Metabolites of 2,3,7,8-Tetrachloro- and Octachloro-dibenzo-*p*-dioxin, and Degradation of PCDDs and PCDFs in a Fly Ash Sample by the White Rot Fungus *Phanerochaete sordida* YK-624

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1. Introduction

The white rot fungus, *Phanerochaete chrysosporium*, has been shown to possess biodegradative capabilities for a wide variety of environmentally persistent xenobiotics to carbon dioxide, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), polycyclic aromatic hydrocarbons, 1,1,1-trichloro-2,2-bis(4-cjlorophenyl)ethane (DDT) and polychlorinated biphenyls.[1-4] Degradation of these pollutants was shown to be mediated by the lignin-degrading system of this fungus. The system includes lignin peroxidase (LiP) and manganese peroxidase (MnP) and H_2O_2-generating system. We reported recently that white rot fungus *Phanerochaete sordida* YK-624 isolated in our laboratory was capable of substantial degradation of a mixture of 10 kinds of the 2,3,7,8-substituted tetra- through octa- chlorodibenzo-*p*-dioxins and chlorodibenzofurans (2,3,7,8-PCDDs/PCDFs), as determined by substrate disappearances.[5] In this study, we embarked on the research to detect the metabolites of 2,3,7,8-TCDD and octachlorodibenzo-*p*-dioxin (OCDD) to verify the degradations of 2,3,7,8-PCDDs/PCDFs by YK-624. Also, degradability of a number of isomers of PCDD and PCDF congeners extracted from a fly ash sample by the fungus was investigated.

2. Materials and Methods

Strain.

Ligninolytic white rot fungus *Phanerochaete sordida* YK-624 strain,[6,7] which was isolated from rotted wood, was used in this study.

Chemicals.

2,3,7,8-TCDD (I) and OCDD (II) were purchased from GL Science Inc, Tokyo Japan. 4,5-Dichloro-1,2-dihdroxybenzene and tetrachloro-1,2-benzoquinone were purchased from Cambridge Isotope Laboratories (Andover, Mass.) and from Aldrich Chem. Co. (Milwaukee, Wis.), respectively. The latter was reduced to tetrachloro-1,2-dihydroxybenzene with sodium dithionite. The diacetyl derivatives were prepared by using Ac_2O and NaOH.

Metabolites of 2,3,7,8-TCDD and OCDD.

Biodegradation method was described earlier.[5] After 6 days of incubation, each substrate in EtOAc (10μl) was added to a culture to a final concentration of 50 ng for I and II. Cultures were oxygenated and glucose was added to the cultures every 3 days. After 10 days, sodium dithionite solution was added to reduce each supposable quinone product (described later) to the cultures. We did not conduct the confirmation of the quinone in this study. To recover unreacted substrates, after adjusting to pH 10 with NaOH, the culture was extracted with hexane and then it was placed in an ultrasonic generator for 10 min. This procedure was repeated two times. The pooled organic phases were dried over anhydrous

Na_2SO_4 and the solution was diluted with hexane until the total volume became exactly 100 ml. ^{13}C-2,3,7,8-TCDD and ^{13}C-OCDD were added to 1 ml of the solution and the concentrations of substrates were analyzed by high resolution gas chromatography and high resolution mass spectrometry (HRGC-HRMS) (selected ion monitoring mode: SIM). Aqueous phase contained mycelia was acidified with HCl to pH 2 and extracted with EtOAc (two times). The mixture was shaken vigorously and extracted by using an ultrasonic generator. The combined organic layer was washed with 5% NaCl, dried over Na_2SO_4, and evaporated under reduced pressure. After acetylation, derivatives were analyzed by HRGC-HRMS (SIM). 2',3,4-Trichlorobiphenyl was used as an internal standard. In these experiments, I and II were also incubated for 10 days with heat-killed controls.

HRGC-HRMS analysis of metabolites
The derivatives and substrates were analyzed by a Finnigan MAT-95 mass spectrometry fitted with a Hewllet Packerd 5890 series II gas chromatograph. A DB-5MS (J & W Scientific, Folsom, Ca.) column (0.32 mm [inner diameter] by 25 m; 0.52 μm film thickness) was used. The initial temperature, 70℃, was maintained for 2 min and then the temperature was increased to 280℃ by 10℃/min and was held for 20 min.

Degradation of PCDDs and PCDFs in fly ash by YK-624
A fly ash sample was obtained from a municipal incinerator plant in Japan, and 500 g of the sample were washed with 2N-HCl, filtered with a Buchner funnel and allowed to dry. The dried sample was soxlet-extracted with toluene for 24 h. The extract was evaporated to small volumes and was dried by slowly passing a stream of purified N_2 over the sample. The residue was dissolved with hexane, washed with concentrated H_2SO_4 and then with water. The hexane layer was dried over Na_2SO_4 and was evaporated to less than 1ml and the solvent exchanged to EtOAc (5 ml).
Degradation method and analytical procedures were described earlier.[5] In this experiment, 10ml of the acetone solution of fly ash extract were added to 2-day-old cultures (triplicate), and the cultures were incubated for 10 days. Controls cultures (duplicate) were performed with same procedure without inoculation.

