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Biodegradation of DDT [1,1,1-Trichloro-2,2-Bis(4-Chlorophenyl)Ethane] by the White Rot Fungus *Phanerochaete chrysosporium*

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Extensive biodegradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by the white rot fungus *Phanerochaete chrysosporium* was demonstrated by disappearance and mineralization of [^{14}C]DDT in nutrient nitrogen-deficient cultures. Mass balance studies demonstrated the formation of polar and water-soluble metabolites during degradation. Hexane-extractable metabolites identified by gas chromatography-mass spectrometry included 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD), 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol (dicofol), 2,2-dichloro-1,1-bis(4-chlorophenyl)ethanol (FW-152), and 4,4′-dichlorobenzophenone (DBP). DDD was the first metabolite observed; it appeared after 3 days of incubation and disappeared from culture upon continued incubation. This, as well as the fact that [^{14}C]dicofol was mineralized, demonstrates that intermediates formed during DDT degradation are also metabolized. These results demonstrate that the pathway for DDT degradation in *P. chrysosporium* is clearly different from the major pathway proposed for microbial or environmental degradation of DDT. Like *P. chrysosporium* ME-446 and BKM-F-1767, the white rot fungi *Pleurotus ostreatus*, *Phellinus weirii*, and *Polyporus versicolor* also mineralized DDT.

Although 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) is a persistent environmental pollutant, it does appear to undergo slow degradation in the environment (1, 15, 18, 23). Studies by Wedemeyer (34–36) and by Alexander and co-workers (12–14, 26, 27, 29, 30) have demonstrated that extensive biodegradation of DDT and DDT metabolites occurs in some bacteria. These studies have led to the elucidation of a pathway for DDT biodegradation (Fig. 1). Although other reactions, most notably conversion of DDT to 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (DDE) may occur during its biodegradation, the major bacterial pathway appears to involve an initial reductive dechlorination of the trichloromethyl group to form 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD), which then undergoes further dechlorination, oxidation, and decarboxylation to form bis(4-chlorophenyl)methane (DDM). DDM may then undergo cleavage of one of the aromatic rings to form *p*-chlorophenylacetic acid, which may also undergo ring cleavage. The products of ring cleavage reactions are then further degraded to Krebs cycle intermediates and, ultimately, oxidized to carbon dioxide to complete the mineralization process. Alternatively, DDM may be converted to DBH (4,4′-dichlorobenzhydrol) or DBP (4,4′-dichlorobenzophenone), DBH also may undergo ring cleavage, or both DBH and DBP may undergo reductive dechlorination of the aromatic ring to form the corresponding nonchlorinated analogs, which then, presumably, readily undergo aromatic ring cleavage and further degradation.

Unlike the situation with bacteria, biodegradation of DDT by fungi has received relatively little attention (30). However, a few studies (2, 3, 9–11, 20, 30), most recently those of Subba-Rao and Alexander (30), have confirmed that a number of fungi can, indeed, degrade DDT. Subba-Rao and Alexander (30) and Engst et al. (9–11) have further suggested that the pathway for DDT degradation for some of the fungi they studied is similar to or the same as the major pathway proposed for bacteria.

We have recently presented evidence that the lignin-degrading system of the white rot fungus *Phanerochaete chrysosporium* is able to mineralize [^{14}C]DDT, as well as a number of other persistent environmental pollutants (5–7). In the present investigation we have identified and documented the presence of a number of the major metabolic intermediates formed during the biodegradation of DDT by this wood-rotting fungus. Furthermore, our studies (5–7) show that DDT degradation, like lignin degradation, is promoted by the onset of idiophasic metabolism in response to nutrient nitrogen starvation. The present study shows that the major metabolic pathway for DDT degradation by this microorganism during idiophasic metabolism is clearly different from the major metabolic pathway that has been shown to occur in bacteria. The major metabolic pathway for DDT biodegradation in *P. chrysosporium* proceeds via a pathway in which DDT is first oxidized to 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol (dicofol), which is then dechlorinated to form 2,2-dichloro-1,1-bis(4-chlorophenyl)ethanol (FW-152). Ultimately, oxidative carbon-carbon bond cleavage results in formation of DBP, which then presumably undergoes aromatic ring cleavage or reductive dechlorination followed by degradation to carbon dioxide.

MATERIALS AND METHODS

Fungi. *P. chrysosporium* BKM-F-1767 and ME-446, *Pleurotus ostreatus*, *Polyporus versicolor*, and *Gleophyllum trabeum* were obtained from the Forest Products Laboratory, U.S. Department of Agriculture, Madison, Wis. The root rot fungus *Phellinus weirii* was a gift from the Forestry Sciences Laboratory, U.S. Department of Agriculture, Pa-

