Degradation of 4-Fluorobiphenyl by Mycorrhizal Fungi as Determined by ^{19}F Nuclear Magnetic Resonance Spectroscopy and ^{14}C Radiolabelling Analysis

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The pathways of biotransformation of 4-fluorobiphenyl (4FBP) by the ectomycorrhizal fungus *Tylospora fibrilosa* and several other mycorrhizal fungi were investigated by using ^{19}F nuclear magnetic resonance (NMR) spectroscopy in combination with ^{14}C radioisotope-detected high-performance liquid chromatography (^{14}C -HPLC). Under the conditions used in this study *T. fibrilosa* and some other species degraded 4FBP. ^{14}C -HPLC profiles indicated that there were four major biotransformation products, whereas ^{19}F NMR showed that there were six major fluorine-containing products. We confirmed that 4-fluorobiphen-4'-ol and 4-fluorobiphen-3'-ol were two of the major products formed, but no other products were conclusively identified. There was no evidence for the expected biotransformation pathway (namely, *meta* cleavage of the less halogenated ring), as none of the expected products of this route were found. To the best of our knowledge, this is the first report describing intermediates formed during mycorrhizal degradation of halogenated biphenyls.

Ecto- and ericoid mycorrhizal fungi isolated from rural locations have been shown to degrade a wide range of environmentally persistent organic pollutants (5–7, 16, 17). These fungi dominate the microbial ecology of heathlands and boreal and temperate forest biomes (20), and their ability to catabolize persistent aromatic contaminants, such as polychlorinated biphenyls (PCBs) (6), atrazine (5, 7), 2,4-dichlorophenoxyacetic acid (5, 7), chlorophenols (16), and 2,4,6-trinitrotoluene (17), makes them suitable target organisms for facilitating bioremediation programs. In addition, host mycorrhizal infections are sustainable as mycorrhizal fungi obtain carbon substrates from their host plant species through symbiosis (10). A range of mycorrhizal fungi and host species (both ectomycorrhizal and ericoid mycorrhizal associations) have been shown to be highly tolerant to industrially polluted soils (11). The sustainability of these organisms may favor their use in bioremediation over white rot fungi, which require constant additions of a carbon substrate (i.e., wood) to the soil to facilitate remediation (8). Meharg et al. (16) provided the first demonstration that the ability of ectomycorrhizal fungi to degrade aromatic contaminants in solution cultures was also exhibited by intact ectomycorrhizal associations. This finding is important because it illustrates that mycorrhizal fungi degrade exogenously applied organic pollutant substances, even though they obtain carbon from their hosts.

There have been few studies in which pathways of biotransformation of PHBs have been attributed to either mycorrhizal or white rot fungi (4, 22), organisms that are very similar in terms of their ability to degrade pollutant substrates. There has been only one study in which biotransformation products of individual PCB congeners for white rot fungi have been identified (4); terminal metabolites for 4,4'-dichlorobiphenyl were identified after incubation with *Phanerochaete chrysosporium*, and two major products were observed. These products were 4-chlorobenzoic acid, the expected product resulting from *meta* cleavage of the less halogenated ring, and 4-chlorobenzyl alcohol. Donnelly and Fletcher (6) demonstrated that mycorrhizal fungi can biotransform PCBs, although no pathways were elucidated.

Organofluorine compounds are being used increasingly in commercial products (13) and are already ubiquitous environmental contaminants. The percentage of fluorine-containing agricultural chemicals has increased from 4 to 9% in the past 15 years; this rate of increase is significantly faster than the rate of increase for nonfluorinated agrochemicals (13). Some important fluorinated organic compounds which are environmentally relevant include Trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)aniline], polyfluorinated biphenyls, Mefluidide [9N-[2,4-dimethyl-5-[[trifluoromethyl)sulfonyl]amino]phenyl]acetamide], and Diflubenzuron [(N-[[4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide]. Aromatic fluorinated compounds are potentially highly toxic; they are also environmentally persistent and accumulate in the biosphere (13). Thus, it is important to determine the fate and behavior of these compounds in suitable model terrestrial systems. ^{19}F nuclear magnetic resonance (NMR) spectroscopy is a technique which potentially allows workers to study such compounds in a nondestructive fashion. This technique is relatively sensitive (detection limit, ca. <50 ng/ml at 9.4T) and has been used to monitor the metabolic fates of fluorinated compounds in mammals (2, 24). More recently, ^{19}F NMR spectroscopy has been used to study the degradation of fluorinated agrochemicals (1, 9, 14, 18, 19).

