APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1996, p. 4323–4328 Vol. 62, No. 12
0099-2240/96/$04.00+0

Degradation of Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans by the White Rot Fungus *Phanerochaete sordida* YK-624

SATOSHI TAKADA,[1]* MATAYOSHI NAKAMURA,[1] TAKAHIKO MATSUEDA,[1] RYUICHIRO KONDO,[2] AND KOKKI SAKAI[2]

Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano Dazaifu Fukuoka, 818-01,[1] *and Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki Higashi-ku Fukuoka, 812-81,*[2] *Japan*

Received 28 May 1996/Accepted 15 September 1996

A method for the degradation of dioxins by white rot fungi was developed. Degradation of a mixture of 10 kinds of tetra- to octachlorodibenzo-*p*-dioxins (polychlorinated dibenzo-*p*-dioxins [PCDDs]) and tetra- to octachlorodibenzofurans (polychlorinated dibenzofurans [PCDFs]), which were chlorinated at 2-, 3-, 7-, and 8-positions of the molecules, by the white rot fungus *Phanerochaete sordida* YK-624 was studied in a stationary low-nitrogen medium. The percent degradation values of PCDDs and PCDFs were approximately 40 (tetrachloro-) to 76% (hexachloro-) and 45 (tetrachloro-) to 70% (hexachloro-), respectively. Metabolites of 2,3,7,8-tetra- and octaCDD formed by *P. sordida* YK-624 included 4,5-dichlorocatechol and tetrachlorocatechol, respectively. These results suggest that white rot fungus is able to substantially degrade both PCDDs and PCDFs. This is the first report of the degradation of highly chlorinated PCDDs and PCDFs by a microorganism.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have been a public concern for several decades because of their strong toxicity in animal tests (31, 32). These hazardous compounds tend to accumulate in the body fat of animals since they are relatively lipophilic and chemically stable (31, 32). PCDDs and PCDFs are unintentionally formed in the process of producing chlorine-containing herbicides (31, 32), in the bleaching of paper pulp by using chlorine compounds (5, 34), and during combustion of domestic and industrial waste (6, 7). They have been released into the environment as recalcitrant contaminants and have been found in many environmental matrices such as air, soil, and plants (6, 7, 10, 18).

Studies of the degradation of PCDDs and PCDFs in the environment have shown these rates to be extremely low (24, 26), the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-tetraCDD) in an outdoor pond and soil being in the order of 1 year (24). Past studies of the degradation of PCDDs (24, 27, 28) and PCDF (15) by microorganisms have been limited to compounds with four or fewer chlorines. The rates of degradation of 2,3,7,8-tetraCDD by bacteria isolated from the environment, such as *Bacillus* spp., *Nocardiopsis* spp., (24), and other strains (28), also appear to be very low, and the rates of metabolism of the chlorinated dibenzo-*p*-dioxins decreased with an increasing number of chlorine substitutions (17, 25).

The white rot fungus *Phanerochaete chrysosporium* has been shown to possess biodegradative capabilities for a wide spectrum of recalcitrant organopollutants, including polycyclic aromatic hydrocarbons (PAHs) (3, 4, 9, 12–14), chlorophenols (2, 12, 16, 39), and polychlorinated biphenyls (PCBs) (4, 36, 43). There have been two reports to date of the degradation of 2,3,7,8-tetraCDD (4) and 2,7-dichlorodibenzo-*p*-dioxin (2,7-DCDD) (40) by *P. chrysosporium*. Although mineralization of 2,3,7,8-tetraCDD by this fungus has been observed, the metabolite was not elucidated (4). 2,7-DCDD was degraded approximately 50% after a 27-day incubation under nitrogen-limited conditions, and the degradation pathway of the compound was proposed (40). *Phanerochaete sordida* has also been shown to degrade environmentally persistent pollutants, including pentachlorophenol (21–23) and PAHs (8), and offers potential for application in pollution management. However, there have been no reports of the degradation of PCDDs and PCDFs by this fungus.

The present study was undertaken with the intent of developing a biodegradation method for PCDDs and PCDFs. By this method, degradation by *P. sordida* YK-624 isolated in our laboratory (19, 20) was performed on a mixture of 10 kinds of 2,3,7,8-substituted tetra- to octaCDDs and tetra- to octachlorodibenzofurans (tetra- to octaCDFs), which included the most toxic compounds among the PCDD and PCDF congeners (31). For comparative purposes, the rates of degradation by the well-studied fungus *P. chrysosporium* were compared with those of *P. sordida* YK-624. One of the metabolites produced by *P. sordida* YK-624 from each 2,3,7,8-tetraCDD and octaCDD was also confirmed.

MATERIALS AND METHODS

Fungi. *P. sordida* YK-624 was isolated from rotted wood obtained from a forest (19, 20). *P. chrysosporium* IFO31249 was purchased from the Institute for Fermentation, Osaka, Japan. These fungi were maintained as slant cultures on potato dextrose agar (Difco Laboratories, Detroit, Mich.) slants. Subcultures were routinely made every 30 to 60 days.

