear gradient from 50% acetonitrile in water to 100% acetonitrile (0–15 min), 100% acetonitrile (15–20 min), a linear gradient from 100 to 50% acetonitrile (20–25 min), and 50% acetonitrile (25–30 min) at a flow rate of 1.0 ml/min. The residual bisphenol A was quantified by a UV detector at 280 nm.

During the degradation of bisphenol A, the activities of ligninolytic enzymes were measured from replicate cultures. After centrifugation of the fungal culture, the supernatant was removed and the activities of lignin peroxidase and manganese-dependent peroxidase were detected by the methods of Tien and Kirk [24] and laccase activity was measured by a method of Ross [19].

All experiments were carried out in triplicate, and the mean values are presented.

RESULTS AND DISCUSSION

Fungal Degradation of Bisphenol A

During 12 h of incubation with 50 mg/l bisphenol A in YMG medium, *Irpex lacteus* could remove 100% of bisphenol A, which was the highest degradation rate among the fungal strains tested (Table 1). Most of the bisphenol A was removed during a further 12-h incubation by *Trametes versicolor* and *T. versicolor* MrP 1, and the other fungi showed somewhat lower degradation rates of bisphenol A, which was likely due to toxicity of bisphenol A [9]. The growth of *I. lacteus* was inhibited by addition of bisphenol A, and 21.7, 56.0, and 73.6% of dry weight of mycelium decreased during 48 h of incubation in YMG medium containing 10, 50, and 100 mg/l bisphenol A, respectively. However, the degradation rate of bisphenol A was highest at 50 mg/l (date not shown), and all the experiments were carried out with 50 mg/l bisphenol A. When the incubation time was shortened, *I. lacteus* could degrade 99.4% of 50 mg l^{-1} bisphenol A within 3 h. Removal of bisphenol A by *I. lacteus* and *T. versicolor* was much faster than 26 strains of various fungal groups, all of which did not belong to white rot fungi [1]. Among 26 strains, 4 strains could remove 100% of 40 ppm bisphenol A effectively after several days of incubation. A white rot fungus, *Pleurotus ostreatus*, also degraded only 80% of 0.4 mM bisphenol A in 12 days [6]. Compared with these fungi, *I. lacteus* and *T. versicolor* are certainly effective degraders of bisphenol A, and their removal rates were also higher than those of bisphenol-A-degrading bacteria isolated from rivers [7, 8]. *I. lacteus* and *T. versicolor* could also efficiently degrade polycyclic aromatic

Table 1. Degradation of 50 mg/l bisphenol A by several white rot fungi in YMG medium.

Fungal strain	Bisphenol A removal (%)	
	12 h	24 h
Irpex lacteus	100.0±0.0	100±0
Trametes versicolor	98.2±0.2	98.6±0.2
Trametes versicolor MrP 1	76.5±0.7	98.1±0.1
Trametes versicolor MrP 13	27.5±10.6	35.5±2.1
Ganoderma lucidum	7.4±3.1	38.5±0.1
Polyporellus brumalis	2.5±3.5	24.0±7.0
Pleurotus eryngii	2.9±4.0	11.8±3.3
Schizophyllum commune	Not detected	45.5±0.7

Table 2. Comparison of the removal mechanisms of bisphenol A by *Irpex lacteus* in 3 h of incubation.

Mechanism	Bisphenol A removal (%)
Biodegradation	98.2±0.5
Biosorption	1.2±0.1
Total removal	99.4±0.6

hydrocarbons and trinitrotoluene [10, 21]. Unexpectedly, the degradation rates *of T. versicolor* MrP strains, especially MrP 13, were not higher than that of wild-type *T. versicolor*. *T. versicolor* MrP strains were constructed by overexpression of a manganese-repressed peroxidase gene (*mrp*), and they showed a higher degradation rate of remazol brilliant blue R and dinitrotoluene [12]. Further study on the biodegrading capability of these transformants is necessary. Since the removal rates of other EDCs such as alkylphenols and phthalates by some fungi showing lower degradation rates of bisphenol A were as high as *I. lacteus* and *T. versicolor* (data not shown), the optimal conditions for bisphenol A degradation by these fungal strains should be also investigated.