3. Results and Discussion

Metabolites of 2,3,7,8-TCDD and OCDD by *P. sordida* YK-624.
The precise pathway for degradation of 2,7-dichlorodibenzo-*p*-dioxin by *P. chrysosporium* was elucidated by Valli et al,[8] who demonstrated that 4-chloro-1,2-benzoquinone was obtained by oxidative cleavage of dioxin ring catalyzed by LiP, but not MnP and then it was reduced to 1-chloro-3,4-dihydroxybenzene. We investigated whether the corresponding metabolites, such as quinone and dihydroxy derivatives, could be obtained in the degradation of I and II by YK-624, though the fungus secreted only MnP.[6,7] The expected metabolites, 4,5-dichloro- (III) and tetrachloro-1,2-dihdroxybenzene (IV), deduced from the study by Valli et al., are shown in Fig. 1. In this experiment, the supposable quinone products were reduced to dihydroxy derivatives. After acetylation, diacetyl derivatives were analyzed by HRGC-HRMS (SIM) and identified comparing with GC retention times, mass fragments (*m/z*) and the ion intensities of authentic standards. For III, mass spectrum *m/z* (relative intensity [%] shown in parentheses) were 177.959 (100.0), 179.956 (62.7), 181.953 (12.3), 219.969 (7.7), 221.966 (2.5), 261.980 (M, 2.1); GC retention time, 13.26 min. For IV, 245.881 (82.6), 247.878 (100.0), 249.875 (47.7), 287.873 (1.2), 289.870 (1.4), 291.867 (1.6), 331.989 (M+2, 4.0); GC retention time, 15.55 min.
These results suggest, therefore, that 2,3,7,8-TCDD (I) and OCDD (II) were substantially metabolized by *P. sordida* YK-624, suggesting that the fungus has essentially the ability of degradation of I and II. No corresponding degradation products were detected in the control cultures.
We have confirmed that *P. chrysosporium* as well as YK-624 was capable of substantial degradation of 2,3,7,8-PCDDs/PCDFs (data not shown), and also examined whether MnP prepared from *P. sordida* YK-624 took part in degradation of 2,3,7,8-PCDDs/PCDFs. However, no degradation was observed (data not shown). The result indicate therefore that another enzyme system to degrade 2,3,7,8-PCDDs/PCDFs might be included in YK-624 under nitrogen-limiting condition.

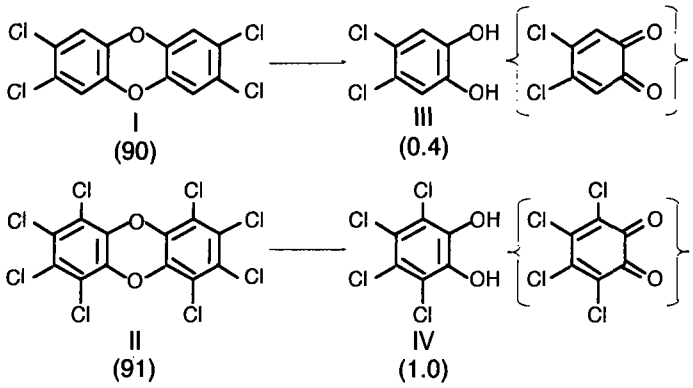

Fig. 1 Metabolites identified from the degradation of 2,3,7,8-TCDD (I) and OCDD (II) by *P. sordida* YK-624 under nitrogen limiting condition (incubated for 10 days). Percent yields are listed below each compound.

Degradation of PCDDs and PCDFs extract in fly ash by *P. sordida* YK-624.

Congener profile of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) obtained from a fly ash sample is shown in Fig. 2. The congener distribution of PCDDs and PCDFs was very convenient for investigating the extent of degradability of YK-624 because no extreme difference of concentrations among congeners was observed. The concentration differences were within the range of 3 times for PCDDs and that of 6 times for PCDFs. Total weights of PCDDs and PCDFs were approximately 12 ng and 11 ng, respectively, and the whole quantity was supplied to a culture in the degradation by YK-624.

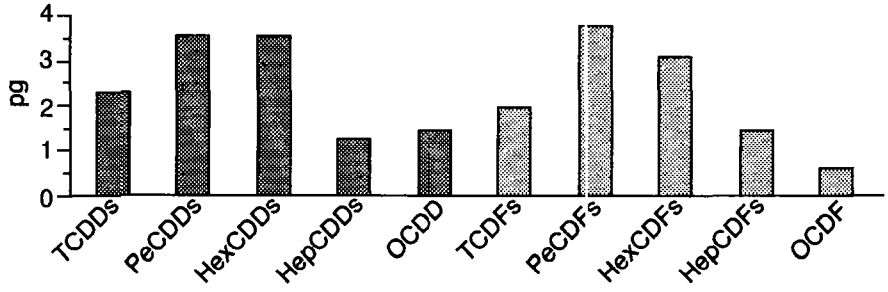

Fig. 2. Concentration profile of PCDD and PCDF congeners in fly ash. Values represent total weight of each congener.

