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ORIGINAL PAPER

A novel metabolic pathway for biodegradation of DDT by the white rot fungi, *Phlebia lindtneri* and *Phlebia brevispora*

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Abstract 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) was used as the substrate for a degradation experiment with the white rot fungi *Phlebia lindtneri* GB-1027 and *Phlebia brevispora* TMIC34596, which are capable of degrading polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated biphenyls (PCBs). Pure culture of *P. lindtneri* and *P. brevispora* with DDT (25 μmol l^{-1}) showed that 70 and 30% of DDT, respectively, disappeared in a low-nitrogen medium after a 21-day incubation period. The metabolites were analyzed using gas chromatography/mass spectrometry (GC/MS). Both fungi metabolized DDT to 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD), 2,2-bis(4-chlorophenyl)acetic acid (DDA) and 4,4-dichlorobenzophenone (DBP). Additionally, DDD was converted to DDA and DBP. DDA was converted to DBP and 4,4-dichlorobenzhydrol (DBH). While DBP was treated as substrate, DBH and three hydroxylated metabolites, including one dihydroxylated DBP and two different isomers of monohydroxylated DBH, were produced from fungal cultures, and these hydroxylated metabolites were efficiently inhibited by the addition of a cytochrome P-450 inhibitor, piperonyl butoxide. These results indicate that the white rot fungi *P. lindtneri* and *P. brevispora* can degrade DBP/DBH through hydroxylation of the aromatic ring. Moreover, the single-ring aromatic metabolites, such as 4-chlorobenzaldehyde,
4-chlorobenzyl alcohol and 4-chlorobenzoic acid, were found as metabolic products of all substrate, demonstrating that the cleavage reaction of the aliphatic-aryl carbon bond occurs in the biodegradation process of DDT by white rot fungi.

Keywords Biodegradation · DDT · White rot fungi · Cytochrome P-450 · Hydroxylation · Metabolite

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Introduction

1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), had been used extensively in the past few decades as an insecticide for the protection of crops and for the control of vector-borne diseases like typhus and malaria (Foght et al. 2001). Though the use of DDT was banned in many developed countries in the 1970s because of its toxicity, hydrophobicity, and bioaccumulation (Kannan et al. 1994; Kelce et al. 1995), in many tropical developing countries, it is still used in

accordance with public health programs. DDT and its metabolites (DDD [1,1-dichloro-2,2-bis(4-chlorophenyl)ethane] and DDE [1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene]) have been found in soil, water, and sediment samples (Aislabie et al. 1997; Simonich and Hites 1995; Bould 1994). Since DDT residues are lipophilic, high levels of DDT and its metabolites have been detected in human adipose tissues, blood plasma, liver, brain, placenta and even in breast milk along the food chain (Dale et al. 1985; Siddiqui et al. 1981; Tanabe et al. 1990). For example, about 35–6900 ng g^{-1} of DDTs have been detected in human milk from different countries (Tanabe et al. 1990). The presence of DDT residues in the environment is a problem of great concern, and reducing their levels in the environment has become an important goal. Thus, in 2001, more than 100 countries signed the Stockholm Convention on Persistent Organic Pollutants (POPs), committing to eliminate the use of the 12 POPs of greatest concern, including DDT.

Due to the extremely slow degradation of DDT in natural environments, the enhancement of its degradation or mineralization process by microorganisms has been gaining popularity in recent decades, and a range of bacteria and white rot fungi have been demonstrated to enhance the degradation process. In bacteria, most reports indicate the reductive dechlorination of DDT to DDD (Langlois 1967; Wedemeyer 1967; Aislabie et al. 1997). Interestingly, some bacteria have been reported to degrade DDT and its metabolites by hydroxylation of the aromatic ring via oxidative attack (Nadeau et al. 1994; Hay and Focht 2000). Additionally, several fungi have been shown to possess biodegradative capabilities for a broad spectrum of environmentally persistent compounds, including DDT (Subba-Rao and Alexander 1985). The majority of the research on DDT degradation by ligninolytic fungi has been carried out by Aust and co-workers using the white rot fungus *Phanerochaete chrysosporium*. They described the degradation of DDT by *P. chrysosporium* in nitrogen-deficient cultures. Approximately 10% of the DDT was mineralized during this period and the remainder appearing as metabolites including DDD, 2,2,2-trichloro-1,1-bis (4-chlorophenyl)ethanol (dicofol), 2,2-dichloro-1, 1-bis(4-chlorophenyl)ethanol (FW-152), and DBP (Bumpus and Aust 1987; Aust 1990; Fernando et al. 1989). However, the metabolic process of DBP in this fungus is still unclear, and the hydroxylation of the aromatic ring of DDT and its intermediate metabolites by white rot fungi have not yet been reported.