Chemicals. Mixtures of ^{12}C- and ^{13}C-containing 2,3,7,8-tetraCDD, 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, octaCDD, 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, and octaCDF (PCDDs-PCDFs) were obtained from Cambridge Isotope Laboratories (Andover, Mass.). For the study of metabolism, 2,3,7,8-tetraCDD and octaCDD were purchased from GL Science Inc., Tokyo, Japan. 4,5-Dichlorocatechol was purchased from Cambridge Isotope Laboratories. The diacetyl derivative prepared by using acetic anhydride and sodium hydroxide was purified by preparative thin-layer chromatography (solvent, hexane-acetone [10:2]). Tetrachloro-1,2-benzoquinone obtained from Aldrich Chem. Co. (Milwaukee, Wis.) was reduced to tetrachlorocatechol with sodium dithionite, and the diacetyl derivative of the compound was purified by recrystalization (ethanol-hexane).

* Corresponding author. Mailing address: Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano Dazaifu Fukuoka, 818-01, Japan. Phone: 81-92-921-9943. Fax: 81-92-928-1203.

Biodegradation method. Low-nitrogen basal III medium described by Tien and Kirk (38) was used in this study. The medium contained 1% glucose, 1.2 mM ammonium tartrate, and 20 mM dimethylsuccinate (pH 4.5). The fungus was incubated on a PDA plate at 30°C for 2 days, and then 5-mm diameter disks were punched from the edge of the mycelium. A disk was put into each 100-ml Erlenmeyer flask containing 10 ml of the low-nitrogen medium. The culture was incubated statically at 30°C under ambient atmosphere. After incubation for 7 days, 1 ml of 10% glucose was added to each inoculated flask and the headspaces were flushed with oxygen, after which 10 μl of a 0.05-mg/ml solution in ethyl acetate of PCDDs-PCDFs (500 pg each) was added, and each flask was sealed with a glass stopper and sealing tape. The cultures were incubated for 3, 7, 10, and 14 days (each in triplicate). For 10- and 14-day incubation samples, 1 ml of 10% glucose was added to the cultures, and the flasks were oxygenated on day 7. At the end of the incubation, 5 ml of hexane and internal standards of ^{13}C-labeled PCDDs-PCDFs (500 pg each) was added to the cultures. To recover the PCDDs and PCDFs adsorbed to the mycelia and to dissolve the mycelia thoroughly, 10 ml of concentrated sulfuric acid was added. The effect of this sulfuric acid treatment was examined in the following manner. After autoclaving of the 10-day-old cultures of the fungus, PCDDs-PCDFs were added. The cultures were kept at 30°C for 7 days and then were treated in a manner similar to that described for the biodegradation method. The results showed that added PC DDs-PCDFs were quantitatively recovered. Hence, sulfuric acid treatment did not result in loss of PCDDs-PCDFs. The cultures were extracted twice with 15 ml of hexane and then 20 ml of hexane. The residue in the flask was extracted with acetone (10 ml) and then a mixture of acetone (5 ml) and hexane (5 ml), and finally with hexane (10 ml) by using an ultrasonic generator (NEY ULTRAsonik 300). All of the hexane extracts were washed with water (two 20-ml portions). The hexane layer was evaporated, and polar compounds were removed with silica gel chromatography (silica gel, 2 g; eluted with 100 ml of hexane). Uninoculated medium controls were treated in the way mentioned above. Concentrations of PCDDs-PCDFs were determined by high-resolution gas chromatography and high-resolution mass spectrometry (HRGC-HRMS) (selected ion-monitoring mode [SIM]), with a Varian model 3400 capillary GC (Quadrex OV-17 column [0.25-mm inside diameter by 25-m length; 0.1-μm film thickness]) coupled to a Finnigan MAT-90 MS. The oven temperature was maintained initially at 120°C for 1 min and was increased to 250°C at 10°C/min and maintained at 250°C for 20 min.

Metabolites of 2,3,7,8-tetraCDD and octaCDD. Inoculated cultures of *P. sordida* YK-624 were prepared under the culture conditions described above. To 6-day-old cultures, 2,3,7,8-tetraCDD or octaCDD in ethyl acetate (10 μl) was added to a final concentration of 50 ng/10 ml. The cultures were incubated as described above. After 10 days, 10 μl of sodium dithionite solution (2 μg/ml) (33, 40) was added to reduce quinone products (described later). We did not conduct the confirmation of the quinone products in this study. To recover unreacted substrates, hexane (10 ml) was added after adjustment to pH 10 with NaOH, and the mixture was shaken vigorously for 20 min. Then, it was placed in an ultrasonic generator for 10 min. This procedure was repeated twice. The pooled organic phases were dried over anhydrous sodium sulfate, and the solution was diluted with hexane to a total volume of 100 ml. ^{13}C-labeled compounds were added to 1 ml of the solution, and the concentrations of substrates were analyzed by HRGC-HRMS. The aqueous phase containing mycelia was acidified with HCl to pH 2 and was extracted with ethyl acetate (two 10-ml portions). The mixture was shaken vigorously for 10 min and was 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 as mentioned above, derivatives were analyzed by HRGC-HRMS (SIM). 2′,3,4-Trichlorobiphenyl was used as an internal standard. A DB-5MS column (J. & W. Scientific) (0.32 mm [inner diameter] by 25 m; 0.52-μm film thickness) was used. The initial temperature, 70°C, was maintained for 2 min, and then the temperature was increased to 280°C by 10°C/min and was held at 280°C for 20 min. The residue containing mycelia after ethyl acetate extraction was treated to recover the substrates adsorbed to the mycelia, with concentrated sulfuric acid to dissolve the hyphae and then hexane extraction and clean up as described above for the biodegradation method, and was analyzed by HRGC-HRMS (SIM). In these experiments, parallel controls were similarly performed with heat-killed cultures.