Considering the high degradation rate of bisphenol A by *I. lacteus*, its removal mechanism was examined. *I. lacteus* degraded a total of 99.4% of bisphenol A from the culture within 3 h. When the fungal mycelium was separated by centrifugation, the residual bisphenol A adsorbed on mycelium was only 1.2% (Table 2). Therefore, most of bisphenol A was removed by fungal metabolism, and this phenomenon was quite similar to the trinitrotoluene degradation by the same fungus [10]. The removal seemed to take place by the action of extracellular degrading enzymes, especially ligninolytic enzymes. Purified laccase from a fungus of family Chaetomiaceae could oxidize 99.2 and 100% of 5 mM bisphenol A, within 3 and 6 h of reaction, respectively [20]. This oxidizing rate of bisphenol A by purified laccase was similar to the removal rate in the culture of *I. lacteus*, in spite of the higher bisphenol A concentration in this study.

Degradation of Bisphenol A by *Irpex lacteus* in Different Conditions

The effects of several environmental conditions on bisphenol A degradation were examined, with *I. lacteus* showing the highest degradation rate. When bisphenol A was added into YMG medium simultaneously with the fungal inoculum, over 99% of bisphenol A was removed within 6 h and 100% was degraded within 24 h (Fig. 1). In contrast, when bisphenol A was added into 5-day preincubated fungal culture, 85 and 98% of bisphenol A disappeared at 6 and 24 h of further incubation, respectively. This difference may be due to the exhaustion of growth substrate in the 5-day preincubated culture. When the growth substrate was additionally provided, increase of mineralization of trinitrotoluene occurred in the culture of the same fungus [11]. Removals of 50 mg/l bisphenol A by *I. lacteus* in nutrient-rich YMG medium and minimal salts medium were compared. In YMG medium, over 99% of bisphenol A was removed within 3 h, which was higher than the 73% removal in the minimal medium. Fungal growth was also better in YMG medium than in minimal salts medium (data not shown). A similar pattern of degradation was also observed in trinitrotoluene degradation by the same fungus [10]. Kang and Kondo [7] reported higher bisphenol A degradation with higher bacterial counts. Therefore, continuous addition of growth substrate may promote sustaining degradation of bisphenol A by fungi in a continuous treatment process.

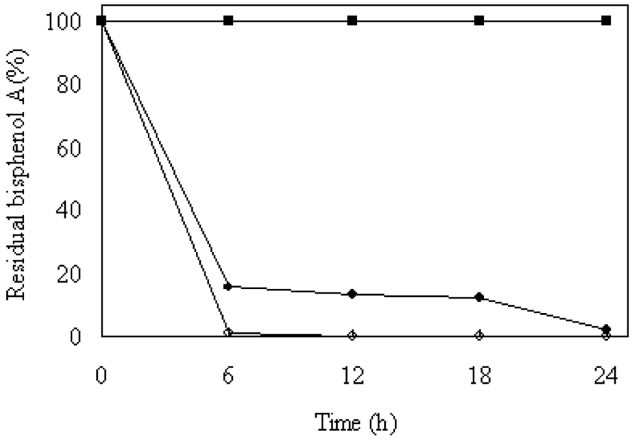

Fig. 1. Effect of preincubation on the biodegradation of 50 mg/l bisphenol A by *Irpex lacteus*.
Symbols: uninoculated control (■), 5-day preincubated culture (●), culture without preincubation (○).