In our previous study, several white rot fungi were chosen for examination based on their ability to degrade dioxin, and it was found that the white rot fungi *Phlebia lindtneri* and *Phlebia brevispora* could hydroxylate polychlorinated dibenzo-*p*-dioxins (PCDDs), 2,7-dichlorodibenzo-*p*-dioxin, 2,3,7-trichlorodibenzo-*p*-dioxin, 1,2,8,9-tetrachlorodibenzo-*p*-dioxin, and 1,2,6,7-tetrachlorodibenzo-*p*-dioxin (Mori and Kondo 2002; Kamei and Kondo 2005). It was also reported that chloronaphthalene, PCBs and PAHs were metabolized by *Phlebia* species (Mori et al. 2003; Kamei et al. 2005). It has been shown that hydroxylation of an aromatic ring is important as the first reaction in the degradation of PCDDs and PAHs. Significant inhibition of the degradation of these substrates was observed when they were incubated with cytochrome P-450 inhibitors. These results indicate that *Phlebia* species have specific activity for the biodegradation of organohalogen compounds via hydroxylation of aromatic rings and that the cytochrome P-450 monooxygenase system plays an important role in the initial hydroxylation of these xenobiotics. These informations led us to pay attention to *Phlebia* species in their capability to degrade DDT.

In this paper we evaluated the ability of *P. lindtneri* and *P. brevispora* to degrade DDT and its intermediate metabolites and describe the newly metabolites from the degradation process of DDT. We also present evidence that a cytochrome P-450 enzyme from fungi is involved in the hydroxylation of DBP.

Materials and methods

Chemicals

DDT and DDD were purchased from Tokyo Kasei Chemical Industry (Tokyo, Japan). DBP was purchased from Aldrich Chemical (Milwaukee, WI, USA). DDA, DBH, piperonyl butoxide and all organic solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Microorganisms and culture condition

Stock cultures of *P. lindtneri* GB-1027 (from the United States Department of Agriculture) and

P. brevispora TMIC34596 (from the Tottori Mycological Institute, Japan) were maintained on 9-cm-diameter potato dextrose agar plates (PDA, Difco) that had been incubated at 30°C. The mycelium mats on the agar plates were transferred to a sterilized blender cup containing 25 ml of sterilized water and were homogenized for 30 s. One milliliter of this homogenate was inoculated into 10 ml of liquid medium (pH 4.5) in 100-ml Erlenmeyer flasks containing 1.0% glucose as a carbon source, 1.2 mM ammonium tartrate as a low nitrogen (LN) medium and 20 mM sodium acetate, as described by Tien and Kirk (1988). The cultures were preincubated statically at 30°C in the ambient atmospheric conditions.