In the present study we examined the ability of ecto- and ericoid mycorrhizal fungi to degrade fluorinated biphenyls in batch cultures. Our objectives were (i) to assess the potential of a number of mycorrhizal fungi to degrade 4-fluorobiphenyl (4FBP) and (ii) to profile the biotransformation

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TABLE 1. Results of incubation of 4FBP with *T. fibrillosa*

Sampling time (day)	Hyphal wt (μ g)	% ^{14}C recovery ^a				
		Medium before acetonitrile extraction	Acetonitrile extract of medium	Fungal hyphae	Total	Control extract ^b
28	338 $\pm$ 20	88.6 $\pm$ 1.9	89.5 $\pm$ 3.6	1.2 $\pm$ 0.1	90.7 $\pm$ 3.2	88.3 $\pm$ 4.9
56	393 $\pm$ 18	84.9 $\pm$ 2.2	86.1 $\pm$ 5.3	1.2 $\pm$ 0.3	87.3 $\pm$ 5.1	93.1 $\pm$ 3.7

^a The levels of ^{14}C recovery are expressed as percentages of the amount of radioactivity applied and are means $\pm$ standard errors based on three replicates.

^b ^{14}C -HPLC analyses of the control preparations indicated that only 4FBP was present in the extracts.

products. ^{19}F NMR spectroscopy, ^{14}C radioisotope-detected high-performance liquid chromatography (^{14}C -HPLC), and gas chromatography-mass spectroscopy (GC-MS) were used to analyze organic solvent extracts of the growth media in order to determine the extent and route of biotransformation of 4FBP.

MATERIALS AND METHODS

Chemicals. The chemicals and reagents used in this study were purchased from Aldrich Chemical Co. (Dorset, United Kingdom) and BDH (Merck Limited) (Leicester, United Kingdom). ^{14}C -labelled 4FBP was prepared by using the Suzuki reaction (21), $[\text{U-}^{14}\text{C}]$ bromobenzene (specific activity, 3.98 MBq mg^{-1} ; Aldrich Chemical Co.), and 4-fluorobenzenboronic acid diluted with unlabelled

4FBP to the required specific activity (chemical purity, >99%). HPLC grade acetonitrile (Aldrich Chemical Co.) was used for extraction.

Batch cultures. Isolates of *Tylospora fibrillosa*, *Thelophora terrestris*, *Suillus variegatus*, *Suillus granulatus*, *Suillus luteus*, *Hymenoscyphus ericae*, and *Paxillus involutus* were maintained on agar plates containing (per 750 ml of distilled water) 0.5 g of $(\text{NH}_4)_2\text{HPO}_4$, 0.3 g of K_2HPO_4 , 0.14 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.025 g of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.003 g of ZnSO_4 , 0.0125 mg of Fe EDTA, 0.0125 mg of citric acid, 10 g of glucose, and 13.3 g of agar; the pH was adjusted to pH 5.5 by using either 2 M HCl or 2 M KOH. The cultures used for the batch studies were prepared by removing 10-mm-diameter plugs from the leading edges of colonies and placing them into petri dishes containing 20 ml of sterilized growth medium (prepared as described above for the agar medium except that the agar was omitted). The batch cultures were incubated at 25°C until growth became apparent. The plugs were then transferred into sterilized 125-ml brown bottles containing 10 ml of the agar-free growth medium. The bottles were then covered with sterilized foil.