RESULTS

Biodegradation methods. The white rot fungus *P. sordida* YK-624, which is isolated from rotted wood, is able to grow and break down lignin in air, although supplemental oxygen improves growth and degradation of lignin and other organic compounds. The biodegradation of the organic compounds has generally been performed with culture flasks sealed with rubber stoppers and purged with oxygen every couple of days. In the early stages of this study, therefore, biodegradation of PCDDs-PCDFs was attempted under aerobic conditions in flasks with cotton plugs. However, significant loss of PCDDs-PCDFs was observed in these cultures. Recoveries of these compounds were tested and compared with those from flasks plugged with cotton plugs or sealed with glass stoppers. The results under these experimental conditions are shown in Fig. 1. In the case of the glass-stoppered flasks, nearly quantitative recoveries of PCDDs-PCDFs were obtained. However, in cotton plugged flasks, the recoveries of both PCDDs and PCDFs were less than 10% for tetraCDD and -CDF and increased with the number of substituted chlorines.

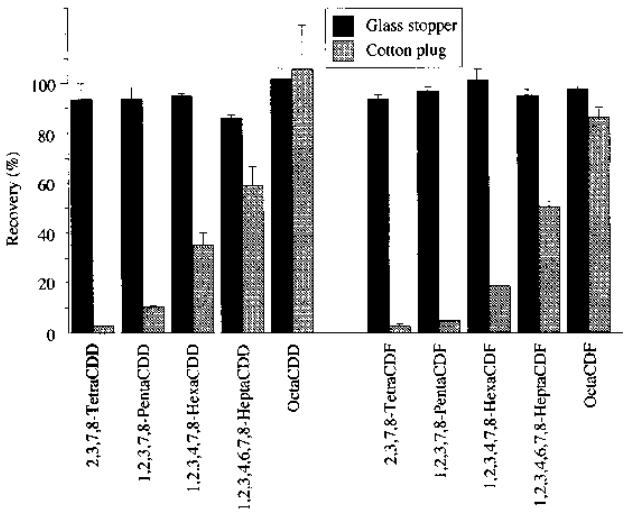

FIG. 1. Recoveries of PCDDs-PCDFs in flasks sealed with glass stoppers and with cotton plugs. A mixture of PCDDs and PCDFs was added to low-nitrogen medium, and the cultures were incubated at 30°C for 7 days, without inoculation. Values are means ± standard deviations for duplicate samples.

Armenante et al. (2) observed that the degradation of 2,4,6-trichlorophenol by *P. chrysosporium* ceased in 5.5 days after exposure to substrate. It is suggested that nutrients such as carbon and nitrogen sources were depleted, and the production of degradation enzyme was reduced. To extend the degradation time, supplementation of glucose (100 mg/10 ml) was

TABLE 1. Degradation of PCDDs and PCDFs by *P. sordida* YK-624 and *P. chrysosporium* IFO31249 with or without supplementation of glucose to the cultures

Compound	% Degradation[a]		
	P. sordida[b]		*P. chrysosporium* with glucose[c]
	Without glucose	With glucose	
2,3,7,8-TetraCDD	22.1 ± 0.0	26.5 ± 1.5	37.1 ± 5.9
1,2,3,7,8-PentaCDD	24.3 ± 1.9	26.7 ± 6.5	53.9 ± 6.8
1,2,3,4,7,8-HexaCDD	35.3 ± 5.0	43.7 ± 4.0	64.9 ± 5.7
1,2,3,4,6,7,8-HeptaCDD	10.3 ± 0.6	38.1 ± 4.3	53.6 ± 7.9
1,2,3,4,6,7,8,9-OctaCDD	6.2 ± 2.0	14.2 ± 4.1	41.1 ± 7.6
2,3,7,8-TetraCDF	28.6 ± 5.6	38.8 ± 1.2	27.3 ± 8.0
1,2,3,7,8-PentaCDF	22.0 ± 1.5	24.2 ± 3.3	38.5 ± 5.7
1,2,3,4,7,8-HexaCDF	41.5 ± 1.6	50.4 ± 2.6	54.7 ± 6.1
1,2,3,4,6,7,8-HeptaCDF	22.0 ± 1.2	39.5 ± 3.2	51.9 ± 7.1
1,2,3,4,6,7,8,9-OctaCDF	13.7 ± 1.0	35.0 ± 2.8	58.6 ± 12.3

[a] All values are means ± standard deviations for triplicate cultures. Percent degradation values have been corrected for controls.

[b] *P. sordida* YK-624 was grown at 30°C for 7 days in low-nitrogen medium, and then a mixture of PCDDs and PCDFs (500 pg each) with or without glucose (1 ml of 10% solution) was added to the cultures, after oxygenation. The cultures were incubated for 7 days.

[c] The cultures were incubated for 14 days in low-nitrogen medium, and then on days 0 and 7 glucose was added to each culture after oxygenation.