Degradation experiment

After preincubation for 5 days, 50 µl of the substrates (5 mM of DDT, DDD, DDA and DBP in *N*,*N*-dimethylformamide) was added to each inoculated flask (final concentration: 25 µmol l^{-1}). The flask was sealed with a glass stopper and sealing tape after the 100% oxygen was added to the headspace of each flask. As a control, the fungal cultures were killed by adding 0.2 g of sodium azide before addition of each substrate. After additional incubation for 7, 14, and 21 days, 0.2 g of sodium azide and internal standard were added to the cultures. The cultures were homogenized with 20 ml of acetone, and the biomass was removed by centrifugation at $3000\times g$ for 10 min. The resulting supernatant was analyzed by high-performance liquid chromatography (HPLC) to quantify the recovery of substrate after filtration with a membrane filter (0.45 µm). HPLC was performed on a Jasco PU-1580 intelligent pump and a Jasco MD-1510 multiwavelength detector fitted with an Inertsil ODS-3 column (150 mm) with an inner diameter of 4.6 mm (GL Science Inc., Tokyo, Japan). The supernatant was eluted with 80% acetonitrile in 0.1% trifluoroacetic acid aqueous solution at a flow rate of 1 ml min^{-1}.

Metabolites detection

For the identification of metabolites, the supernatant was evaporated to remove acetone, was acidified to pH 2.0 with 0.1 N HCl and extracted three times with ethyl acetate. The organic fraction was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The concentrate was analyzed by gas chromatography/mass spectrometry (GC/MS). Trimethysilyldiazomethane or acetic anhydride/pyridine was used for methyl or acetyl derivatization analysis (de Souza Bergo et al. 2009). GC/MS was performed on an HP 6890 GC system linked to an HP 5973 mass selective detector and a 30-m fused DB-5MS column (0.25 mm inside diameter, 0.25 µm film thickness; J&W Scientific, Folson, CA, USA). The oven temperature was programmed at 80°C for 3 min, followed by a linear increase to 300°C at 20°C min^{-1} and held at 300°C for 5 min.

Cytochrome P-450 inhibitor experiments

After preincubation for 5 days, the cytochrome P-450 inhibitor piperonyl butoxide (PB) (final concentration: 1.0 mmol l^{-1}) was added to the culture of *P. lindtneri*. The cultures with inhibitor were incubated for 2 h, and then DDT and DBP (25 µmol l^{-1}) were added. The cultures were incubated for 14 days, and recovery was measured using the methods described for the degradation experiments.

All experiments in this study were performed in triplicate. The results are reported as the average of triplicate determinations.

Results

The time courses for the degradation of DDT in LN medium by treatment with *P. lindtneri* and *P. brevispora* are shown in Fig. 1. About 36 and 9% of the added DDT had disappeared after the 7-day incubation period, and 70 and 30% of the DDT had disappeared after the 21-day incubation period in cultures of *P. lindtneri* and *P. brevispora*, respectively. In an HPLC analysis, DDD was detected in the cultures of both fungi after 7 days of incubation, with about 8.4 µmol l^{-1} (34%) and 4 µmol l^{-1} (16%) of the initial were transformed into DDD by *P. lindtneri* and *P. brevispora* during the 21-day incubation period, respectively. The concentration of DDT was stable in the killed control cultures during the incubation period.

To obtain trace metabolites of DDT by *P. lindtneri* and *P. brevispora*, culture extracts were analyzed with GC/MS by comparing their retention times and mass spectra with those of available standard

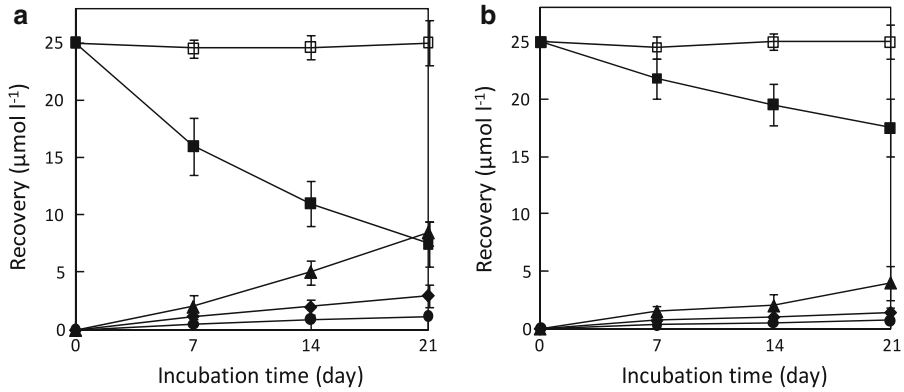

Fig. 1 Time course for metabolism of DDT (*closed squares*) and production of DDD (*closed triangles*), DDA (*closed diamonds*) and DBP (*closed circles*) by *P. lindtneri* (**a**) and *P. brevispora* (**b**) for 21-day incubation period. Recovery of DDT (*open squares*) from azide-killed control cultures are shown. Values are means ± SD of triplicate samples