Incubation of 4FBP with the mycorrhizal fungi. After 7 days 100 μg of ^{14}C -4FBP dissolved in 30 μl of acetone was added to give a final concentration of 10 $\mu\text{g ml}^{-1}$ and an activity of 6.5 MBq ml^{-1} in the culture medium. The bottles were then sealed by using steel crown caps fitted with sterilized Teflon-faced liners, wrapped twice in foil, and then incubated at 25°C. The controls included (i) preparations containing no 4FBP and (ii) preparations without fungal plugs. Three replicates were sampled on days 28 and 56. The incubation media were passed through a solid-phase extraction column (C_{18} ; 100 mg:1 ml) dropwise and were eluted twice with 0.5 ml of acetonitrile. The two eluates were kept separate to avoid unnecessary dilution. The acetonitrile used to elute the solid-phase extraction column was also used to wash the incubation chamber and the fungal plug prior to elution. The radioactivity in the aliquots of media before extraction and after extraction and in the acetonitrile extract was counted with a Packard Tri-Carb 2500 TR liquid scintillation counter calibrated with quenched standards. After the surface moisture was removed with a tissue, each washed fungal plug was weighed and then digested with a chromic acid digestion mixture. The CO_2 that evolved was trapped in 2 ml of aqueous 4 M KOH by using the method of Dalal (3). The KOH trap was removed, a 500- μl aliquot was mixed

FIG. 1. Representative ^{14}C -HPLC profiles for extracts obtained after incubation of 4-FBP with *T. fibrillosa*. Peak P is the 4FBP peak, and peaks M1 to M4 are metabolite peaks. (A) Profile obtained after the first elution with acetonitrile. (B) Profile obtained after the second elution.

FIG. 2. Representative ^{19}F NMR spectra for extracts obtained after incubation of 4FBP with *T. fibrillosa*. Peak P is the 4FBP peak, and peaks F1 through F6 are metabolite peaks. (A) Spectrum obtained after the first elution with acetonitrile. (B) Spectrum obtained after the second elution.

with 2 ml of Hi-ionic scintillation fluid and the radioactivity was counted with the liquid scintillation counter as described above. The acetonitrile extracts were then analyzed by using ^{14}C -HPLC, ^{19}F NMR spectroscopy, and GC-MS. The other fungal species were investigated by using the same procedure, but only the results obtained after incubation of *T. fibrillosa* are described in detail in this paper.

^{19}F NMR spectroscopy. The ^{19}F NMR spectroscopy analysis was carried out by using 500- μl portions of acetonitrile extracts to which 100 μl of D_2O and a known amount of internal standard (2-fluorobiphenyl in acetonitrile) had been added. CFCl_3 was used as an external reference to adjust the chemical shift to 0 ppm. The ^{19}F - ^1H couplings were eliminated by using inverse-gated proton decoupling. $^{19}\text{F}\{^1\text{H}\}$ NMR spectra were recorded with a Bruker model DPX400 spectrometer that was operated at a ^{19}F observation frequency of 376.4 MHz and was equipped with a 5-mm ^{19}F - ^1H probe head; 90° pulses and a 1,965.41-Hz spectral width were used. Typically, 1,024 scans were collected, which yielded 32,768 data points with an acquisition time of 3.48 s. A further delay of 20 s between pulses was added to allow full T_1 relaxation. The free induction decays were multiplied by an exponential apodization function corresponding to a 1-Hz broadening prior to Fourier transformation.

HPLC. HPLC analyses were performed by using a Hewlett-Packard model 1050 system (including an autosampler and a UV detector), a Berthold model LB506-C1 radiodetector, and LAURA HPLC software (Lablogic Limited). The pump A mobile phase was water with the pH adjusted to 2.5 with formic acid, and the pump B mobile phase was acetonitrile. The analysis was carried out by using a 100- μl sample that was diluted to a volume of 500 μl with the pump A mobile phase, a Spherisorb C_{18} column (250 by 46 mm; particle size, 5 μm ;

HPLC Technology) at room temperature, and a flow rate of 1.0 ml/min. The linear gradient used for the analysis of extracts was as follows: at zero time, 80% pump A mobile phase and 20% pump B mobile phase; and at 40 min, 30% pump A mobile phase and 70% pump B mobile phase.