For the investigation of effects of carbon and nitrogen sources on bisphenol A degradation, several different carbon substrates and nitrogen compounds were added to minimal salts medium, and bisphenol A degradations were examined. Addition of starch and glucose showed the highest removal of 50 mg/l bisphenol A, 73 and 70%, respectively, within 3 h (Fig. 2A). The culture of *I. lacteus* on other carbon substrates exhibited less than 50% removal. Among nitrogen sources, yeast extract and tryptone showed 71.9 and 70.1% removal, respectively (Fig. 2B). Since the maximal activity of ligninolytic enzymes of different fungi may occur with different carbon and nitrogen sources and environmental conditions [15, 25], more carbon and nitrogen sources and different concentrations of each compound should be tested in bisphenol A removal for the application of fungal degradation to treatment of bisphenol-A-containing wastewater.

Activities of Ligninolytic Enzymes During Bisphenol A Degradation

Extracellular ligninolytic enzymes are known to be involved in various recalcitrant compounds including bisphenol A [18, 20]. During bisphenol A degradation by *I. lacteus* in YMG medium, activities of typical ligninolytic enzymes,

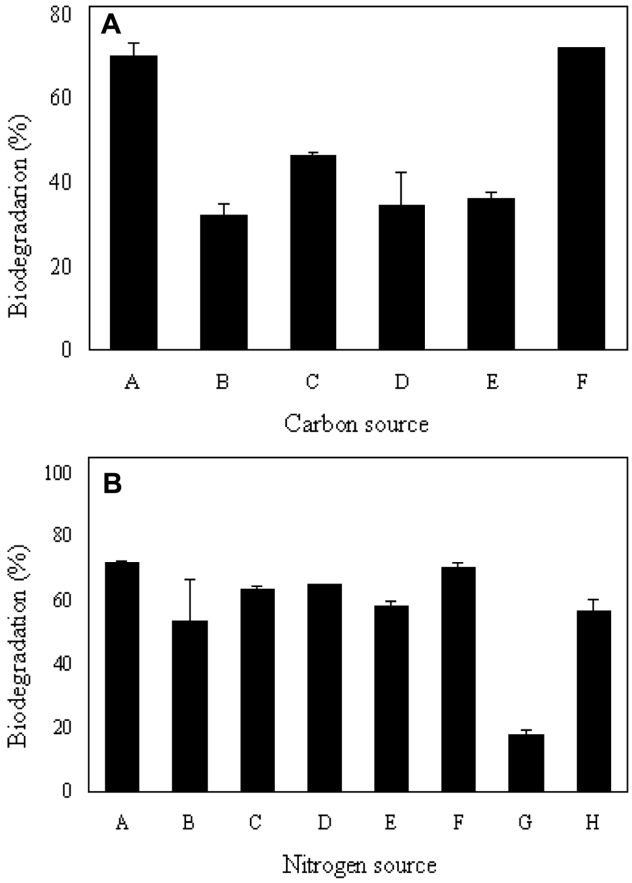

Fig. 2. Degradation of 50 mg/l bisphenol A in 3-h incubation by *Irpex lacteus* with various carbon sources (**A**) (A, glucose; B, sucrose; C, fructose; D, glycerol; E, lactose; F, starch) and nitrogen sources (**B**) (A, yeast extract; B, $NaNO_3$; C, peptone; D, asparagine; E, ammonium tartrate; F, tryptone; G, ammonium nitrate; H, ammonium sulfate).