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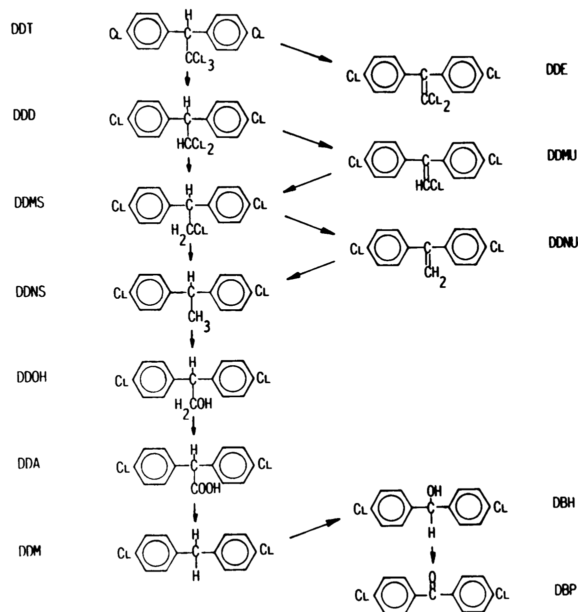

FIG. 1. Primary biodegradative pathway for the metabolism of DDT in microorganisms (adapted from references 9–11, 12–14, 26, 27, 29, and 30). Abbreviations: DDMS, 1-chloro-2,2-bis(4-chlorophenyl)ethane; DDNS, 2,2-bis(4-chlorophenyl)ethane; DDOH, 2,2-bis(4-chlorophenyl)ethanol; DDA, 2,2-bis(4-chlorophenyl)acetic acid; DDMU, l-chloro-2,2-bis(4-chlorophenyl)ethene; DDNU, 1,1-bis (4-chlorophenyl)ethene.

cific Northwest Station, Corvallis, Oreg. *Polyporus versicolor* was maintained on yeast agar, and the other fungi used in this study were maintained on malt agar. All fungi were kept at room temperature until used. Subcultures were routinely made every 30 to 60 days.

Chemicals. Dicofol and FW-152 were gifts from Rohm & Haas Co., Spring House, Pa. DDT and DDT metabolites were purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. [^{14}C]dicofol (9.78 mCi/mmol) was also a gift from Rohm & Haas Co. [^{14}C]DDT (40 and 85 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, Ill., and [^{14}C]methoxychlor [1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane] (5.08 mCi/mmol) was purchased from Pathfinder Laboratories Inc., St. Louis, Mo. The purity of radiolabeled chemicals was monitored by thin-layer chromatography and high-performance liquid chromatography (HPLC). When necessary, these compounds were repurified by thin-layer chromatography. The purity of radiolabeled compounds used in these experiments was 98% or greater.

Gas-liquid chromatography. A gas-liquid chromatograph (GLC) (model 3700; Varian, Sunnyvale, Calif.) equipped with an electron capture detector and a digital integrator (model 3390; Hewlett-Packard Co., Palo Alto, Calif.) was used in this study. DDT, DDT metabolites, and their derivatives were separated on a glass column (1.8 m by 2 mm) packed with 3% OV-1 on Gas Chrom Q (100/120 mesh; Applied Science Laboratories, State College, Pa.). The column temperature was 220°C, the injector temperature was 240°C, and the detector temperature was 350°C. Nitrogen was used as the carrier gas.

Mass spectrometry. Mass spectra of DDT, DDT metabolites, and their acetate derivatives were obtained with a gas chromatograph-mass spectrometer (model 5985; Hewlett-Packard Co.) equipped with a 3% OV-1 column. The ionization voltage was 70 eV. Typically, a temperature program from 170 to 220°C was used to separate these compounds.

Culture conditions and analytical procedures. Fungi were incubated at 37 to 39°C in the liquid culture medium described by Kirk et al. (17). This medium is composed of 56 mM glucose, 1.2 mM ammonium tartrate, trace minerals, and thiamine in 20 mM dimethylsuccinate buffer (pH 4.2). Cultures of *P. chrysosporium* were established by inoculating the medium with spores as described previously (17). Inoculations of *Phellinus weirii*, *Polyporus versicolor*, *Pleurotus ostreatus*, and *G. trabeum* were prepared by adding approximately 25 ml of sterile H_2O to a slant culture in a culture tube (25 by 150 mm). The mycelia were suspended by agitation with a sterile inoculation loop. A 1-ml portion of this suspension was then used to inoculate the medium with each of these fungi. Each 250-ml culture flask was equipped with a gas exchange manifold and contained 10 ml of the nutrient nitrogen-deficient medium. Each culture also contained 1.25 nmol of [^{14}C]DDT (50 nCi), which was added in 10 μl of acetone. The cultures were sealed and incubated for a total of 30 days. During the first 3 days of incubation, cultures were allowed to grow in air. After 3 days and at 3-day intervals thereafter, cultures were flushed with oxygen. Evolution of $^{14}CO_2$ from [^{14}C]DDT was assayed at 3-day intervals as previously described (7). DDT disappearance and metabolite formation were assayed as previously described (7). Glucose concentration was measured by the procedure described by Nelson (24). Mycelium dry weight was determined after the mycelia were collected and dried on tared filters (pore site, 0.22 μm; Millipore Corp., Bedford, Mass.). All studies were performed in triplicate or quadruplicate.