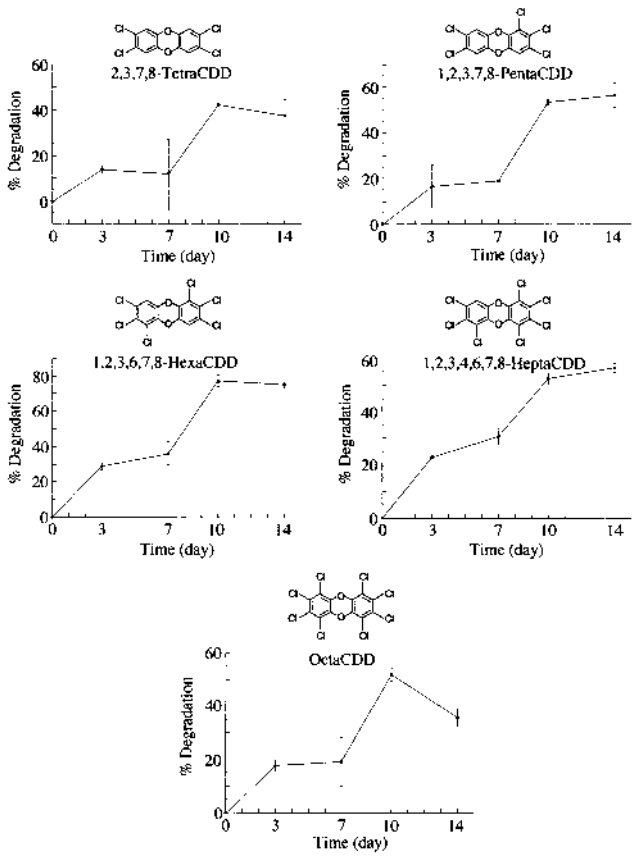

FIG. 2. Degradation of PCDDs by *P. sordida* YK-624 under low-nitrogen medium. Datum points are means ± standard deviations ($n = 3$). On days 0 and 7, glucose was added to each culture, and headspaces were purged with oxygen.

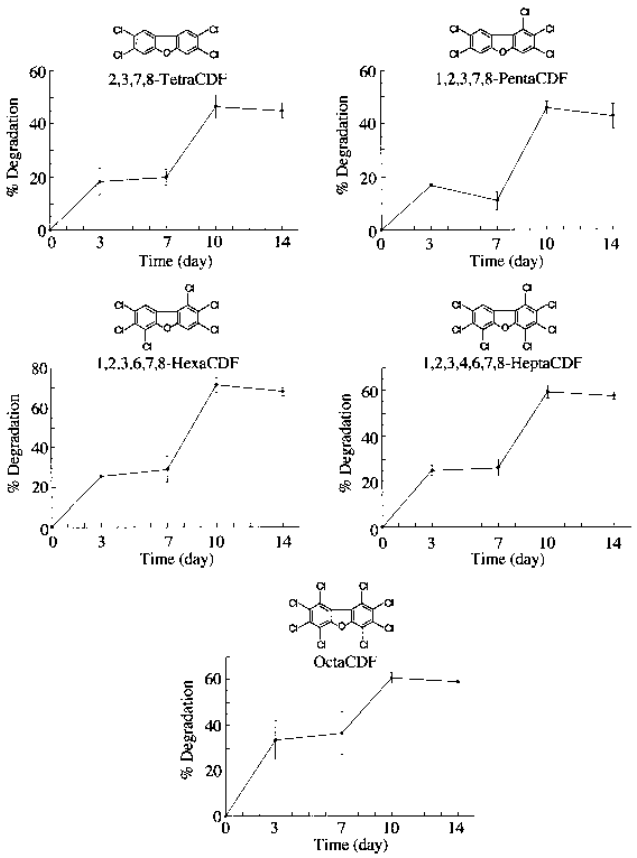

FIG. 3. Degradation of PCDFs by *P. sordida* YK-624 under low-nitrogen medium. Datum points are means ± standard deviations ($n = 3$). On days 0 and 7, glucose was added to each culture, and headspaces were purged with oxygen.

tested. The results are shown in Table 1. For all compounds, the glucose-supplemented culture led to a higher percent degradation. Similar results were obtained by Yadav and Reddy (44), who found that the degradation of 2,4-dichlorophenoxyacetic acid by *P. chrysosporium* was enhanced by the addition of glucose (14 mg/10 ml) compared with the culture without glucose.

Degradation of PCDDs and PCDFs by *P. sordida* YK-624. Time courses for the degradation of PCDDs and PCDFs by the fungus YK-624 are shown in Fig. 2 (PCDDs) and Fig. 3 (PCDFs). All of the PCDDs and PCDFs were partially degraded. The percent degradation values were promoted by the addition of glucose and oxygenation on days 0 and 7; however, the effect continued only for 3 days, as indicated by the fact that the slopes of percent degradation of days 0 to 3 and 7 to 10 were steeper than those of days 3 to 7 and 10 to 14. During the incubation time, the mycelial weight showed a similar tendency to increase until 10 days and plateau. In both PCDDs and PCDFs, hexaCDD and -CDF showed the highest degradation values, i.e., ~75% and ~70%, respectively. The lowest degradation value for PCDDs was ~40% of tetraCDD, and that for PCDFs was ~45% of tetra- as well as pentaCDF.

Metabolites of 2,3,7,8-tetraCDD and octaCDD formed by *P. sordida* YK-624. The results of HRGC-HRMS (SIM) of the diacetyl derivative of 4,5-dichlorocatechol for a metabolite of 2,3,7,8-tetraCDD and tetrachlorocatechol for a metabolite of octaCDD are shown in Tables 2 and 3, respectively. The retention times of each derivative coincided with the authentic standards, and the metabolites were confirmed by comparing the *m/z* and the ion intensities for several peaks near the molecular ion, base peak ion, and others. These results suggest, therefore, that the fungus essentially has the ability to degrade tetraCDD and octaCDD. No corresponding degradation products were detected in the control cultures. Of tetraCDD and octaCDD, 0.4 and 1%, respectively, were estimated to have been metabolized to a quinone and/or dihydroxy derivative from the values of quantitative analysis of diacetyl derivatives.