All isomers of each PCDD and PCDF congener in a fly ash sample were evidently observed to be degraded by YK-624 (data not shown) and no obvious differences to the number of the position of chlorine substituents were obtained. Results of degradation of total isomers of each PCDD and PCDF congener in a fly ash are shown in Fig. 3. All of the PCDD and PCDF congeners were equally degraded in which the rates were from 26 % (peCDDs) to 35% (hepCDDs) for PCDDs and from 29% (TCDFs) to 37 % (hexCDFs) for PCDFs. Similar results were shown by our preivious work; all 2,3,7,8-PCDDs/PCDFs were degraded by YK-624, and the degradation rates of PCDDs and PCDFs were approximately 40 (tetra-) to 76 (hexa-)% and 45(tetra-) to 70 (hexa-)%, respectively.[5] Consequently, *P. sordida* YK-624 was found to be a promising microorganism possessing remarkable biodegradative properties for all of the PCDDs and PCDFs.

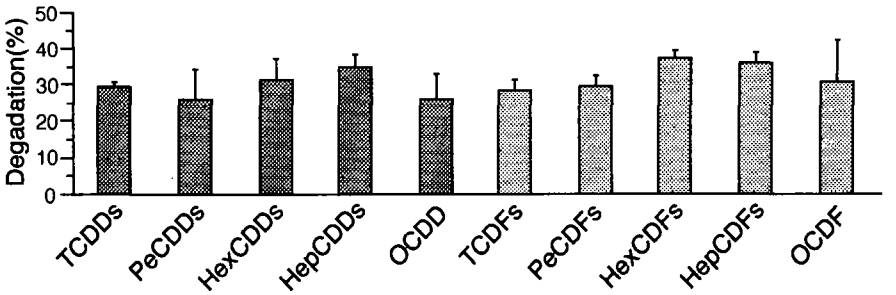

Fig. 3. Degradation of PCDDs and PCDFs extracted from a fly ash sample by *P. sordida* YK-624 under low-nitrogen medium. Incubation time was 10 days. All data were collected for the control cultures.

4. Conclusion

The metabolites of 2,3,7,8-tetraCDD and OCDD by *P. sordida* YK-624 were confirmed in this study, suggesting that the fungus was capable of substantial degradation of 2,3,7,8-PCDDs/PCDFs as reported previously. In addition, *P. sordida* YK-624 substantially degraded all of the isomers of each PCDD and PCDF congeners in fly ash and showed no obvious differences to the number of and the position of chlorine substituents of molecules. To our knowledge, this is the first report on extensive degradations of PCDDs and PCDFs by microorganism.

5. References

1) Bumpus J. A., M. Tien, D. Wright, and S. D. Aust (1985): Oxidation of Persistent Environmental Pollutants by White Rot Fungus. Sci. **228**, 431-1436.
2) Bumpus, J. A., and S. D. Aust (1987): Biodegradation of DDT [1,1,1-trichloro-2,2-bis(4-chlorobiphenyl)ethane] by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **53**,2001-2008.
3) Hammel, K. E., W. Z. Gai, B. Green, and M. A. Moen (1992): Oxidative degradation of phenanthrene by the ligninolytic fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **58**.1832-1838.
4) Dietrich, D., W. J. Hickey, and R. Lamar (1994): Degradation of 4,4'-dichlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and 2,2',4,4',5,5'-hexachlorobiphenyl by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **61**,3904-3909.
5) Takada, S., M. Nakamura, T. Matsueda, Y. Kurokawa, R. Kondo, and K. Sakai (1994) : Degradation of PCDDs/PCDFs by ligninolytic fungus *Phanerochaete sordida* YK-624. Proceedings of 14th International Symposium on Chlorinated Dioxins and Related Compounds, Kyoto Japan. **22**, 195-198.
6) Kondo, R., K. Kurashiki, and K. Sakai (1994): In vitro bleaching of hardwood kraft pulp by extracellular enzymes excreted from white rot fungi in a cultivation system using a membrane filter. Appl. Environ. Microbiol. **60**,921-926.
7) Kondo, R., K. Harazono, and K. Sakai (1994): Bleaching of hardwood kraft pulp with manganese peroxidase secreted from *Phanerochaete sordida* YK-624. Appl. Environ. Microbiol. **60**,4359-4363.
8) Valli H., H. Wariishi, and M. H. Gold (1992) : Degradation of 2,7-Dichlorodibenzo-*p*-dioxin by the Lignin-degrading Basidomycete *Phanerochaete chrysosporium*. J. Bacteriol. **174**, 2131-213.