Studies on the degradation of recalcitrant environmental pollutants by white-rot fungi

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

専　　攻：バイオサイエンス　　氏　名：王　剣橋
Course　：Department of Bioscience　　Name　：Jianqiao Wang

論文題目：白色腐朽菌による難分解性環境汚染物質の分解に関する研究
Title of Thesis　：Studies on the degradation of recalcitrant environmental pollutants by white-rot fungi

Abstract :

White-rot fungi can degrade lignin, a complex high-molecular-weight aromatic polymer, as well as a wide spectrum of recalcitrant organopollutants, including polycyclic aromatic hydrocarbons, polychlorinated biphenyl, polychlorinated dioxins, polychlorinated phenols and endocrine disrupting compounds. The white-rot fungus *Phanerochaete sordida* YK-624, isolated from rotted wood, showed much higher ligninolytic activity and selectivity than typical white-rot fungi *P. chrysosporium* and *Trametes versicolor*. In the present study, the biodegradation of mycotoxin aflatoxin B_1 (AFB), actamiprid (ACE) as a neonicotinoid insecticide, and endocrine-disrupting compounds (EDCs) by the white-rot fungus *P. sordida* YK-624 or ligninolytic enzymes were investigated.

1) Detoxification of AFB and ACE by the *P. sordida* YK-624 or ligninolytic enzymes

AFB is a potent mycotoxin with mutagenic, carcinogenic, teratogenic, hepatotoxic, and immunosuppressive properties. It is a frequent contaminant of many food products and one of the most potent naturally occurring mutagens and carcinogens known. Manganese peroxidase (MnP) from *P. sordida* YK-624 was able to degrade AFB, and the maximum degradation (86.0%) of AFB was observed by the reaction mixture containing 5 nkat MnP for 48 h treatment. The addition of Tween 80 accelerated the AFB degradation by MnP. Using umu test, it was also confirmed that MnP substantially removed the mutagenic activity of AFB. It became clear that MnP reaction system can transform AFB to AFB-8,9-dihydrodiol by ^{1}H NMR and ESI-MS analyses of a metabolite. These results suggest that AFB was oxidized to AFB-8,9-epoxide, and then hydrolyzed to AFB-8,9-dihydrodiol.

ACE belongs to the neonicotinoid class of systemic broad-spectrum insecticides, which are the most highly effective and largest-selling insecticides word-wide for crop protection. Under ligninolytic and non-ligninolytic conditions, 45% and 30% of ACE were eliminated, respectively, after 15 d of incubation. ESI-MS and NMR analyses of a metabolite identified in the culture supernatant suggested that ACE was *N*-demethylated to (E)-N^1-[(6-chloro-3-pyridyl)-methyl]-N^2-cyano -acetamidine, which has a much lower toxicity than ACE. In addition, the effect of the cytochrome P450 inhibitor piperonyl butoxide (PB) on the elimination of ACE was investigated. These results

suggest that cytochrome P450 plays an important role in the *N*-demethylation of ACE by *P. sordida* YK-624.

2) Effective removal of ECDs by lignin peroxidase (LiP) from *P. sordida* YK-624

Five endocrine disruptors, *p*-*t*-octylphenol (OP), bisphenol A (BPA), estrone, 17ß-estradiol (E_2), and ethinylestradiol (EE_2) were eliminated by LiP from *P. sordida* YK-624 (YK-LiP1) more effectively than LiP from *P. chrysosporium* (Pc-LiP), and OP and BPA were disappeared almost completely in the reaction mixture containing YK-LiP1 after a 24 h treatment. Particularly, the removal of estrogenic activities of E_2 and EE_2, which show much higher estrogenic activities than other EDCs such as BPA and OP, were removed following 24 h treatment with YK-LiP1. Moreover, 5,5′-bis(1,1,3,3-tetramethylbutyl)-[1,1′-biphenyl]-2,2′-diol and 5,5′-bis-[1-(4-hydroxy-phenyl)-1-methyl-ethyl]-biphenyl-2,2′-diol were identified as the main metabolite from OP or BPA, respectively. These results suggest that YK-LiP1 is highly effective in removing of EDCs by the oxidative polymerization of these compounds.

3) Metabolism of BPA by *P. sordida* YK-624

BPA is one of the representative compounds of the ECDs and the highest volume chemicals produced worldwide. As a result, BPA is often detected in many soil and water environments. The metabolisms of BPA by the white-rot fungus *P. sordida* YK-624 under ligninolytic and non-ligninolytic conditions were demonstrated.

Under ligninolytic condition, both 1 mM and 0.1 mM BPA were effectively decreased within a 24 h treatment and two metabolites were detected. Two metabolites (5,5′-bis-[1-(4-hydroxy-phenyl) 1-methyl-ethyl]-biphenyl-2,2′-diol and 4-(2-(4-hydroxy-phenyl)propan-2-yl)-2-(4-(2-(4-hydroxyphenyl) propan-2-yl)phenoxy)phenol) were identified by ESI-MS and NMR analyses. These results suggest that BPA was oxidized to BPA phenoxy radicals by ligninolytic enzymes and then dimerized at extracellular region under ligninolytic condition. Unfortunately, these BPA dimers are possible to be decomposed to toxic BPA by various reactions. Therefore, the transformation techniques of BPA without polymerization such as non-ligninolytic condition are necessary.

Under non-ligninolytic condition, approximately 80% of BPA was eliminated after 7 d of incubation. BPA was converted to 4-(2-(4-hydroxyphenyl)propan-2-yl)benzene-1,2-diol (hydroxy-BPA) by *P. sordida* YK-624. In addition, much lower transformation activity of BPA was observed in cultures containing PB. And, 66% of hydroxy-BPA was eliminated after 7 d of incubation. HR-ESI-MS and NMR analyses of the metabolites isolated from the culture broth indicated that hydroxy-BPA was metabolized to methoxy-BPA and to dimethoxy-BPA by sequential methylation events. These estrogenic activities of these metabolites were much lower than that of BPA. These results suggest that BPA is firstly monooxygenated to hydroxy-BPA by cytochrome P450, and then methylated by *P. sordida* YK-624 under non-ligninolytic condition.