

Biodegradation of polyfluorinated biphenyl in bacteria

David Hughes · Benjamin R. Clark ·
Cormac D. Murphy

Received: 8 April 2010 / Accepted: 23 August 2010 / Published online: 10 September 2010
© Springer Science+Business Media B.V. 2010

Abstract Fluorinated aromatic compounds are significant environmental pollutants, and microorganisms play important roles in their biodegradation. The effect of fluorine substitution on the transformation of fluorobiphenyl in two bacteria was investigated. *Pseudomonas pseudoalcaligenes* KF707 and *Burkholderia xenovorans* LB400 used 2,3,4,5,6-pentafluorobiphenyl and 4,4'-difluorobiphenyl as sole sources of carbon and energy. The catabolism of the fluorinated compounds was examined by gas chromatography–mass spectrometry and fluorine-19 nuclear magnetic resonance spectroscopy (^{19}F NMR), and revealed that the bacteria employed the upper pathway of biphenyl catabolism to degrade these xenobiotics. The novel fluorometabolites 3-pentafluorophenyl-cyclohexa-3,5-diene-1,2-diol and 3-pentafluorophenyl-benzene-1,2-diol were detected in the supernatants of biphenyl-grown resting cells incubated with 2,3,4,5,6-pentafluorobiphenyl, most likely as a consequence of the actions of BphA and BphB. 4-Fluorobenzoate was detected in cultures incubated with 4,4'-difluorobiphenyl and ^{19}F NMR analysis of the supernatant from *P. pseudoalcaligenes* KF707 revealed the presence of additional water-soluble fluorometabolites.

Keywords PCB · Fluorometabolites · NMR · Mass spectrometry

Introduction

Organofluorine compounds are rare in nature, but are used in an extensive array of applications (refrigerants, propellants, agrochemicals, pharmaceuticals) and consequently they are of environmental concern (Key et al. 1997; Murphy et al. 2009). The transformation and degradation of anthropogenic compounds by microorganisms is highly significant, since biodegradation is an attractive method of waste treatment. Furthermore, it is important to understand the transformation products that may potentially occur in the environment, particularly if they are highly toxic. For example, fluoroacetate's toxicity is a consequence of its *in vivo* transformation to 2*R*,3*R*-fluorocitrate, which is a reversible inhibitor of aconitase and an irreversible inhibitor of citrate transport across mitochondrial membranes (Kirsten et al. 1978).

In comparison to research conducted on the microbial degradation of polychlorinated biphenyls (PCBs), very few studies have been undertaken to investigate the biodegradation of fluorinated biphenyls. Several studies have investigated the aerobic microbial catabolism of fluoroaromatic compounds, such as fluorobenzoate and fluorophenol (Boersma et al. 2004; Brooks et al. 2004; Ferreira et al. 2008), and demonstrated that the fluorinated compounds can

D. Hughes · B. R. Clark · C. D. Murphy (✉)
School of Biomolecular and Biomedical Science
and Centre for Synthesis and Chemical Biology,
University College Dublin, Belfield, Dublin 4, Ireland
e-mail: Cormac.d.murphy@ucd.ie

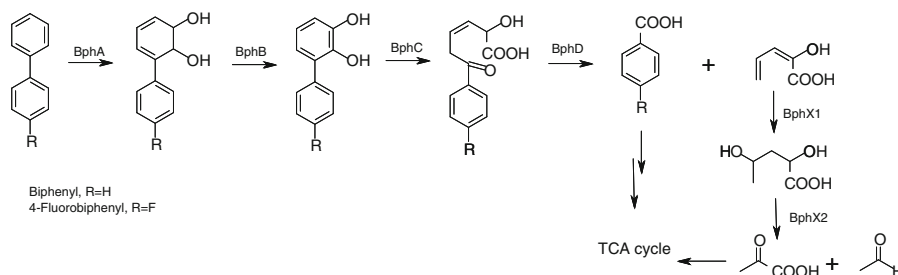


Fig. 1 Degradation of biphenyl and 4-fluorobiphenyl in *P. pseudoalcaligenes* KF707. The enzymes of the 'upper' pathway are: *BphA* biphenyl 2,3-dioxygenase, *BphB* dehydrogenase, *BphC* 2,3-dihydroxybiphenyl 1,2-dioxygenase, *BphD* 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase. The