In these experiments, 82% of tetraCDD as well as octaCDD was recovered from extracellular culture fluids, and as the substrate adsorbed to the mycelia, 9% of tetraCDD and 8% of octaCDD were recovered from the dissolved mycelia solutions by adding concentrated sulfuric acid to the final residue after extraction of metabolites.

Degradation of PCDDs and PCDFs by *P. chrysosporium* IFO31249. The degradation of PCDDs-PCDFs by *P. chrysosporium* IFO31249 was also carried out for 14 days under conditions similar to those for YK-624 (Table 1). The results show almost the same degradation rate as that for YK-624 (Fig. 2 and 3).

DISCUSSION

The degradation method was developed carefully to avoid the evaporation of dioxins and consequent human exposure. Nondegraded dioxins are recovered by this method, which is applicable for other toxic compounds such as PCBs and other toxic halogenated aromatic compounds. By using this method,

TABLE 2. Result of HRGC-HRMS (SIM) of an acetyl derivative (4,5-dichloro-1,2-diacetoxybenzene) of the 2,3,7,8-tetraCDD metabolites by *P. sordida* YK-624[a]

Mass fragment ion	*m/z*[b]	No. of atom					Exact mass	Standard intensity (%)[c]	Metabolite intensity (%)[c]
		C	H	^{35}Cl	^{37}Cl	O			
$M^+ - (COCH_2)_2$	178[d]	6	4	2	0	2	177.959	100.00	100.00
$M^+ + 2 - (COCH_2)_2$	180	6	4	1	1	2	179.956	60.31	62.71
$M^+ + 4 - (COCH_2)_2$	182	6	4	0	2	2	181.953	8.63	12.26
$M^+ - COCH_2$	220	8	6	2	0	3	219.969	10.30	7.66
$M^+ + 2 - COCH_2$	222	8	6	1	1	3	221.966	5.91	2.50
M^+	262	10	8	2	0	4	261.980	1.16	2.13
$M^+ + 2$	264	10	8	1	1	4	263.977	0.70	0.94

[a] A culture was incubated in low-nitrogen medium with 50 ng of the substrate for 10 days. Retention time of 4,5-dichloro-1,2-diacetoxybenzene on DB-5MS (J. & W. Scientific), 13.26 min.
[b] In the mass spectrum of the acetyl derivative, there were no fragment ions showing strong intensity (greater than ~50%), except for *m/z* 178 and 180.
[c] Values were converted into percentages on the basis of the peak area of base peak (*m/z* = 178).
[d] Base peak.

the risk of human exposure could be reduced to a minimum level.

As shown in Fig. 1, the recoveries of PCDDs and PCDFs in cotton-plugged flasks increased with the number of substituted chlorines. This tendency depends on physicochemical properties, such as vapor pressures and the solubilities of these compounds. In fact, the relationships correlated positively with the vapor pressures of PCDDs, i.e., they were as follows (in order of solubility and vapor pressure): 2,3,7,8-tetraCDD, 7.91 pg/ml (1) and 4.5×10^{-7} Pa (29); 1,2,3,4,7,8-hexaCDD, 4.42 pg/ml and 5.1×10^{-9} Pa; 1,2,3,4,6,7,8-heptaCDD, 2.4 pg/ml and 7.5×10^{-10} Pa; and octaCDD, 0.4 pg/ml and 1.1×10^{-10} Pa (11, 29). The solubilities of PCDDs are extremely low. The 500 pg of these compounds used in this experiment did not dissolve in 10 ml of the aqueous culture and remained largely in solid or vapor states. Consequently, biodegradation was carried out in flasks by sealing with glass stoppers and, in addition, sealing with sealing tape in order to protect against the loss of these compounds.

P. sordida YK-624 and *P. chrysosporium* IFO31249 were capable of substantial degradation of the mixtures of the 2,3,7,8-substituted tetra- to octa-CDDs and CDFs tested, as determined by substrate disappearances. The degradation by YK-624 was additionally supported by identification of a corresponding metabolite from 2,3,7,8-tetraCDD and octaCDD. These observations are significant from the viewpoint of the potential of white rot fungi for degradation of dioxins in the environment. This is particularly important, since contamination by mixtures of chemicals is much more common than contamination by single compounds.

It is worth noting that these fungi showed no clear structural dependence for degradation of PCDDs and PCDFs but also degraded tetra- to octachloro substituents, suggesting that the degradation of these substrates might be a free-radical process showing little specificity. Many researchers have attributed the degradation of chemicals by white rot fungi to a free-radical mechanism (12, 14, 40, 43). Indeed, dibenzo-*p*-dioxin cation radical has been observed following lignin peroxidase (LiP) oxidation (12, 14). Recently, Yadav et al. observed the substantial degradation of PCB mixtures (Aroclors 1242, 1254, and 1260) by *P. chrysosporium* (43). They reported that, unlike the degradation of PCDDs and PCDFs reported in the present work, the relative degradability of a PCB congener decreases with an increase in the number of chlorine substitutions on the biphenyl nucleus. An explanation of the discrepancy between PCB and dioxin degradation may be that radical reactions proceed more readily in the presence of an oxygen atom(s) of the PCDD and PCDF molecules. In this context, Valli et al. (40) suggested that the pathway for C-O-C bond cleavage in 2,7-DCDD is via cation formation at the angular position adjacent to the ether bridge of the molecule. The cation then undergoes nucleophilic attack of H_2O at this position.