Derivatizations. Dicofol and FW-152 were acetylated by placing 50 mg of dicofol or 1 mg of FW-152–2 ml of acetic anhydride in a 4-ml Reacti-vial (Pierce Chemical Co., Rockford, Ill.). Concentrated sulfuric acid (2 drops) was then added, and the vials were sealed with Teflon-lined caps and incubated for 15 min at 60°C. Following incubation, the unreacted anhydride was discharged by carefully and slowly adding the reaction mixture to 2 ml of water in a 100-ml Erlenmeyer flask. These solutions were extracted with two 15-ml portions of hexane. The hexane layers were combined, dried with magnesium sulfate, filtered, and evaporated under argon. The residues were then dissolved in 2 ml of hexane and stored at room temperature.

Hexane extracts of cultures of *P. chrysosporium*, which had been incubated with DDT, were placed in a round-bottom flask. Hexane was removed by evaporation, and the residue was dissolved in 2 ml of acetic anhydride, transferred to a 2-ml Reacti-vial, and acetylated as described above.

HPLC. HPLC of DDT metabolites was performed by using a system equipped with an Altex model 110A pump (Anspec Co., Inc., Ann Arbor, Mich.), an injector (Rheodyne Inc., Cotati, Calif.), a reverse-phase column (4.6 by 250 mm; R-Sil C-18; Alltech Associates Inc., Deerfield, Ill.), and a Schoeffel model 770 detector (Kratos Div., ABI Analytical, Ramsey, N.J.). Isocratic elution was performed with 80% methanol at a flow rate of 1 ml/min. The retention time of DDT and DDT metabolites was established by monitoring the elution of authentic standards at 238 nm. In a typical experiment, four cultures (10 ml) of *P. chrysosporium* which had been incubated with [^{14}C]DDT (1.25 nmol per culture) were pooled, 25 ml of acetonitrile was added, and the mixture was homogenized in a Potter Elvehjem tissue homogenizer equipped with a Teflon pestle.

The homogenized material was then extracted with two 50-ml portions of hexane, which were pooled, dried over magnesium sulfate, and filtered. Hexane was then removed by evaporation under argon. Following hexane extraction, the aqueous phase was acidified to pH 2.0 with concentrated HCl and extracted with two 50-ml portions of methylene chloride. The methylene chloride extracts were then pooled, dried over magnesium sulfate, and filtered. Methylene chloride was removed by evaporation under argon. The hexane extracts were then dissolved in a small amount (ca. 1 ml) of hexane, and the methylene chloride extracts were dissolved in a small amount (ca. 1 ml) of methanol. In some experiments, material that precipitated during concentration was removed by filtration with a Pasteur pipette containing a small amount of glass wool. Aliquots of 20 μl were typically used for injection into the HPLC. Fractions (1 ml) were collected in scintillation vials. Safety Solve (Research Products International Corp., Mount Prospect, Ill.) (10 ml) was added to each fraction, and radioactivity was determined by liquid scintillation spectrometry.

Mass balance experiments. Following incubation of [^{14}C]DDT in nutrient nitrogen-deficient cultures of *P. chrysosporium*, cultures were extracted with hexane and methylene chloride as described above. Following these extractions, particulate matter (i.e., mycelium) was separated from the aqueous fraction by filtration. Safety Solve (10 ml) was added to the recovered mycelium in a scintillation vial. Safety Solve (10 ml) was also added to 1-ml aliquots of the hexane, methylene chloride, and aqueous fractions. The radioactivity of all fractions was determined by liquid scintillation spectrometry.

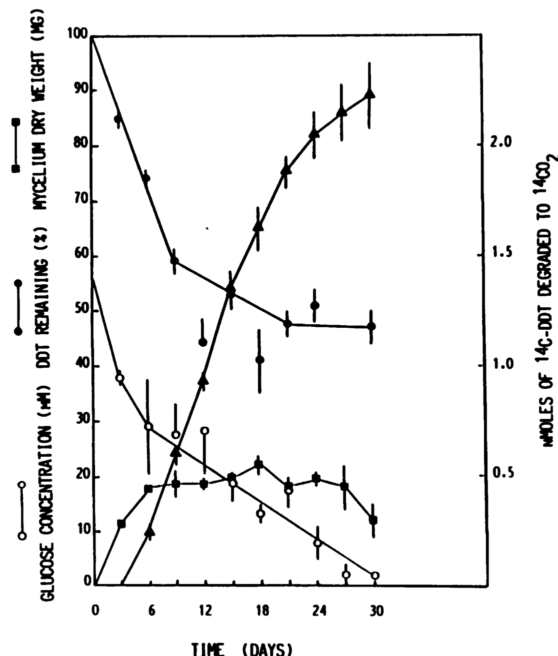

FIG. 2. DDT disappearance (●), DDT mineralization (▲), glucose utilization (○), and dry-weight change [■] in nutrient nitrogen-deficient culture of *P. chrysosporium* ME-446. Each 250-ml culture flask contained 10 ml of the nutrient nitrogen-deficient medium described in Materials and Methods. The initial concentration of DDT in each flask was 4.8 μM. For mineralization studies, cultures also contained 50 nCi of [^{14}C]DDT.