'lower' pathway is composed of the enzymes of benzoate catabolism plus *BphX1* and *X3*, which are 2-hydroxypenta-2,4-dienoate hydratase and 4-hydroxy-2-oxovalerate hydrolase, respectively. The degradation of 4-fluorobenzoate is incomplete in *P. pseudoalcaligenes*

act as substrates for the enzymes that normally transform the non-fluorinated derivatives. Improvement in analytical technology has enabled the ready detection of fluorometabolites, in particular fluorine-19 nuclear magnetic resonance spectroscopy (^{19}F NMR), which allows the direct detection of fluorometabolites in microbial cultures without the need for purification (Boersma et al. 2001; Cobb and Murphy 2009). Fluorobiphenyl can be degraded by fungi and bacteria along the classical aromatic degradative pathways; thus, fungi transform 4-fluorobiphenyl to hydroxylated and conjugated products (Green et al. 1999; Amadio and Murphy 2010), and the bacterium *Pseudomonas pseudoalcaligenes* KF707, degrades 2- and 4-fluorobiphenyl to 2- and 4-fluorobenzoate (Murphy et al. 2008), via the upper pathway of biphenyl degradation (Fig. 1). No investigation has yet been conducted on the effect of additional fluorine substitution on the biodegradability of fluorobiphenyl, and here we describe experiments on the growth and catabolism of 4,4'-difluorobiphenyl and 2,3,4,5,6-pentafluorobiphenyl on *P. pseudoalcaligenes* KF707 and *Burkholderia xenovorans* LB400. Both bacteria have been studied extensively for their abilities to degrade biphenyl and polychlorinated biphenyl (Gibson et al. 1993; Arnett et al. 2000).

Materials and methods

Culture conditions

Pseudomonas pseudoalcaligenes KF707 and *Burkholderia xenovorans* LB400 were obtained from

Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and maintained on tryptone soya agar. The bacteria were exposed to biphenyl by including crystals of this compound inside the lid of the petri dish. Batch cultures of the bacteria were established in 250 ml Erlenmyer flasks containing 50 ml of mineral medium and fluorobiphenyl crystals (12.5 mg; Fluorochem, Derbyshire, UK) that were incubated on a shaker table at 27–30°C. Growth of the bacteria was monitored using standard plate count on nutrient agar after serial dilution of the culture fluid.

Resting cell experiments were conducted with cells from biphenyl-grown cultures that were harvested after 48–72 h incubation, washed with distilled water and resuspended with mineral medium containing either 4,4'-difluorobiphenyl or 2,3,4,5,6-pentafluorobiphenyl. The cells were incubated for 24 h and the supernatants analysed for the presence of fluorinated transformation products, after centrifugation to remove the biomass.

Identification of intermediates

Culture supernatants (1 ml) were extracted with chloroform (1 ml), and the organically extractable compounds derivatized to the trimethylsilyl ester using *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, Sigma) after the chloroform was removed under a stream of nitrogen. An Agilent 6890 gas chromatograph coupled to a 5973 mass selective detector was used to identify metabolites. An aliquot of the derivatized sample (1 μl) was injected onto a HP-5MS column and the oven temperature held at 120°C for 2 min then raised to 300°C at 10°C/min.

The mass spectrometer was operated in the scan mode. Water-soluble products were similarly analysed after lyophilising an aliquot of supernatant (200 μ l) and derivatising by MSTFA.

For ^{19}F NMR analysis, culture supernatant (15 ml) was extracted with chloroform, and the organic layer reduced to dryness, redissolved in 0.7 ml of CDCl_3 (Sigma) and spectra recorded using a Varian 400 MHz spectrometer. The aqueous fraction was lyophilised and resuspended in 0.7 ml of D_2O for NMR analysis. The shifts are reported relative to CFCl_3 .

For the identification of the metabolite with ^{19}F NMR shift $\delta -136.8$ ppm, culturing and resting cell experiments were carried out as described above. The supernatant (20 ml) was extracted with chloroform, acidified to pH 3, and re-extracted with ethyl acetate. The aqueous fraction was lyophilized and analysed by ^1H and ^{19}F NMR.

A standard sample of fluorolactate was synthesised biochemically by incubating sodium fluoropyruvate (80 mM) plus NADH with lactate dehydrogenase (10 units) in a final volume of 0.5 ml, for 3 h at room temperature; D_2O (200 μ l) was added and ^{19}F NMR analysis conducted directly on the reaction mixture.