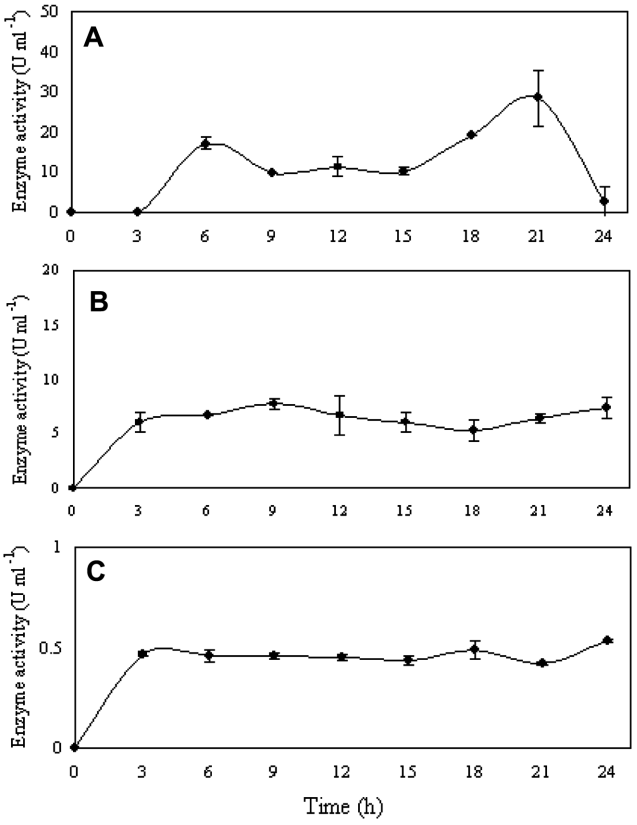

Fig. 3. Activities of ligninolytic enzymes, lignin peroxidase (**A**), manganese peroxidase (**B**) and laccase (**C**), of *Irpex lacteus* during degradation of 50 mg/l bisphenol A in YMG medium.

lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase, were measured. MnP showed rapid increase of activity within 3 h of incubation and maintained a stable level until 24 h (Fig. 3). Although the activity level was somewhat low, laccase also showed a similar pattern of activity. In contrast, LiP did not exhibit activity until 3 h, in which most bisphenol A was oxidized, and showed activity peaks at 6 and 21 h. Therefore, MnP and laccase might be involved in bisphenol A degradation by *I. lacteus*. Those two enzymes have also been reported to oxidize bisphenol A in different fungi [6, 20]. Furthermore, purified laccase or MnP could also rapidly oxidize bisphenol A [6]. Optimization of production of laccase and MnP in the culture of *I. lacteus* or *T. versicolor* is necessary for efficient bisphenol A removal [4]. Since bisphenol A oxidation by laccase or MnP can be increased by addition of mediators [9] or addition of some additives such as polyethylene glycol to maintain enzyme stability [14] or substrate analogs [2], and immobilized biomass or enzymes can be used in oxidizing reactions [13], these applications should be tested for the better enzymatic transformations of bisphenol A by the *I. lacteus* or *T. verscolor* used in this study.

Acknowledgments

This subject is supported by the Korea Ministry of Environment as "The Eco-technopia 21 project." The authors are thankful to Drs. J. Sung in Kangwon National University and K. Kim in Kangnung National University for providing the fungal strains.

REFEENCES

1. Chai, W., Y. Handa, M. Suzuki, M. Saito, N. Kato, and C. Horiuchi. 2005. Biodegradation of bisphenol A by fungi. *Appl. Biochem. Biotechnol.* **120:** 175–182.
2. Chakraborty, J. and T. Dutta. 2006. Isolation of a *Pseudomonas* sp. capable of utilizing 4-nonylphenol in the presence of phenol. *J. Microbiol. Biotechnol.* **16:** 1740–1746.