RESULTS

Figure 2 shows that DDT was degraded by nutrient nitrogen-deficient cultures of *P. chrysosporium*. Degradation was measured by DDT disappearance and [^{14}C]DDT mineralization. Glucose utilization and fungal growth results, as measured by mycelium dry weight, are also presented in Fig. 2. During the first 3 days of incubation, vigorous growth occurred, as evidenced by the fact that approximately 32% of the glucose originally present was consumed during this time, resulting in production of a clearly visible mycelial mat. The fungus achieved 50% of its maximal mass during this period. A substantial amount of DDT (15%) disappeared during the first 3 days of incubation. However, degradation does not appear to be extensive as no mineralization of [^{14}C]DDT was observed during this time and DDD was the only metabolite observed. During the 30-day incubation period, approximately 50% of the DDT was degraded, as measured by DDT disappearance. Mineralization of [^{14}C]DDT began to occur between days 3 and 6 and continued to occur throughout the incubation period (30 days). As discussed below, the presence of a number of other DDT metabolites was first observed during this time.

Mineralization appears to be first order with respect to DDT concentration. For example, in an earlier study (7) we found that when the initial concentration of [^{14}C]DDT was 0.125 μM, roughly 4% of the [^{14}C]DDT originally present was mineralized during the 30-day incubation period. In the present study (Fig. 2), in which the initial [^{14}C]DDT concentration was 38-fold higher (4.8 μM), again approximately 4 to 5% of the [^{14}C]DDT was mineralized. Indeed, in other studies we found that the initial rate (i.e., the rate observed during days 3 to 18) of [^{14}C]DDT mineralization was first order with respect to [^{14}C]DDT concentration over a range from 0.125 to 17 μM. These findings are similar to those of Eaton (8), who showed that ^{14}C-labeled polychlorinated biphenyl mineralization was also proportional to ^{14}C-labeled polychlorinated biphenyl concentration.

The rate of DDT disappearance appeared to decline as the concentration of glucose decreased (Fig. 2). In studies in which additional glucose was added after 31 and 61 days of incubation, it was shown that greater than 99% of the DDT originally present was degraded after 75 days of incubation (6). Furthermore, neither DDD nor DDE accumulated as metabolites.

Mineralization of [^{14}C]dicofol (a [^{14}C]DDT metabolite) and [^{14}C]methoxychlor (a [^{14}C]DDT analog) in nutrient nitrogen-deficient cultures of *P. chrysosporium* BKM-F-1767 was also studied. The rate and extent of mineralization of both of these compounds were substantially greater than the rate and extent of mineralization observed for [^{14}C]DDT (Fig. 3). Like [^{14}C]DDT, [^{14}C]dicofol and [^{14}C]methoxychlor were not observed to undergo mineralization during the first 3 days of incubation. However, mineralization of all of these compounds began between days 3 and 6 and continued throughout the incubation period.

It is now apparent that the lignin-degrading system of *P. chrysosporium* is responsible, at least in part, for the ability of this microorganism to mineralize DDT as well as a wide variety of xenobiotics (4–8, 16, 28). To determine whether other white rot fungi possess similar abilities, we studied three other species of white rot fungi for their ability to mineralize [^{14}C]DDT. Table 1 shows that in nutrient nitrogen-deficient cultures *Phellinus weirii*, *Pleurotus ostreatus*, and *Polyporus versicolor* mineralized 5.7 ± 0.9, 5.9 ± 4.2, and 5.3 ± 3.2%, respectively, of the total [^{14}C]DDT present during the 30-day incubation period. In this experiment,

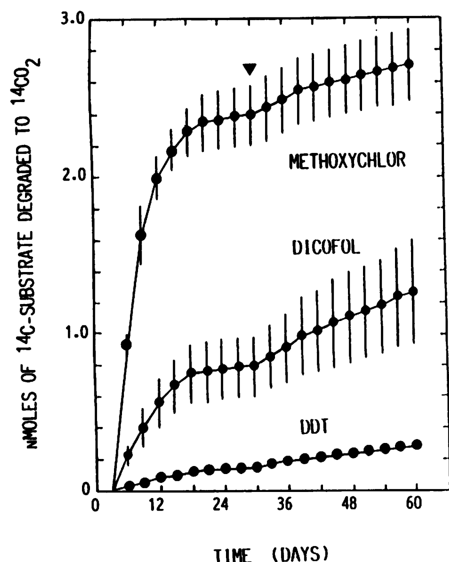

FIG. 3. Mineralization of [^{14}C]dicofol, [^{14}C]methoxychlor, and [^{14}C]DDT in nutrient nitrogen-deficient cultures of *P. chrysosporium* BKM-F-1767. Each culture contained 5.0 nmol of ^{14}C-labeled chemical. For [^{14}C]DDT, the width of error bars is less than the diameter of the circles. The arrow indicates the point at which supplemental glucose (56 mM) was added to each culture.

which was conducted at room temperature (22 to 27°C), *P. chrysosporium* BKM-F-1767 mineralized 13.1 ± 2.1% of the [^{14}C]DDT initially present. In all cases, vigorous growth occurred during the first 3 days of incubation, as evidenced by the appearance of a mycelial mat. However, in no case was [^{14}C]DDT mineralization observed at this time. In cultures of *P. chrysosporium* and *Phellinus weirii*, [^{14}C]DDT mineralization began to occur between days 3 and 6. In cultures of *Pleurotus ostreatus* and *Polyporus versicolor*, [^{14}C]DDT mineralization began to occur between days 6 and 9. In all cultures [^{14}C]DDT mineralization continued to occur throughout the 30-day incubation period.