Results and discussion

Growth of bacteria on 4,4'-difluorobiphenyl and 2,3,4,5,6-pentafluorobiphenyl. After 24 h incubation an increase in turbidity was observed in all culture flasks, and the cultures grown in 2,3,4,5,6-pentafluorobiphenyl turned yellow, which is indicative of *meta*-cleavage products. Because the substrates are not very soluble in water, it was not possible to accurately follow the growth of the bacteria by measuring optical density, thus plate counting was employed. The cell counts of the bacterial cultures are shown in Fig. 2, and confirm that the bacteria could employ both compounds as growth substrates. GC-MS analysis of the pertrimethylsilylated culture supernatants of *P. pseudoalcaligenes* KF707 indicated the presence of a compound that eluted at 3.21 min that had the expected mass spectrum of derivatised pentafluorobenzoate (m/z 284) in flasks containing 2,3,4,5,6-pentafluorobiphenyl as the carbon source; 4-fluorobenzoate (m/z 212) was detected in both cultures grown on 4,4'-difluorobiphenyl.

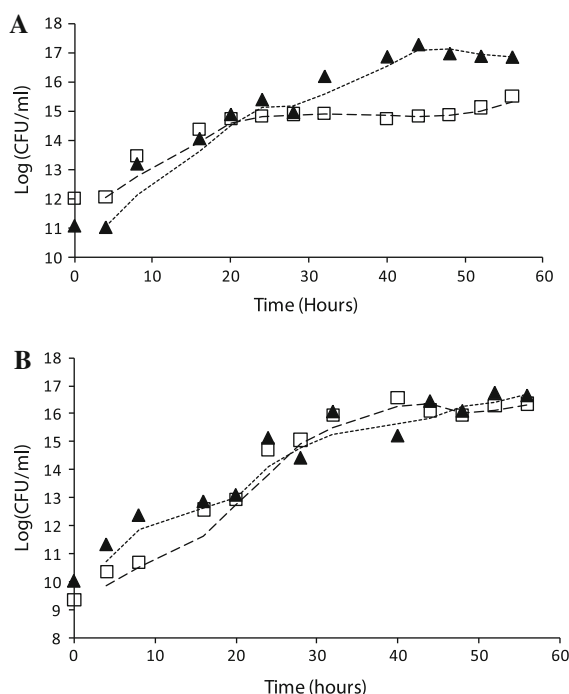


Fig. 2 Growth of *P. pseudoalcaligenes* KF707 (a) and *B. xenovorans* (b) on 4, 4'-difluorobiphenyl (square) and 2,3,4,5,6-pentafluorobiphenyl (triangle)

Resting cultures

In order to investigate the fluorometabolites that accumulate in the culture supernatants, both bacteria were grown in biphenyl-containing medium for 48–72 h. The cells were harvested, washed with water and resuspended in fresh medium (50 ml) containing either 4,4'-difluorobiphenyl or 2,3,4,5,6-pentafluorobiphenyl. After a further 24–48 h incubation a sample of culture was centrifuged and the supernatant analysed by ^{19}F NMR and GC-MS. The ^{19}F NMR spectrum of 2,3,4,5,6-pentafluorobiphenyl has resonances at -143.3 (dd, $J = 22.8, 8.2$ Hz, F2/F6), -155.7 (t, $J = 20.9$ Hz, F4) and -162.4 ppm (ddd, $22.8, 20.9, 8.2$, F3/5). After incubation with the resting cultures of both bacteria these signals disappeared and new resonances at -139.8 (dd, $J = 23.1, 8.1$ Hz), -155.8 (t, $J = 20.9$ Hz) and -163.0 (ddd, $J = 23.1, 20.9, 8.1$ Hz) ppm, which had similar splitting patterns to the resonances of the starting substrate, were observed in the organic soluble fraction, together with two sets of smaller signals at -139.7 (dd, $J = 22.7, 8.2$ Hz), -155.2 (t, $J = 20.7$ Hz) and -162.7 (ddd, $J = 22.7, 20.7, 8.0$ Hz) ppm (Fig. 3), and -139.5