The precise pathway for degradation of 2,7-DCDD by *P. chrysosporium* was elucidated by Valli et al. (40), who demonstrated that 4-chloro-1,2-benzoquinone and 2-hydroxy-1,4-benzoquinone were obtained by oxidative cleavage of the dioxin

TABLE 3. Result of HRGC-HRMS (SIM) of an acetyl derivative (3,4,5,6-tetrachloro-1,2-diacetoxybenzene) of the octaCDD metabolites by *P. sordida* YK-624[a]

Mass fragment ion	*m/z*[b]	No. of atom					Exact mass	Standard intensity (%)[c]	Metabolite intensity (%)[c]
		C	H	^{35}Cl	^{37}Cl	O			
$M^+ - 2 - (COCH_2)_2$	246	6	2	4	0	2	245.881	74.31	82.58
$M^+ - (COCH_2)_2$	248[d]	6	2	3	1	2	247.878	100.00	100.00
$M^+ + 2 - (COCH_2)_2$	250	6	2	2	2	2	249.875	44.34	47.73
$M^+ - 2 - COCH_2$	288	8	2	3	1	3	287.873	2.47	1.23
$M^+ - COCH_2$	290	8	2	2	2	3	289.870	2.63	1.45
$M^+ + 2 - COCH_2$	292	8	2	1	3	3	291.867	1.25	1.57
$M^+ + 2$	332	10	6	3	1	4	331.899	2.50	4.05

[a] A culture was incubated in low-nitrogen medium with 50 ng of the substrate for 10 days. Retention time of 3,4,5,6-tetrachloro-1,2-diacetoxybenzene on DB-5MS (J. & W. Scientific), 15.55 min.
[b] In the mass spectrum of the acetyl derivative, there were no fragment ions showing strong intensity (greater than ~50%), except for *m/z* 246, 248, and 250.
[c] Values were converted into percentages on the basis of the peak area of base peak (*m/z* = 248).
[d] Base peak.

ring of 2,7-DCDD catalyzed by LiP. The former was reduced to 1-chloro-3,4-dihydroxybenzene. We examined whether corresponding metabolites for 2,3,7,8-tetraCDD and octaCDD were formed. If a similar mechanism occurs in the degradation of 2,3,7,8-tetraCDD and octaCDD by *P. sordida* YK-624, although the fungus secretes only manganese peroxidase (MnP) (19, 20), it is supposed that 4,5-dichloro-1,2-benzoquinone and/or 4,5-dichlorocatechol for 2,3,7,8-tetraCDD and tetrachloro-1,2-benzoquinone and/or tetrachlorocatechol for octaCDD would be obtained as metabolites. In this context, use of the SIM mode in HRGC-HRMS analysis permits the detection of subpicogram levels of compounds and also offers structural and quantitative information. Furthermore, because the abundance ratio of ^{35}Cl and ^{37}Cl approximately equals 3:1, characteristic mass fragments are obtained in mass spectra of the compounds containing chlorine atoms, allowing confirmation of the metabolites for 2,3,7,8-tetraCDD and octaCDD. By this method, we confirmed the presence of the chlorocatechols cleaved at the C-O-C moiety of 2,3,7,8-tetraCDD and the octaCDD skeleton by YK-624.

Bacterial degradation of 2,3,7,8-tetraCDD was found to be slow, and traces of polar metabolites were produced (24, 28). Slow cometabolisms of mono-, di-, and triCDDs by biphenyl-utilizing bacteria have been reported elsewhere (17, 27). Dihydroxy and/or hydroxy metabolites were formed in their systems. Of PCDFs, the degradative pathway of 3-monoCDF by a *Pseudomonas* sp. has been reported elsewhere (15). The degradation was initiated by an angular attack of a dioxygenase enzyme, yielding chlorotrihydroxybiphenyls from initially formed dihydrodiols, and the chlorohydroxy compounds were transformed to 3-chlorosalicylic acid.

Our results are particularly notable from the viewpoint of the difference of enzymatic constituents in ligninolytic systems in the white rot fungi used in this study. *P. chrysosporium* secretes LiP and MnP and H_2O_2-generating enzymes, which are induced during secondary metabolism under ligninolytic conditions (37, 42). Many researchers have implicated these peroxidases of this fungus as essentially responsible for oxidative degradation of a variety of environmentally persistent compounds, such as PAHs (12, 14, 41), chlorophenols (12, 16, 39), and lignin (12, 37). In addition, some studies have revealed that the presence of another enzyme system taking part in breakdown of PAHs by this microorganism in nitrogen-limited cultures. For example, Hammel et al. reported that the extracellular LiPs of the fungus were not able to oxidize phenanthrene in vitro and, therefore, are also unlikely to catalyze the first step of phenanthrene degradation in vivo (14). Similar results were obtained by Dhawale et al., i.e., they indicated that phenanthrene was degraded by a mutant of *P. chrysosporium* which totally lacked LiP and MnP activities (9). With regard to the role of the LiP from *P. chrysosporium* in the degradation of phenanthrene, Tatarko and Bumps (35) showed that 9-phenanthrenol, an intermediate formed during phenanthrene degradation under nonligninolytic cultures, is oxidized by LiP to phenanthrene-9,10-quinone.