Table 1 Mass balance for recovery of substrates and metabolites from the fungal cultures after 21 days of incubation

Treatment	Substrates	Degradation rate (%)	Recovery of substrates and metabolites (μmol l^{-1})								
			DDT	DDD	DDA	DBP	DBH	a	b	c	Total (%)
P. lindtneri	DDT	70 ± 2.1	7.5 ± 0.3	8.4 ± 0.4	3.0 ± 0.5	1.2 ± 0.1	–	0.1	0.5	0.2	20.9 (83.6)
	DDD	81 ± 3.4		4.8 ± 0.1	8.2 ± 0.7	2.7 ± 0.6	–	0.2	0.9	0.5	17.3 (69.2)
	DDA	45 ± 1.8			13.8 ± 1.1	3.3 ± 0.3	2.1 ± 0.2	0.2	1.0	0.3	20.7 (82.8)
	DBP	84 ± 4.7				4.0 ± 0.4	8.2 ± 0.8	0.3	1.6	0.7	14.8 (59.2)
P. brevispora	DDT	30 ± 1.6	17.5 ± 1.5	4.0 ± 0.2	1.4 ± 0.1	0.7 ± 0.1	–	–	–	–	23.6 (94.4)
	DDD	26 ± 3.0		18.4 ± 2.0	1.8 ± 0.4	0.5 ± 0.02	–	–	0.2	–	20.9 (83.6)
	DDA	32 ± 2.5			17.1 ± 0.9	1.3 ± 0.2	1.1 ± 0.3	–	0.4	0.1	20.0 (80.0)
	DBP	40 ± 4.8				14.9 ± 1.0	2.9 ± 0.5	0.1	0.6	0.2	18.7 (74.8)

a 4-chlorobenzaldehyde, *b* 4-chlorobenzyl alcohol, *c* 4-chlorobenzoic acid

"–" undetectable

The initial concentrations of all substrates are 25 μmol l^{-1}

Values are means ± SD of triplicate samples

compounds. The GC/MS analysis of the extracts from the culture supernatants of both fungi revealed a metabolite with retention time at 15.22 min, which was identified as DBP. After methylation, another metabolite with a retention time of 16.05 min was detected from both fungi. This spectrum is consistent with the methyl derivative of authentic DDA. After the 21-day incubation period, about 3.0 and 1.2 μmol l^{-1} of DDA and DBP were obtained in the culture of *P. lindtneri*, while 1.4 and 0.7 μmol l^{-1} of DDA and DBP were obtained in the culture of *P. brevispora*, respectively (Fig. 1). The results show clearly that degradation of DDT by *P. lindtneri* and *P. brevispora* had occurred. No metabolic product of DDT was observed in the azide-killed control cultures.

To investigate the degradation and metabolites of DDD, DDA and DBP, *P. lindtneri* and *P. brevispora* were incubated with 25 μmol l^{-1} of each substrate at 30°C for 21 days. The cultures were extracted and analyzed by GC/MS. As shown in Table 1, after 21 days of incubation, about 81, 45 and 84% of DDD, DDA and DBP had disappeared from the culture of *P. lindtneri*, respectively. In contrast, lower degradation activity was observed in *P. brevispora*, as only 26, 32 and 40% of DDD, DDA and DBP disappeared from the culture of *P. brevispora*, respectively. When DDD was used as a degradation substrate, DDA and

DBP were detected in the cultures of both fungi as metabolic products of DDD. While DBP and the other metabolite had retention times (15.8 min) and mass spectra identical to those of authentic 4,4-dichlorobenzhydrol (DBH), they were detected in the cultures of both fungi when DDA was added as a degradation substrate. The lack of stoichiometric mass balance might suggest that these substrates were metabolized to other products, which are undetectable by GC/MS analysis.