GC-MS. GC-MS analyses were carried out by using a Kratos model MS-80RFA mass spectrometer under electron ionization conditions. Samples were introduced via split injection onto a type HP-1 cross-linked methyl silicone capillary column (12 m by 0.2 mm; 0.33 μm). The column temperature was kept at 170°C for 1 min and then increased at a rate of $20^\circ\text{C}/\text{min}$ to 250°C . Eluate from the column was directed into the mass spectrometer ion source. The mass spectrometer was scanned repetitively from 400 to 50 m/z at a speed of 1 s/decade. The detector output was recorded electronically for subsequent processing. The standards and samples were analyzed both underivatized and following derivatization with bis(trimethylsilyl)trifluoroacetamide (BSTFA) to form trimethylsilyl ethers of unprotected hydroxyl functions. Derivatization was carried out by taking a 100- μl aliquot and gently blowing it to dryness in a small reactivial. Then 25 μl of BSTFA-pyridine (1:1) was added to the dried residue. The vial was sealed and then incubated at 60°C for 30 min before aliquots were analyzed.

RESULTS

Incubation of 4FBP with *T. fibrillosa*: mass balance and distribution of radiolabel. The total levels of recovery (Table 1) of the radiolabel were more than 85% for all of the replicates and

FIG. 3. Representative GC-MS data for acetone extracts obtained after incubation of 4FBP with ectomycorrhizal fungi. (A) Total ion count for the GC trace. (B) MS profiles for 4FBP (P), underivatized monohydroxy-4-fluorobiphenyl (G1), BSTFA-derivatized 4'-hydroxy-4-fluorobiphenyl (G2), and BSTFA-derivatized 3'-hydroxy-4-fluorobiphenyl (G3).

TABLE 2. ^{14}C -HPLC data for 4FBP incubated with *T. fibrillosa*

Sampling time (day)	% of applied radioactivity ^a					
	Peak P	Peak M1	Peak M2	Peak M3	Peak M4	Total
28	52.6 $\pm$ 3.1	14.1 $\pm$ 1.1	13.2 $\pm$ 2.3	4.3 $\pm$ 0.2	1.6 $\pm$ 0.4	85.8 $\pm$ 3.1
56	5.2 $\pm$ 0.6	21.3 $\pm$ 1.3	36.5 $\pm$ 3.9	13.4 $\pm$ 2.3	6.2 $\pm$ 1.9	82.6 $\pm$ 5.2

^a The values are means $\pm$ standard errors based on three replicates. Peak P was the 4FBP peak, and peaks M1 through M4 were metabolite peaks. The RT for peaks P, M1, M2, M3, and M4 were 36.2, 27.6, 18.9, 11.1, and 10.4 min, respectively.

the control preparations. KOH traps were excluded from this study because of problems with contamination, as determined in preliminary studies; hence, it was not possible to determine whether mineralization was occurring. However, as the levels of recovery for the replicates and controls were equivalent, our findings indicate that mineralization was not occurring. No major losses were observed throughout the experiment as each replicate was sealed and opened only when it was sampled. Any minor losses were attributed to volatile compounds (parent and/or possible biotransformation products) in the headspace that were lost when the bottles were opened. Volatilization of 4FBP was previously shown to be a major source of such losses during the development stages of this work and during previous studies of 4FBP degradation in soil (9).

Increases in the levels of radiolabel in the acetonitrile extracts compared to the growth media prior to extraction were observed. These increases were due to washing of the bottles with acetonitrile as part of the extraction procedure. As a consequence, any 4FBP and biotransformation products were included in the total amount of radiolabel counted, which resulted in elevated levels of recovery.

The levels of radiolabel in the fungal hyphae were found to be no more than 1.5% of the total activity applied (Table 1). The relative levels of radioactivity did not vary significantly over time. Hyphal weight increased from day 28 to day 56, indicating that the hyphae were not dormant.