In nutrient nitrogen-sufficient (12 mM ammonium tartrate) cultures, *Phellinus weirii*, *Pleurotus ostreatus*, and *P. chrysosporium* each mineralized less than 0.1% of the [^{14}C]DDT initially present during the 30-day incubation. In contrast, cultures of *Polyporus versicolor* mineralized 2.8 ± 0.01% of the [^{14}C]DDT initially present in nitrogen-sufficient cultures. In all cases, however, mineralization was less than that observed under nutrient nitrogen-deficient conditions. The brown rot fungus *G. trabeum* was also studied. Mineralization of [^{14}C]DDT was less than 0.1% in nutrient nitrogen-deficient or sufficient cultures.

Two strains of *P. chrysosporium* ME-446 and BKM-F-1767, were compared for their ability to mineralize [^{14}C]DDT at 39°C and at room temperature (22 to 27°C) (Table 1). *P. chrysosporium* is a mesophilic fungus whose optimal temperature for growth and lignin degradation is 39°C (17). However, both strains mineralized [^{14}C]DDT equally well at 39°C and at room temperature (Table l). Furthermore, there was little or no temperature-dependent difference in the ability of either strain to mineralize [^{14}C]DDT.

In mineralization studies (Fig. 2; Table 1) the extent of [^{14}C]DDT mineralization was found to be somewhat variable. In experiments with *P. chrysosporium* it was found that, in general, approximately 4 to 13% of the [^{14}C]DDT originally present was mineralized during the 30-day incubation. However, in some cultures the extent of mineralization was as great as 17%.

Identification of DDT metabolites. During the first 3 days of incubation, a metabolite having the same retention time as DDD during GLC was observed in hexane extracts of nutrient nitrogen-deficient cultures of *P. chrysosporium* incubated with DDT. Subsequent mass-spectral analysis of this material showed a parent ion of *m/e* 318 and a base peak of *m/e* 235, as well as other major fragments (*m/e* 200, 199, 176, and 165), confirming a structural assignment as DDD (22, 31). During the remainder of the incubation period, the concentration of DDD decreased such that it was barely detectable after 30 days of incubation. After 6 days of incubation, another DDT metabolite having a retention time identical to that of DBP was observed. The concentration of this material reached a maximum after 12 days of incubation and gradually declined for the rest of the incubation period. Mass-spectral analysis of this material revealed the presence of a parent ion of *m/e* 250, a base peak of *m/e* 139, a fragment ion of *m/e* 111, indicative of the *p*-chlorophenyl ion, and a fragment ion of *m/e* = 215, indicative of the 4-chlorobenzophenone fragment. These findings are consistent with a structural assignment as DBP (22, 31). However, because DBP is a known thermal decomposition product of dicofol in some GLC systems (22), acetylated DDT biodegradation products (which are more resistant to thermal degradation) were also examined. These studies confirmed the presence of a compound which comigrated with authentic dicofol acetate. The mass spectrum (Fig. 4) of this material strongly resembled that of DDE, having a parent ion of *m/e* 316, as well as other fragments (*m/e* 246, 233, and 176) diagnostic of this DDT metabolite (22, 31). However, a base peak of *m/e* 43 (characteristic of acetates) was found, rather than a base peak of *m/e* 246, expected for DDE. Also, a fragment of *m/e* 293, which is not found in mass spectra of DDE, occurred in mass spectra of this material. These studies are consistent with a structural assignment as dicofol acetate. It has been shown by others (21) that dicofol acetate undergoes zinc-catalyzed thermolytic decomposition to form DDE; it is therefore not surprising to find that the mass spectra of these two compounds are very similar, although clearly not identical. The presence of a strong peak of *m/e* 251 indicates that cleavage to form the 4,4′-dichlorobenzhydrol ion is a major alternative fragmentation pattern. The presence, in the dicofol acetate mass spectrum, of fragment ions of *m/e* 139 and 111, which are also diagnostic for 4,4′-dichloro-

TABLE 1. Mineralization of [^{14}C]DDT by white rot fungi[a]

Organism	Incubation temp[b]	Amt of [^{14}C]DDT degraded to $^{14}CO_2$	% [^{14}C]DDT degraded to $^{14}CO_2$
P. chrysosporium ME-446	RT	128.2 ± 31.9	10.3 ± 2.6
P. chrysosporium BKM-1767	RT	163.6 ± 26.8	13.1 ± 2.1
Pleurotus ostreatus	RT	73.3 ± 52.3	5.9 ± 4.2
Phellinus weirii	RT	71.0 ± 11.2	5.7 ± 0.9
Polyporus versicolor	RT	66.6 ± 40.5	5.3 ± 3.2
P. chrysosporium ME-446	39°C	168.9 ± 53.0	13.5 ± 4.2
P. chrysosporium BKM-1767	39°C	131.8 ± 72.9	10.5 ± 5.8

[a] Each result is the mean ± standard deviation of three or four determinations.