DBH and several metabolites were detected in the culture of *P. lindtneri* with DBP. The mass spectrum of metabolite A (with a retention time of 17.54 min) had a molecular ion peak at *m/z* 282 (Cl = 35.0)(molecular mass of DBP [250] + 32 mass) and fragment ions at *m/z* 265 (M-OH), 247 (M-Cl), 169 (M-C_6H_6Cl), 143 (M-C_6H_4Cl–CO), 139 (M-$C_6H_4O_2Cl$), 111 (C_6H_4Cl) and 77 (C_6H_5) (Fig. 2a). The occurrence of an *m/z* 282 indicated the formation of two hydroxyl substituents. After acetyl derivatization, the metabolite disappeared and new peaks that had a weak molecular ion peak at *m/z* 366 (molecular mass of metabolite A [282] + 84 mass) and fragment ions at *m/z* 324 (M-$COCH_2$), 282 (M-$COCH_2$–$COCH_2$), 264 (282-OH_2) and 229 (264-Cl) were obtained. This result suggested that metabolite A represents dihydroxylated DBP. Metabolites B and C (retention times at 17.00 and 17.17 min, respectively) had identical mass spectra (Fig. 2b), which had a molecular ion peak at *m/z* 268 (molecular mass of DBH [252] + 16 mass) and fragment ions at *m/z* 233 (M-Cl), 215 (M-Cl–OH_2), 155 (M-C_6H_6Cl), 139 (M-C_6H_6OCl), 111, 77 and 59. After acetyl derivatization, metabolites B and C disappeared, and two peaks with a weak molecular ion peak at *m/z* 352 (molecular mass of metabolites B and C [268] + 84 mass) and fragment ions at *m/z* 310 (M-$COCH_2$), 292 (M-$COCH_2$–OH_2), 268 (M-$COCH_2$–$COCH_2$), 250 (268-OH_2) and 215 (250-Cl) were obtained. This result suggested that metabolites B and C were different isomers of monohydroxylated DBH. On the other hand, from the culture of *P. brevispora* with DBP, DBH and dihydroxylated DBP were observed, but monohydroxylated DBH was not detected.

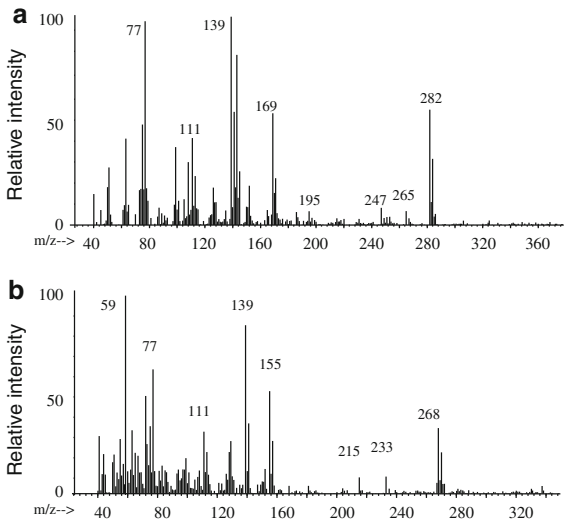

Fig. 2 Mass spectra of metabolite A (**a**) and metabolites B, C (**b**) produced from the culture of *P. lindtneri* with DBP

Additionally, the single-ring aromatic metabolites were observed as metabolites from both fungal cultures with all substrates (Table 1). These metabolites were identified as 4-chlorobenzaldehyde (retention time, 9.20 min), 4-chlorobenzyl alcohol (retention time, 10.10 min) and 4-chlorobenzoic acid (retention time, 10.63 min) on the basis of a GC/MS comparison with authentic compounds.