On the other hand, *P. sordida* YK-624, which exhibited remarkable bleaching ability with kraft pulp, secreted mainly MnP, a trace of phenol oxidase, and no LiP during the bleaching process (19, 20). Rüttimann-Johnson et al. reported recently that only MnP activity was detected in the supernatant liquid of *P. sordida* culture (30). Valli et al. reported that the first step of breakdown of 2,7-DCDD was mediated by LiP, not by MnP, resulting in generation of 4-chloro-1,2-benzoquinone and 2-hydroxy-1,4-benzoquinone (40). We also examined whether MnP prepared from *P. sordida* YK-624 takes part in the degradation of PCDDs and PCDFs under conditions of the crude enzyme in the presence of $MnSO_4$ with continuous addition of H_2O_2 in malonate buffer (19); however, no degradation was observed. This result supports the report by Valli et al. and also strongly indicates that YK-624, which has only MnP as a major ligninase, contains another enzyme system to degrade PCDDs and PCDFs.

ACKNOWLEDGMENTS

We gratefully acknowledge R. Mori for reviewing the manuscript and H. Muto for valuable suggestions. We thank Yoichi Kurokawa and Kazumi Fukamachi for technical assistance.

This work was supported in part by grant-in-aid for Scientific Research, B-6453177, from the Japanese Ministry of Education.

REFERENCES

1. **Adams, W. J., and K. M. Blaine.** 1986. A water solubility determination of 2,3,7,8-TCDD. Chemosphere **15:**1397–1400.
2. **Armenante, P. M., N. Pal, and G. Lewandowski.** 1994. Role of mycelium and extracellular protein in the biodegradation of 2,4,6-trichlorophenol by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **60:**1711–1718.
3. **Bogan, B. W., and R. T. Lamar.** 1995. One-electron oxidation in the degradation of creosote polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **61:**2631–2635.
4. **Bumpus, J. A., M. Tien, D. Wright, and S. D. Aust.** 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science **228:**1434–1436.
5. **Buser, H.-R., L.-O. Kjeller, S. E. Swanson, and C. Rappe.** 1989. Methyl-, polymethyl-, and alkylpolychlorodibenzofurans identified in pulp mill sludge and sediments. Environ. Sci. Technol. **23:**1130–1137.
6. **Czuczwa, J. M., and R. A. Hites.** 1984. Environmental fate of combustion-generated polychlorinated dioxins and furans. Environ. Sci. Technol. **18:**444–450.
7. **Czuczwa, J. M., and R. A. Hites.** 1986. Airborne dioxins and dibenzofurans: sources and fates. Environ. Sci. Technol. **20:**195–200.
8. **Davis, M. W., J. A. Glaser, J. W. Evans, and R. T. Lamar.** 1993. Field evaluation of the lignin-degrading fungus *Phanerochaete sordida* to treat creosote-contaminated soil. Environ. Sci. Technol. **27:**2572–2576.
9. **Dhawale, S. W., S. S. Dhawale, and D. Dean-Ross.** 1992. Degradation of phenanthrene by *Phanerochaete chrysosporium* occurs under ligninolytic as well as nonligninolytic conditions. Appl. Environ. Microbiol. **58:**3000–3006.
10. **Facchetti, S., A. Balasso, C. Fichtner, G. Frare, A. Leoni, C. Mauri, and M. Vasconi.** 1987. Chapter 16. Studies on the absorption of TCDD by plant species, p. 225–239. *In* C. Rappe, G. Choudhary, and L. H. Keith (ed.), Chlorinated dioxins and dibenzofurans in perspective. Lewis Publishers, Inc., Chelsea, Mich.
11. **Friesen, K. J., L. P. Sarana, and G. R. B. Webster.** 1985. Aqueous solubility of polychlorinated dibenzo-*p*-dioxins determined by high pressure liquid chromatography. Chemosphere **14:**1267–1274.
12. **Hammel, K. E.** 1992. Oxidation of aromatic pollutants by lignin-degrading fungi and their extracellular peroxidases, p. 41–60. *In* H. Sigel and A. Sigel (ed.), Metal ions in biological systems, vol. 28. Degradation of environmental pollutants by microorganisms and their metalloenzymes. Marcel Dekker, Inc., New York.
13. **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.
14. **Hammel, K. E., B. Kalyanaraman, and T. K. Kirk.** 1986. Oxidation of polycyclic aromatic hydrocarbons and dibenzo[*p*]dioxins by *Phanerochaete chrysosporium* ligninase. J. Biol. Chem. **261:**16948–16952.
15. **Harms, H., H. Wilkes, V. Sinnwell, R.-M. Wittich, K. Figge, W. Francke, and P. Fortnagel.** 1991. Transformation of 3-chlorodibenzofuran by *Pseudomonas* sp. HH69. FEMS Microbiol. Lett. **81:**25–30.
16. **Joshi, D. K., and M. H. Gold.** 1993. Degradation of 2,4,5-trichlorophenol by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **59:**1779–1785.
17. **Klecka, G. M., and D. T. Gibson.** 1980. Metabolism of dibenzo-*p*-dioxin and chlorinated dibenzo-*p*-dioxins by a *Beijerinckia* species. Appl. Environ. Microbiol. **39:**288–296.
18. **Koester, C. J., and R. A. Hites.** 1992. Wet and dry deposition of chlorinated dioxins and furans. Environ. Sci. Technol. **26:**1357–1382.
19. **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.
20. **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.
21. **Lamar, R. T., and D. M. Dietrich.** 1990. In situ depletion of pentachloro-