[b] Cultures were grown without agitation and in the dark at room temperature (RT) or 39°C.

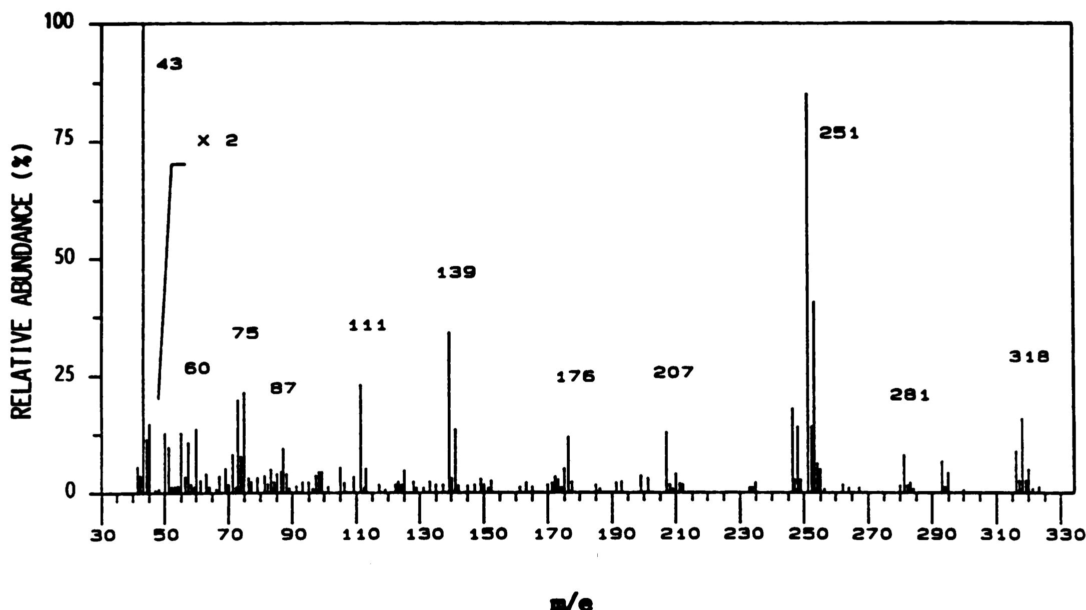

FIG. 4. Mass spectrum of an acetylated DDT metabolite. A structural assignment of dicofol acetate was made on the basis of interpretation (see text) of mass-spectral data and because this material comigrated with authentic dicofol acetate during GLC.

benzhydrol, supports this interpretation. Lastly, the fragment ion of *m/e* 293 was apparently formed by the loss of the trichloromethyl moiety. Thus these findings confirm a structural assignment as dicofol acetate; they also confirm that dicofol was the underivatized DDT metabolite.

Mass-spectral studies were supported by studies in which [^{14}C]DDT metabolites were separated by HPLC (Fig. 5). In these studies radiolabeled DDT metabolites extracted from nutrient nitrogen-deficient cultures of *P. chrysosporium*

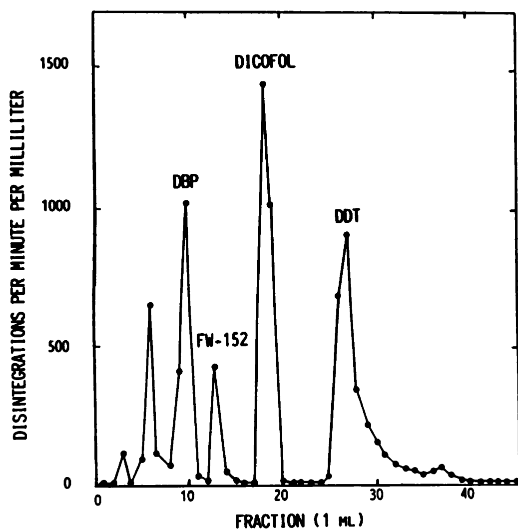

FIG. 5. Chromatogram (HPLC) of hexane-extractable DDT metabolites. [^{14}C]DDT (1.25 nmol, 50 nCi) was incubated with nutrient nitrogen-deficient cultures of *P. chrysosporium* BKM-F-1767. After 30 days, supplemental glucose (56 mM) was added and the incubation was continued for another 30 days, after which the cultures (four) were extracted with hexane and prepared for HPLC as described in Materials and Methods.

were shown to comigrate with authentic dicofol and with DBP.

We have previously shown that DDD is a major *P. chrysosporium* metabolite of DDT and, indeed, was the only metabolite observed during the first 3 days of incubation (5, 7). This, coupled with the fact that it disappeared fairly rapidly after the cultures became ligninolytic (after 3 to 4 days), prompted us to look for metabolites of DDD. Except for DBP, none of the metabolites of DDD in the DDT degradation pathway proposed for bacteria were found. However, a metabolite whose acetylated derivative comigrated with authentic FW-152 acetate during GLC was found. Like the situation for dicofol acetate, the mass spectrum of FW-152 acetate was very similar to that of DDE, except that a small amount of a parent ion of *m/e* 376 was observed, thus documenting the structural assignment of the underivatized metabolite as FW-152. A radiolabeled DDT metabolite which comigrated with authentic FW-152 was also found in HPLC studies (Fig. 5).