3. Colborn, T., D. Dumanoski, and J. Myers. 1996. *Our Stolen Future*. Dutton, New York, U.S.A.
4. Gohel, V., D. Jiwan, P. Vyas, and H. Chhatpar. 2005. Statistical optimization of chitinase production by *Pantoea dispersa* to enhance degradation of Crustacean chitin waste. *J. Microbiol. Biotechnol.* **15:** 197–201.
5. Han, M., H. Choi, and H. Song. 2005. Purification and characterization of laccase from the white rot fungus *Trametes versicolor*. *J. Microbiol.* **43:** 555–560.
6. Hirano, T. H., Y. Honda, T. Watanabe, and M. Kuwahara. 2000. Degradation of bisphenol A by the lignin-degrading enzyme, manganese peroxidase, produced by the white-rot basidiomycete, *Pleurotus ostreatus*. *Biosci. Biotechnol. Biochem*. **64:** 1958–1962.
7. Kang, J. H. and F. Kondo. 2002. Bisphenol A degradation by bacteria isolated from river water. *Arch. Environ. Contam. Toxicol.* **43:** 265–269.
8. Kang, J. H., N. Ri, and F. Kondo. 2004. *Streptomyces* sp. strain isolated from river water has high bisphenol A degradability. *Lett. Appl. Microbiol*. **39:** 178–180.
9. Kang, J. H., Y. Katayama, and F. Kondo. 2006. Biodegradation or metabolism of bisphenol A: From microorganism to mammals. *Toxicology* **217:** 81–90.
10. Kim, H. and H. Song. 2000. Transformation of 2,4,6-trinitrotoluene by white rot fungus *Irpex lacteus*. *Biotechnol. Lett.* **22:** 969–975.
11. Kim, H. and H. Song. 2003. Transformation and mineralization of 2,4,6-trinitrotoluene by the white rot fungus *Irpex lacteus*. *Appl. Microbiol. Biotechnol*. **61:** 150–156.
12. Kim, Y. 2005. Cloning and expression analysis of manganese-dependent peroxidase and Mn-repressed peroxidase, overexpression of its gene by genetic transformation. Dissertation for the degree of M.Sc., Dept. Microbiol., Graduate School, Kangwon National University.
13. Kim, J., H. W. Ryu, D. J. Jung, T. H. Lee, and K.-S. Cho. 2005. Styrene degradation in a polyurethane inoculated with *Pseudomonas* sp. IS-3. *J. Microbiol. Biotechnol.* **15:** 1207–1213.
14. Kim, Y.-J. and J. Nicell. 2006. Laccase-catalyzed oxidation of bisphenol A with the aid of additives. *Process Biochem*. **41:** 1029–1037.
15. Lee, Y., C. Park, B. Lee, E. Han, T. Kim, J. Lee, and S. Kim. 2006. Effect of nutrients on the production of extracellular enzymes for decolorization of reactive blue 19 and reactive black 5. *J. Microbiol. Biotechnol.* **16:** 226–231.
16. Maffini, M., B. Rubin, C. Sonnenschein, and A. Soto. 2006. Endocrine disruptors and reproductive health: The case of bisphenol-A. *Mol. Cell. Endocrinol*. **254–255:** 179–186.
17. Modaressi, K., K. Taylor, J. Bewtra, and N. Biswas. 2005. Laccase-catalyzed removal of bisphenol-A from water: Protective effect of PEG on enzyme activity. *Wat. Res*. **39:** 4309–4316.
18. Reddy, C. A. 1995. The potential for white-rot fungi in the treatment of pollutants. *Curr. Opin. Biotechnol.* **6:** 320–328.
19. Ross, I. K. 1982. The role of laccase in carpophore initiation in *Coprinus congregatus*. *J. Gen. Microbiol.* **128:** 2763–2770.
20. Saito, T., K. Kato, Y. Yokogawa, M. Nishida, and N. Yamashita. 2004. Detoxification of bisphenol A and nonylphenol by purified extracellular laccase from a fungus isolated from soil. *J. Biosci. Bioeng.* **98:** 64–66.
21. Song, H. 1997. Biodegradation of aromatic hydrocarbons by several white-rot fungi. *J. Microbiol.* **35:** 66–71.
22. Staples, C., P. Dorn, G. Klecka, S. O'Block, and L. Harris. 1998. A review of the environmental fate, effects, and exposures of bisphenol A. *Chemosphere* **36:** 2149–2173.
23. Susiarjo, M., T. Hassold, E. Freeman, and P. Hunt. 2006. Bisphenol A exposure *in utero* disrupts early oogenesis in the mouse. *PLoS Genet.* **3:** 1–8.
24. Tien, K. and T. Kirk. 1998. Lignin peroxidase of *Phanerochaete chrysosporium*. *Methods Enzymol.* **161:** 813–817.
25. Zhang, F., J. Knapp, and K. Tapley. 1999. Decolourisation of cotton bleaching effluent with wood rotting fungus. *Wat. Res.* **33:** 918–928.