Biotransformation of 4FBP by *T. fibrillosa*. ^{14}C -HPLC, GC-MS, and ^{19}F NMR spectroscopy analyses of the extracts indicated that *T. fibrillosa* was able to biotransform 4FBP to a number of products (Fig. 1 through 3).

The ^{19}F NMR spectroscopic analysis of the extracts resulted in seven peaks with chemical shifts (δ) of -116.29 , -116.71 , -116.90 , -116.99 , -117.36 , -117.52 , and -118.06 ppm; these peaks were designated peaks F1, F2, P, and F3 through F6, respectively. The ^{14}C -HPLC analysis of the extracts resulted in five peaks at retention times (RT) of 36.2, 27.6, 18.9, 11.1, and 10.4 min; these peaks were designated peaks P and M1 through M4, respectively. The GC-MS analysis revealed two components with GC retention times of 2.8 and 5.7 min and molecular ions at m/z 172 and 188; these components were designated peaks P and G1, respectively. After derivatization, two additional peaks were observed; these peaks had RT of 5.9 and 6.3 min and were designated peaks G2 and G3, respec-

tively. Peaks G2 and G3 produced identical patterns of ions at m/z 245 and 260 and differed only in their relative intensities.

Peak P, which had a chemical shift (δ) of -116.90 , and HPLC RT of 36.2 min, and a GC RT of 2.8 min, was confirmed to be an undegraded parent by performing another analysis after an authentic standard was added to the extract.

As shown by both the ^{19}F NMR spectra and the ^{14}C -HPLC profiles there were relative increases in the products formed compared to the remaining 4FBP from day 28 to day 56. This was less evident from the GS-MS data, as not all of the products were observed and a quantitation analysis was not carried out. The different numbers of biotransformation products revealed by ^{19}F NMR and ^{14}C -HPLC indicated that in the case of the latter analysis two of the peaks were coeluting products (possibly isomers). This conclusion was supported by the quantitation data (Tables 2 and 3).

We confirmed that 4'-hydroxy-4-fluorobiphenyl (4OH) and 3'-hydroxy-4-fluorobiphenyl (3OH) were components of the mixture by adding authentic standards to the extracts and re-analyzing the preparations. The 4OH standard coeluted with peak M1 (RT, 27.6 min) in the HPLC profile and had the same chemical shift as peak F6 ($\delta = -118.06$ ppm) in the NMR spectrum. Similarly, the 3OH standard coeluted with peak M1 (RT, 27.6 min) in the HPLC profile and had the same chemical shift as peak F3 ($\delta = -116.99$ ppm) in the NMR spectrum. Peak G1 (RT, 5.7 min) in the GC-MS profile was shown to be monohydroxy-4-fluorobiphenyl by the addition of authentic 3OH and 4OH standards. For each standard similar RT and similar mass spectra were observed. Peaks G2 (RT, 5.9 min) and G3 (RT, 6.3 min) also represented 3OH and 4OH, respectively, as revealed by addition of derivatized authentic standards. Similar RT and mass spectra were observed. We noted that derivatization with BSTFA gave low yields. This may have been a consequence of the high levels of water present in the extracts.

We could not identify the other biotransformation products (peaks M2 through M4, F1, F2, F4, and F5). Several attempts to identify these products were made with a number of authentic standards (Table 4). These standards were chosen on the basis of previously published descriptions of biotransformation of polyhalogenated biphenyls by bacteria and fungi. None of the standards coeluted when HPLC was performed or had peaks which coincided with the product peaks in the ^{19}F NMR

TABLE 3. ^{19}F NMR data for 4FBP incubated with *T. fibrillosa*

Sampling time (day)	% of applied 4FBP						
	Peak F1	Peak F2	Peak P	Peak F3	Peak F4	Peak F5	Peak F6
28	10.9 $\pm$ 1.8	14.0 $\pm$ 2.7	49.1 $\pm$ 3.6	0.0	13.9 $\pm$ 2.4	0.0	0.0
56	15.9 $\pm$ 2.4	13.7 $\pm$ 1.5	4.7 $\pm$ 0.3	8.5 $\pm$ 2.1	25.3 $\pm$ 3.5	4.4 $\pm$ 1.1	9.2 $\pm$ 1.2
							Total
							88.0 $\pm$ 5.8
							81.7 $\pm$ 4.9

^a The values are means $\pm$ standard errors based on three replicates. Peak P was the 4FBP peak, and peaks F1 through F6 were metabolite peaks. The chemical shifts for peaks F1, F2, P, F3, F4, F5, and F6 were -116.29 , -116.72 , -116.90 , -116.99 , -117.36 , -117.52 , and -118.06 ppm, respectively.