These studies suggest that during the first 3 days of incubation, *P. chrysosporium* cultures are able to catalyze the reductive dechlorination of DDT to DDD under these culture conditions. Upon continued incubation the ability to hydroxylate both DDT and DDD was induced, enabling the fungus to form dicofol and FW-152, respectively, as well as a number of other metabolites. The fungus also began to mineralize DDT during this time. It is interesting that the onset of hydroxylase activity coincided with the onset of idiophasic metabolism and with the biosynthesis of the lignin-degrading peroxidases and the concomitant ability of the fungus to degrade lignin (32). It is also interesting that this type of hydroxylation, that is, hydroxylation of benzylic carbons, is common during lignin biodegradation (32).

Mass balance experiments. Mass balance experiments were performed after [^{14}C]DDT (1.25 nmol) had been incubated in nutrient nitrogen-deficient cultures of *P. chrysosporium* for 12 days. These studies demonstrated that 6.9% of the radioactivity recovered at that time was water soluble, 14.2% was found in the methylene chloride fraction, 70.6%

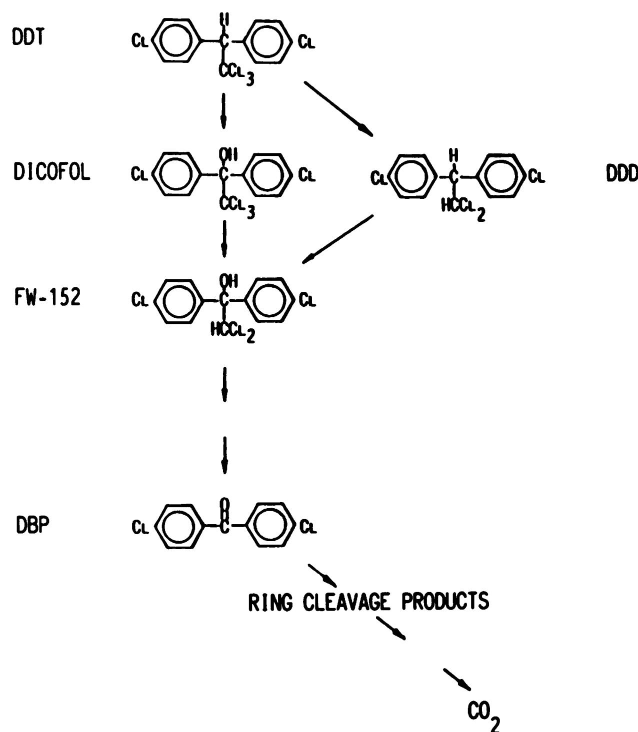

FIG. 6. Proposed pathway for DDT degradation in *P. chrysosporium.*

was present in the hexane fraction, and 8.2% had been evolved as $^{14}CO_2$. Less than 0.1% was present in the mycelium. The total mass recovery was 92.7%. Unmetabolized DDT, as well as a number of DDT metabolites including dicofol, FW-152, DDD, DBP, and at least two unidentified metabolites, were present in the hexane extract as determined by HPLC and GLC.

DISCUSSION

These studies show that the wood-rotting fungus *P. chrysosporium* is able to cause extensive degradation of DDT in nutrient nitrogen-deficient cultures. Degradation was demonstrated by DDT disappearance, metabolite identification, mass balance analysis, and [^{14}C]DDT mineralization studies. DDT metabolites identified included dicofol, DDD, FW-152, and DBP, suggesting that in this fungus, DDT degradation proceeds via the pathway outlined in Fig. 6. Except for DDD and DBP, none of the metabolites common to the major bacterial DDT degradation pathway were found in extracts of *P. chrysosporium* incubated with [^{14}C]DDT. This suggests that the general scheme presented in Fig. 6 is the sole or at least the major DDT-biodegradative pathway in this fungus. The identity of a number of additional metabolites remains to be determined.

Previous studies in our laboratory (5–7) and by others (4, 8, 16, 28) have shown that metabolism of many hard-to-degrade xenobiotics including DDT is dependent upon the lignin-degrading system of *P. chrysosporium* induced during idiophasic metabolism in nutrient nitrogen-deficient media. Since other white rot fungi probably degrade lignin in the same or a similar manner as *P. chrysosporium* does and may be phylogenetically more closely related to each other than to non-lignin-degrading fungi (25), it was of interest to show that other white rot fungi could also mineralize DDT. Furthermore, mineralization of [^{14}C]DDT, like [^{14}C]lignin mineralization, was repressed in nutrient nitrogen-sufficient cultures of these fungi. This, coupled with the fact that a time lag of at least 3 days was observed for each species before mineralization of [^{14}C]DDT occurred, suggests that like lignin mineralization, DDT mineralization may be an idiophasic event in these fungi.