phenol from contaminated soil by *Phanerochaete* spp. Appl. Environ. Microbiol. **56:**3093–3100.
22. **Lamar, R. T., J. W. Evans, and J. A. Glaser.** 1993. Solid-phase treatment of a pentachlorophenol-contaminated soil using lignin-degrading fungi. Environ. Sci. Technol. **27:**2566–2571.
23. **Lamar, R. T., M. J. Larsen, and T. K. Kirk.** 1990. Sensitivity to and degradation of pentachlorophenol by *Phanerochaete* spp. Appl. Environ. Microbiol. **56:**3519–3526.
24. **Matsumura, F., J. Quensen, and G. Tsushimoto.** 1983. Microbial degradation of TCDD in a model ecosystem. Environ. Sci. Res. **26:**191–219.
25. **Morgan, P., and R. J. Watkinson.** 1989. Microbiological methods for the cleanup of soils and ground water contaminated with halogenated organic compounds. FEMS Microbiol. Rev. **63:**277–300.
26. **Muir, D. C. G., A. L. Yarechewski, R. L. Corbet, G. R. B. Webster, and A. E. Smith.** 1985. Laboratory and field studies on the fate of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin in soil and sediments. J. Agric. Food Chem. **33:**518–523.
27. **Parsons, J. R., and M. C. M. Storms.** 1989. Biodegradation of chlorinated dibenzo-*p*-dioxins in batch and continuous cultures of strain JB1. Chemosphere **19:**1297–1308.
28. **Philippi, M., J. Schmidt, H. K. Wipf, and R. A. Hütter.** 1982. A microbial metabolite of TCDD. Experientia **38:**659–661.
29. **Rordorf, B. F.** 1985. Thermodynamic and thermal properties of polychlorinated compounds: the vapor pressures and flow tube kinetics of ten dibenzopara-dioxins. Chemosphere **14:**885–892.
30. **Rüttiman-Johnson, C., D. Cullen, and R. T. Lamar.** 1994. Manganese peroxidases of white rot fungus *Phanerochaete sordida*. Appl. Environ. Microbiol. **60:**599–605.
31. **Safe, S.** 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. **21:**51–88.
32. **Schecter, A. J., J. J. Ryan, M. Gross, N. C. A. Weerasinghe, and J. D. Constable.** 1987. Chapter 3. Chlorinated dioxins and dibenzofurans in human tissues from Vietnam, 1983–1984, p. 35–50. *In* C. Rappe, G. Choudhary, and L. H. Keith (ed.), Chlorinated dioxins and dibenzofurans in perspective. Lewis Publishers, Inc., Chelsea, Mich.
33. **Suzuki, F., S. Trenbeath, R. D. Gleim, and C. J. Sih.** 1978. Total synthesis of adriamycinone, regiospecific synthesis of anthracyclinones via base-catalyzed cyclazations. J. Am. Chem. Soc. **100:**2272–2273.
34. **Swanson, S. E., C. Rappe, J. Malmström, and K. P. Klingstad.** 1988. Emissions of PCDDs and PCDFs from the pulp industry. Chemosphere **17:**681–691.
35. **Tatarko, M., and J. A. Bumpus.** 1993. Biodegradation of phenanthrene by *Phanerochaete chrysosporium*: on the role of lignin peroxidase. Lett. Appl. Microbiol. **17:**20–24.
36. **Thomas, D. R., K. S. Carswell, and G. Georgiou.** 1992. Mineralization of biphenyl and PCBs by the white rot fungus *Phanerochaete chrysosporium*. Biotech. Bioeng. **40:**1395–1402.
37. **Tien, M., and T. K. Kirk.** 1983. Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science **221:**661–663.
38. **Tien, M., and T. K. Kirk.** 1988. Lignin peroxidase of *Phanerochaete chrysosporium*. Methods Enzymol. **161:**238–249.
39. **Valli, K., and M. H. Gold.** 1991. Degradation of 2,4-dichlorophenol by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. J. Bacteriol. **173:**345–352.
40. **Valli, K., H. Wariishi, and M. H. Gold.** 1992. Degradation of 2,7-dichlorodibenzo-*p*-dioxin by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. J. Bacteriol. **174:**2131–2137.
41. **Vazquez-Duhalt, R., D. W. S. Westlake, and P. M. Fedorak.** 1994. Lignin peroxidase oxidation of aromatic compounds in systems containing organic solvents. Appl. Environ. Microbiol. **60:**459–466.
42. **Wariishi, H., K. Valli, and M. H. Gold.** 1991. *In vitro* depolymerization of lignin by manganese peroxidase of *Phanerochaete chrysosporium*. Biochem. Biophys. Res. Commun. **176:**269–275.
43. **Yadav, J. S., J. F. Quensen III, J. M. Tiedje, and C. A. Reddy.** 1995. Degradation of polychlorinated biphenyl mixtures (Aroclors 1242, 1254, and 1260) by white rot fungus *Phanerochaete chrysosporium* as evidenced by congener-specific analysis. Appl. Environ. Microbiol. **61:**2560–2565.
44. **Yadav, J. S., and C. A. Reddy.** 1993. Mineralization of 2,4-dichlorophenoxyacetic acid (2,4-D) and mixtures of 2,4-D and 2,4,5-trichlorophenoxyacetic acid by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **59:**2904–2908.