The effect of the cytochrome P-450 inhibitor piperonyl butoxide on the fungal degradation of DDT and DBP were investigated. The effects of piperonyl butoxide on the metabolism of DDT and DBP by *P. lindtneri* are summarized in Table 2. When DDT was treated as substrate, almost no change of degradation rate for DDT was observed after addition of piperonyl butoxide, however, the levels of the DBP increased, about 1.6 times higher than of DBP in the culture without P-450 inhibitor treatment, while less of production of single-ring aromatic metabolites were observed from inhibitor treated culture. On the other hand, degradation rate of DBP decreased approximately 18% in inhibitor treated culture when DBP as treated substrate. In addition, the most noticeable difference was the production of metabolites between the no-inhibitor and inhibitor treated samples. No hydroxylated metabolite of DBP was found in inhibitor-treated culture. Similarly, levels of single-ring aromatic metabolites decreased about 60%. This result indicates that the hydroxylation of the DBP aromatic ring might be mainly catalyzed by fungal cytochrome P-450 enzymes.

Discussion

This study showed that the white rot fungi *P. lindtneri* and *P. brevispora* can cause the degradation of DDT in

Table 2 Effect of cytochrome P-450 inhibitor piperonyl butoxide (PB) on the metabolism of DDT and DBP by *P. lindtneri* after 14 days of incubation

Treatment	DDT degradation (%)	DDT metabolites (μmol l^{-1})			DBP degradation (%)	DBP metabolites (μmol l^{-1})			
		DDD	DBP	Single-ring aromatic metabolites[a]		DBH	Dihydroxy-DBP	Monohydroxy-DBH	Single-ring aromatic metabolites[a]
Without PB	56.1 ± 3.4	5.5 ± 0.4	2.8 ± 0.3	0.7 ± 0.1	78.0 ± 4.5	7.1 ± 0.4	+	+	2.2 ± 0.2
With PB	57.8 ± 2.1	4.9 ± 0.3	7.3 ± 0.7	0.3 ± 0.04	60.3 ± 3.7	6.2 ± 0.6	−	−	0.8 ± 0.07

"+" detectable;"−" undetectable

[a] The single-ring aromatic metabolites included 4-chlorobenzaldehyde, 4-chlorobenzyl alcohol and 4-chlorobenzoic acid

Values are means ± SD of triplicate samples

a nitrogen-deficient medium. In *P. lindtneri*, the DDT metabolites identified included DDD, DDA, DBP, DBH, hydroxylated DBP, and hydroxylated DBH. In *P. brevispora*, the DDT metabolites identified included DDD, DDA, DBP, DBH and hydroxylated DBP. These results suggest that DDT might have been reductively dechlorinated to DDD, which was then degraded to DDA by dechlorination and oxidation. The introduction of a carboxyl group at C-1 of DDA would be expected to make the carbon–carbon bond of the ethane group more labile to cleavage and give rise to DBP. The metabolism of DBP occurred by two pathways: hydroxylation to form dihydroxylated DBP and reduction to form DBH. In *P. lindtneri*, DBH was subsequently hydroxylated to monohydroxylated DBH. It was considered that the hydroxyl-DBP/DBH might be further metabolized to single-ring aromatic compounds. On the basis of the information obtained from the results, a degradation pathway in *P. lindtneri* and *P. brevispora* is proposed (Fig. 3).

The mechanisms of microbial attack have already been described. Most reports indicate that DDT is reductively dechlorinated to DDD. A number of studies on DDT metabolism in microorganisms have shown that under aerobic conditions the dehydrochlorination of DDT predominates, whereas under anaerobic conditions reductive dechlorination is the dominant reaction (Johnsen 1976; Essac and Matsumura 1980; Lal and Saxena 1982). Fungi also seem to play a significant role in the degradation of DDT. In the present study, fungi have been shown to possess degradative capabilities for DDT. The results of Subba-Rao and Alexander (1985) showed that several species of fungi are able to convert DDT to some common DDT metabolites, including DDE, DDD, DDM, DBH, and DBP. Bumpus and Aust (1987), working on DDT degradation by white rot fungi, indicated that DDT degradation in *P. chrysosporium* occurred by reductive dechlorination and hydroxylation of the ethane group to DDD, dicofol and FW152, which was degraded by sequential steps involving reductive dechlorination and oxidation of the ethane group to DBP. However, the further metabolic process of DBP in fungi is still unclear at present, though the fungus *Aspergillus niger* have been shown to convert DBP into 4-chlorobenzophenone by reductive dechlorination of the aromatic ring (Subba-Rao and Alexander 1985). Although the formation of 4-chlorobenzophenone was not detected as metabolite of DBP in our study, it was not excluded that 4-chlorobenzophenone was rapidly metabolized to other metabolites.