A number of strains of *P. chrysosporium* have been found or developed for use in the study of lignin biodegradation. Of particular interest are strains which produce relatively high levels of H_2O_2-requiring ligninases, with respect to other strains. The data in Fig. 1 and previous biodegradation studies reported by workers in our laboratory (5–7) were obtained with strain ME-446. Because of its increased ligninase production, strain BKM-F-1767 is now used. However, comparison of the relative abilities of ME-446 and BKM-F-1767 to mineralize [^{14}C]DDT showed that they mineralized this xenobiotic to the same extent. At least two reasonable interpretations of these data are possible: (i) the ligninases of *P. chrysosporium* may not be involved in [^{14}C]DDT mineralization; or (ii) the ligninases of *P. chrysosporium* do not participate in the rate-limiting steps which regulate [^{14}C]DDT mineralization. Given the evidence that the lignin-degrading enzymes of *P. chrysosporium* (i) are induced in the same time frame as [^{14}C]DDT mineralization, (ii) are nonspecific, and (iii) participate in the biodegradation of other xenobiotics (16, 28), the latter hypothesis is favored.

Early studies of DDT degradation in fungi showed that the soil fungi *Trichoderma viride* (20) and *Mucor alterans* (2, 3) could degrade [^{14}C]DDT to hexane- and water-soluble metabolites. Dicofol and DDD were identified as DDT metabolites in cultures of *T. viride* (20), whereas a number of unidentified metabolites were observed in cultures of *M. alterans* (2, 3). Also, Engst et al. discovered that *Fusarium oxysporum* degraded DDT via a pathway very similar to that proposed for bacteria (9–11). More recently, Subba-Rao and Alexander (30) have studied cometabolism of DDT by several species of fungi. Their results show that certain species of fungi are able to convert DDT to some common DDT metabolites as well as a number of unidentified metabolites. For example, *Aspergillus flavus* ATCC 11495 converted DDT to DBH, DDD, and DDE, whereas *Thanatephorus cucumeris* converted DDT to DBP, DDD, and DDE. An interesting aspect to their work was the finding that although eight species of fungi did not cometabolize DDT, they did readily degrade DDT metabolites such as 2,2-bis(4-chlorophenyl)acetic acid, DDM, and DBH. It was suggested by these authors that the resistance of DDT to degradation may be attributed to the trichloromethyl moiety of the molecule. Thus the ability of *P. chrysosporium* to catalyze the initial oxidation of DDT by hydroxylation of the benzylic carbon of DDT to form dicofol, a tertiary alcohol, might be expected to have a profound effect upon the ability of the molecule to undergo subsequent oxidative degradation, because introduction of a hydroxyl group at C-1 of DDT would be expected to make the trichloromethyl group more labile to cleavage and/or subsequent metabolism. In general, tertiary alcohols are difficult to oxidize. However, substitution of the benzylic hydrogen atom of DDT to form dicofol results in formation of a tertiary alcohol whose hydroxyl-bearing carbon is bonded to four bulky and electrophilic groups, a condition which would be expected to favor subsequent carbon-carbon bond cleavage to relieve steric crowding. Indeed, the trichloromethyl group of dicofol is extraordinarily susceptible to carbon-carbon bond cleavage

to form DBP. For example, incubation of dicofol at pH 8.2 for 24 h at 20 to 30°C has been shown to cause 100% conversion to DBP (33). Similarly, relatively mild temperatures (125 to 140°C) in the presence of zinc also cause conversion of dicofol to DBP (21). It is not yet known whether oxidative cleavage of the trichloromethyl group of dicofol to form DBP is a major step in the biological degradation of DDT by *P. chrysosporium*. However, because FW-152, the product of aliphatic reductive dechlorination of dicofol, was found as a major metabolite, it seems reasonable that the pathway between dicofol and DBP in *P. chrysosporium* might proceed in a manner similar to that observed in bacterial systems, in which the trichloromethyl carbon undergoes successive reductive dechlorinations followed by oxidation to the carboxylic acid, which then undergoes decarboxylation to form DBP. However, we have not found the expected carboxylic acid metabolite [2-hydroxy-2,2-bis(*p*-chlorophenyl)acetic acid] that would support such a conclusion.

The DDT metabolite DBP undergoes reductive dechlorination of the aromatic ring to form 4-chlorobenzophenone in the fungus *Aspergillus niger* (30). Another fungus, which causes false smut in rice, catalyzed aromatic ring cleavage of DDM to form *p*-chlorophenylacetic acid (30). We have not yet identified aromatic ring cleavage products or reductive dechlorination products in cultures of *P. chrysosporium* incubated with [^{14}C]DDT. Presumably these products are readily metabolized and do not accumulate.

Matsumura (19) has presented a "microbiologic and environmental" pathway for DDT degradation which is, in part, very similar to the pathway outlined in Fig. 6. However, to our knowledge, *P. chrysosporium* is the first microorganism shown to make extensive use of such a pathway in the complete degradation of DDT in axenic culture. Further elucidation of the details of the pathway between DDT and CO_2 in this microorganism are in progress.

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