TABLE 4. HPLC RT and ^{19}F NMR chemical shifts for authentic standards for expected biotransformation products

Biotransformation standard	^{19}F NMR chemical shift (ppm)	HPLC RT (min)
4FBP	-116.90	36.2
4-Fluorobiphen-4'-ol	-118.06	27.6
3-Fluorobiphen-3'-ol	-116.99	27.6
4-Fluorocinnamic acid	-110.95	16.8
4-Fluorobenzoyl propionic acid	-105.99	13.1
4-Fluorobenzyl alcohol	-116.63	9.2
4-Fluorobenzoic acid	-107.02	12.7

spectra. As a result, the products were not identified. Nevertheless, we could eliminate processes such as *meta* cleavage as a biotransformation pathway. A key characteristic of the products was that they were increasingly polar, as demonstrated by the ^{14}C -HPLC profiles. Also, the site of biotransformation in 4FBP is probably on the nonfluorinated ring, as larger ^{19}F NMR chemical shifts would have been observed if a substitution or other modification had occurred on the fluorinated ring.

Incubation of 4FBP with other mycorrhizal fungi. Six other mycorrhizal fungi were also studied. These fungi included the ectomycorrhizal organisms *T. terrestris*, *S. variegatus*, *S. granulatus*, *S. luteus*, and *P. involutus* and the ericoid mycorrhizal organism *H. ericae*. Three of these fungi biotransformed 4FBP, and three exhibited no degradative ability under the conditions used in this study. The organisms which degraded 4FBP were *T. terrestris* (>65% of the extract was biotransformation products), *S. variegatus* (>50%) and *H. ericae* (>20%), as determined by ^{14}C -HPLC and ^{19}F NMR spectroscopy. The products formed were found to be identical to the products previously observed with *T. fibrillosa* namely, four peaks as determined by ^{14}C -HPLC and six peaks in the ^{19}F NMR spectra. The order of efficiency of 4FBP biotransformation under the conditions used in this study was as follows: *T. fibrillosa* > *T. terrestris* > *S. variegata* > *H. ericae*.

DISCUSSION

The results of our study are similar to the results obtained previously for aromatic xenobiotic compounds and white rot and mycorrhizal fungi (4, 6, 22, 23, 25). In this paper we show that *T. fibrillosa* and other mycorrhizal fungi are able to degrade 4FBP to significant extents (>80%). The negligible amount (<2%) of the radiolabel incorporated into the fungal hyphae was not consistent with previously published results obtained for 4,4'-dichlorobiphenyl when *P. chrysosporium* was used; in the latter case incorporation of >10% of the radiolabel was reported (4). Dietrich et al. (4) proposed that the proportion of a radiolabel in hyphae was due to a partitioning effect and not due to covalent binding to cellular macromolecules. The differences between the levels of radiolabel detected in the present study and the levels detected by Dietrich et al. (4) may be explained by the acetonitrile washing procedure (prior to digestion) which we used. It was expected that the acetonitrile would extract a significant proportion of the radiolabel if it were loosely bound in the hyphal matrix. The compounds used by Dietrich et al. (4) were di-, tetra-, and hexachlorobiphenyls, which had greater lipophilicities than 4FBP and therefore would be expected to partition to a greater extent than 4FBP. This supports the hypothesis that the compounds partition rather than covalently bind to the fungal hyphae.