Nadeau et al. (1994) showed that the PCB-degrading bacterium *Alcaligenes eutrophus* A5 oxidizes DDT at the unsubstituted carbons on the aromatic ring to produce 2,3-dihydrodiol-DDT intermediate. It is proposed that this is a dioxygenase type of attack resulting in the transient production of a DDT dihydrodiol. The 2,3-dihydrodiol-DDT would be degraded to 2,3-dihydroxy-DDT by a dehydrogenase. 2,3-dihydroxy-DDT would be further metabolized through *meta*-ring cleavage to form the yellow ring fission product, which would then be catabolized to 4-chlorobenzoic acid (4-CBA). The bacterium *Ralstonia eutropha* A5 grown on biphenyl was also shown to degrade DDD to form single-ring aromatic compounds via hydroxylation of the aromatic ring and subsequent *meta*-ring cleavage (Hay and Focht 2000). However, there is

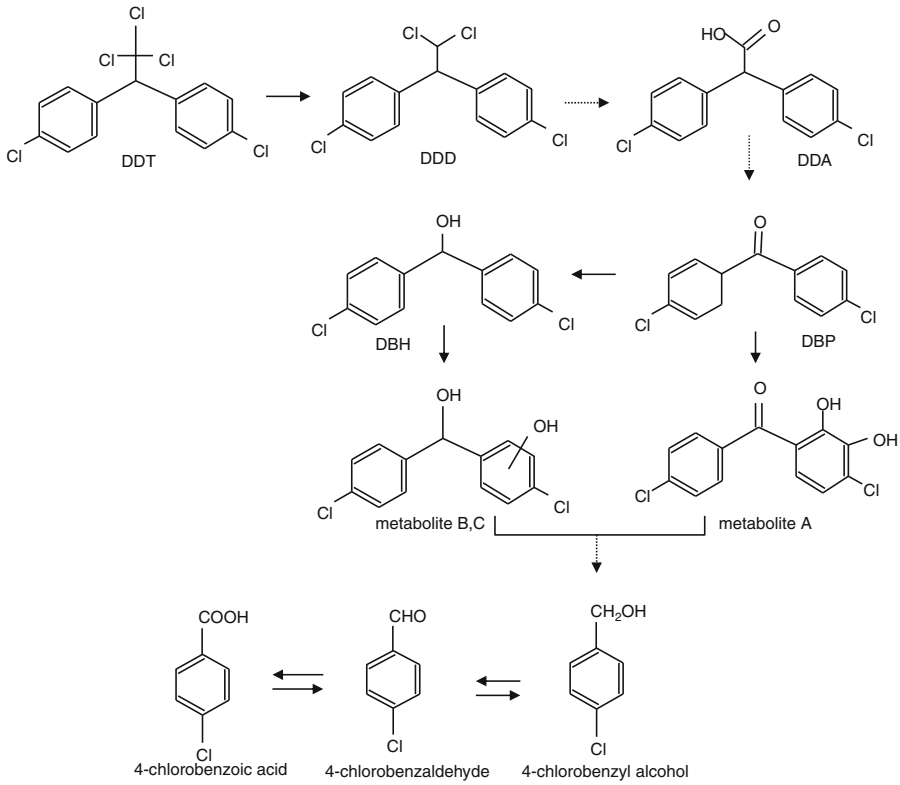

Fig. 3 Proposed pathway for the degradation of DDT by *P. lindtneri* and *P. brevispora*. The metabolites B, C were not detected from the culture of *P. brevispora* with DBP. *Dotted arrows* indicate involvement of more than one step

limited information on the transformation of DDT and its metabolites via hydroxylation at the aromatic ring and ring cleavage reaction by white rot fungi at present. In this study, the hydroxylation products of DDT at the aromatic ring, such as hydroxy-DDT and hydroxy-DDD, were not observed from fungal cultures. However, an interesting aspect to this work was the finding of the formation of hydroxy-DBP/DBH, which was considered to undergo aromatic ring cleavage. The results suggest that hydroxylation of aromatic ring of DDT and its intermediate metabolites by *Phlebia* species might be effected by the substitute group on the benzylic carbon. On the other hand, the presence of single-ring aromatic compounds such as 4-chlorobenzaldehyde, 4-chlorobenzyl alcohol, and 4-chlorobenzoic acid proved that the cleavage reaction of the aliphatic-aryl carbon bond has occurred in the degradation process of DDT by both fungi, but it is unclear whether these single-ring aromatic compounds resulted from the aromatic ring cleavage reaction.

Cytochrome P-450 enzymes play significant role in the metabolism of various organic pollutants have been reported (Mori and Kondo 2002; Mori et al. 2003; Kamei et al. 2005). However, no reports have been found in the literature concerning cytochrome P-450 enzymes involved in the metabolism of DDT by white rot fungi. Our study showed the inhibition of DBP degradation at least partly due to the addition of the cytochrome P-450 inhibitor piperonyl butoxide, and the formation of hydroxylated DBP/DBH from DBP was likely associated with cytochrome P-450 monooxygenase because the production of these compounds was efficiently inhibited by the addition of piperonyl butoxide. Therefore, it is assumed that in *Phlebia* species, the DBP/DBH is catalyzed by cytochrome P-450 monooxygenase to form corresponding monohydroxylated products, which were then oxidation to dihydroxylated products by the same enzyme again. This estimation would be supported by the result that mono- and dihydroxylated products were detected in both fungal cultures. Based on these results, we proposed that in *Phlebia* species, the mechanism for hydroxylation of aromatic ring differ from in bacteria (Nadeau et al. 1994; Hay and Focht 2000), and the cytochrome P-450 monooxygenase system plays an important role in the

hydroxylation and following ring cleavage reaction on DBP/DBH molecular, though there are almost no inhibition effect on the degradation rate of DDT.

Recently, new and classic families of fungal peroxidases were reviewed in detail by Hofrichter et al. (2010). It is reported that the peroxygenase secreted by the wood rot fungi efficiently transferring oxygen from peroxides to various organic substrates including aromatic, heterocyclic and aliphatic compounds (Ullrich and Hofrichter 2005; Anh et al. 2007). Although the precise enzymological details of xenobiotic degradation in white rot fungi have not been established, mounting evidence suggests that the lignin-degrading system is responsible. Bumpus et al. (1985) showed that mineralization of both [^{14}C]DDT and [^{14}C]lignin were promoted in nitrogen-starved cultures and suppressed in nitrogen-sufficient cultures by *P. chrysosporium*. A few species of *Phlebia* are known to produce lignin-degrading extracellular enzyme system including lignin peroxidases, manganese peroxidases, and laccase (Hofrichter et al. 2001; Vares et al. 1995; Leontievsky et al. 1997). However, it needs further research to prove if that the extracellular enzyme systems take part in the process of DDT degradation by selected fungi in our study.

In this report, metabolism of DDT and its intermediate metabolites in white rot fungi, *P. lindtneri* and *P. brevispora* was studied. To our knowledge, this is the first study describing the hydroxylation occurred at the aromatic ring of DDT metabolites by white rot fungi. This result is important because of the possibilities of using fungi for the decontamination and detoxification of organochlorine-polluted environments. The use of microorganisms for bioremediation requires an understanding of all the physiological and biochemical aspects involved in pollutant transformation. Future research includes identification of other intermediate metabolites, identification and isolation of an enzyme system involved in the degradation of DDT and molecular approaches for application in the organochlorine-polluted environments.

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