Other mycorrhizal species that have been shown to have the ability to degrade 4FBP under the conditions used in this study include the ectomycorrhizal fungi *T. terrestris* and *S. variegatus* and the ericoid mycorrhizal fungus *H. ericae*. The remaining three ectomycorrhizal fungi species examined, *S. luteus*, *P. involutus*, and *S. granulatus*, exhibited no catabolic activity toward 4FBP.

White rot fungi and mycorrhizal fungi have been shown to be capable of readily biotransforming the lower substituted PCBs (6, 25). In particular, Donnelly and Fletcher (6) used 21 fungal species, including *S. granulatus* and *H. ericae*, to catabolize a number of dichlorinated biphenyls. They found that *S. granulatus* was better than *H. ericae* for degrading PCBs. In the present study we found that *S. granulatus* did not biotransform 4FBP, whereas *H. ericae* converted >16% of the 4FBP present to products. These two species were much less efficient than *T. fibrillosa*, *T. terrestris*, or *S. variegatus*, organisms which biotransformed more than 50% of the 4FBP supplied. These results show that there are significant differences among species with respect to biphenyl degradation.

Microbial biotransformation pathways for conversion of halogenated biphenyls (PHBs) have been well-documented (12, 15). It is common for the process to be initiated by ring hydroxylation, followed by ring opening via *meta* cleavage. Such biotransformations occur through exposure of PHBs to microbial species, especially bacteria which exhibit dioxygenase enzyme activity (12). Fungi predominately hydroxylate aromatic compounds via a monooxygenase system, such as cytochrome P-450 enzymes, but can also cleave aromatic rings via *meta* cleavage (12). Also, white rot fungi, which are thought to possess degradative capabilities similar to those of mycorrhizal fungi, produce a lignin peroxidase in response to nitrogen starvation (10). There are, however, no documented pathways for biotransformation of polyhalogenated biphenyls by mycorrhizal species, but such a pathway has been found in the white rot fungus *P. chrysosporium* (4). Production of 4-chlorobenzoic acid, a major product formed through the process of *meta* cleavage, was observed, indicating that biotransformation proceeded via typical routes. Dietrich et al. (4) have suggested that an alternative pathway may be important as a benzyl alcohol product was also found. Mineralization studies have also been carried out with a range of xenobiotic compounds, including PHBs, and the results indicate that biotransformation pathways for both mycorrhizal and white rot fungi do include ring fission and the ultimate production of carbon dioxide (4, 23, 25).

The complete pathway for biotransformation of 4FBP by *T. fibrillosa* was not determined in this study. However, there were a number of clear indications concerning the biotransformation processes involved. The ^{14}C -HPLC profiles indicated that all of the products formed were more polar than 4FBP and hence that functional groups, such as hydroxyl and carboxylic acid, had been introduced into the molecule. The relatively small chemical shift changes in the ^{19}F NMR spectra indicated that little change occurred on the fluorinated ring. The presence of the monohydroxylated metabolites 4OH and 3OH was confirmed by using HPLC, ^{19}F NMR spectroscopy, and GC-MS, and the results indicated that biotransformation involved monooxygenation.

Although biotransformation of 4FBP could be expected to proceed through formation of a catechol, followed by ring cleavage, we found that this does not occur. Standard samples of various expected products of *meta* cleavage were added to the extract, but during reanalyses these compounds showed no correspondence with the observed biotransformation products (Table 4).

It is possible that the mycorrhizal fungi used in this study are not capable of *meta* cleavage and only insert oxygen (in the form of hydroxyl groups) into the nonfluorinated ring. As fungi are known to predominantly use monooxygenase systems for degradation of aromatic rings, we propose that sequential hydroxylation occurs in mycorrhizal fungi under the conditions used in this study.

The observation that mycorrhizal species do not exhibit *meta* cleavage is important as it means that these organisms are not able to fully degrade the substrate and hence effect mineralization. In a real terrestrial scenario in which the microbial biomass is very diverse, this is less important as other species in the indigenous bacterial and fungal community may continue degradation of the xenobiotic compound. However, the ability to initiate biotransformation is a key stage in the overall degradation process, and this fact confirms the potential importance of ericoid and ectomycorrhizal fungi in bioremediation.

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