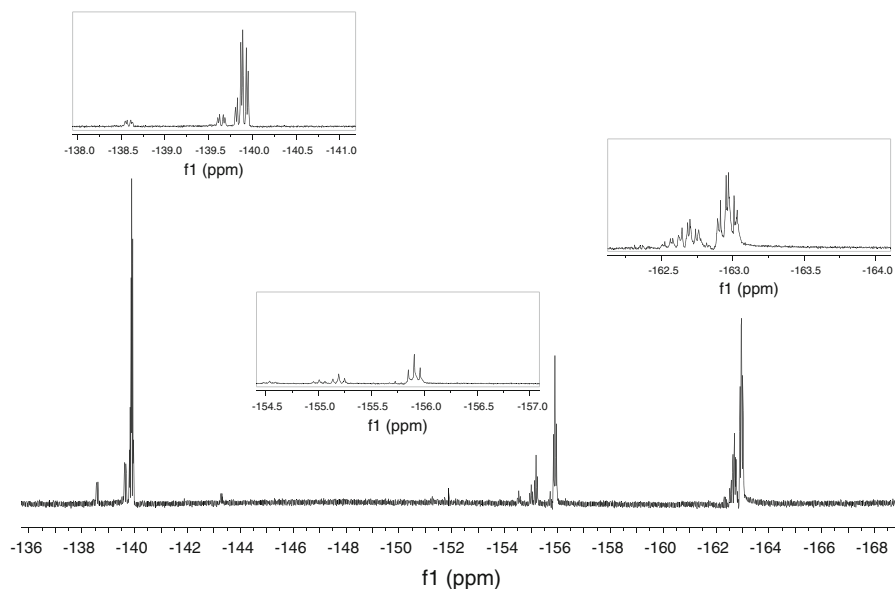
(dd, $J = 22.7, 8.0$ Hz), -155.0 (t, $J = 20.6$ Hz), and -162.6 (ddd, $J = 22.7, 20.6, 7.1$ Hz). Integration of resonances suggested that these three compounds were present in a ratio of approximately 7:2:1. GC–MS analysis of the trimethylsilyl derivatives (Fig. 4) demonstrated the presence of a compound eluting at 9.5 min with m/z 422, which is the expected mass of the trimethylsilylated 3-(2,3,4,5,6-pentafluorophenyl)-cyclohexa-3,5-diene-1,2-diol. A compound present in much larger amounts eluted at 10 min with a mass (m/z 420) of that expected for 3-(2,3,4,5,6-pentafluorophenyl)-benzene-1,2-diol. Therefore, it is most likely that the more prominent resonances in the ^{19}F NMR spectrum are those of the re-aromatised catechol, while one set of the smaller signals are from the dihydrodiol. The identity of the third compound observed in the ^{19}F NMR spectrum is unknown. Thus in the resting cells, the pentafluorobiphenyl is readily transformed via the first two enzymes of the upper biphenyl-degrading pathway (Fig. 4d).

Transformation of 4,4'-difluorobiphenyl by *B. xenovorans* LB400 was examined by ^{19}F NMR analysis of the supernatant after lyophilisation and reconstitution in D_2O , which revealed the presence of a single fluorinated compound at -110.3 ppm (tt, $J = 9.0, 5.5$ Hz) (Fig. 5). The chemical shift and coupling were highly similar to that of 4-fluorobenzoate (Boersma et al. 2004), and GC–MS analysis of the silylated supernatant and comparison with a standard confirmed the presence of this compound. No other

fluorinated compounds were observed, which is unusual, as no fluoride ion was detected in the ^{19}F NMR analysis ($\delta -120$ ppm). This suggests that the fluorinated compounds formed from the other fluorinated ring do not accumulate in the culture supernatant. *P. pseudoalcaligenes* KF707 also transformed 4,4'-difluorobiphenyl to a range of fluorometabolites (Fig. 6a), some of which have been observed previously during experiments with 4-fluorobiphenyl, e.g. 4-fluorocyclohexadiene-*cis,cis*-1,2-diol-1-carboxylate ($\delta_{\text{F}} -116.5$, dq, $J = 12.0, 5.9$ Hz), which is a transformation product of 4-fluorobenzoate, and putative 5-fluoro-4-hydroxy-2-oxovaleric acid -227.1 (td, $J = 46.5, 21.5$ Hz) (Murphy et al. 2008). Previously unobserved signals were detected at -127.8 (dd, $J = 38.4, 26.9$), -136.8 (dq, $J = 34.6, 2.3$ Hz), -183.5 (dt, $J = 45.8, 13.6$ Hz), -187.8 (ddt, $J = 46.9, 36.6, 16.0$ Hz) and -228.8 (dt, $J = 47.4, 31.2$ Hz) ppm. The latter signal has the same chemical shift and coupling constants as fluorolactate, which can arise from fluoropyruvate through the action of lactate dehydrogenase (Meyer and O'Hagan 1992). The identity of this metabolite was confirmed by comparison with an authentic standard of fluorolactate generated by incubating fluoropyruvate with lactate dehydrogenase. Furthermore, KF707 resting cells incubated with fluoropyruvate yielded the same compound.

Also of note is the resonance at -136.8 ppm (dq, $J = 34.6, 2.3$ Hz), which has not previously been

Fig. 3 ^{19}F NMR spectrum of supernatant from cultures of *B. xenovorans* LB400 incubated with pentafluorobiphenyl. The insets show the splitting patterns of the signals



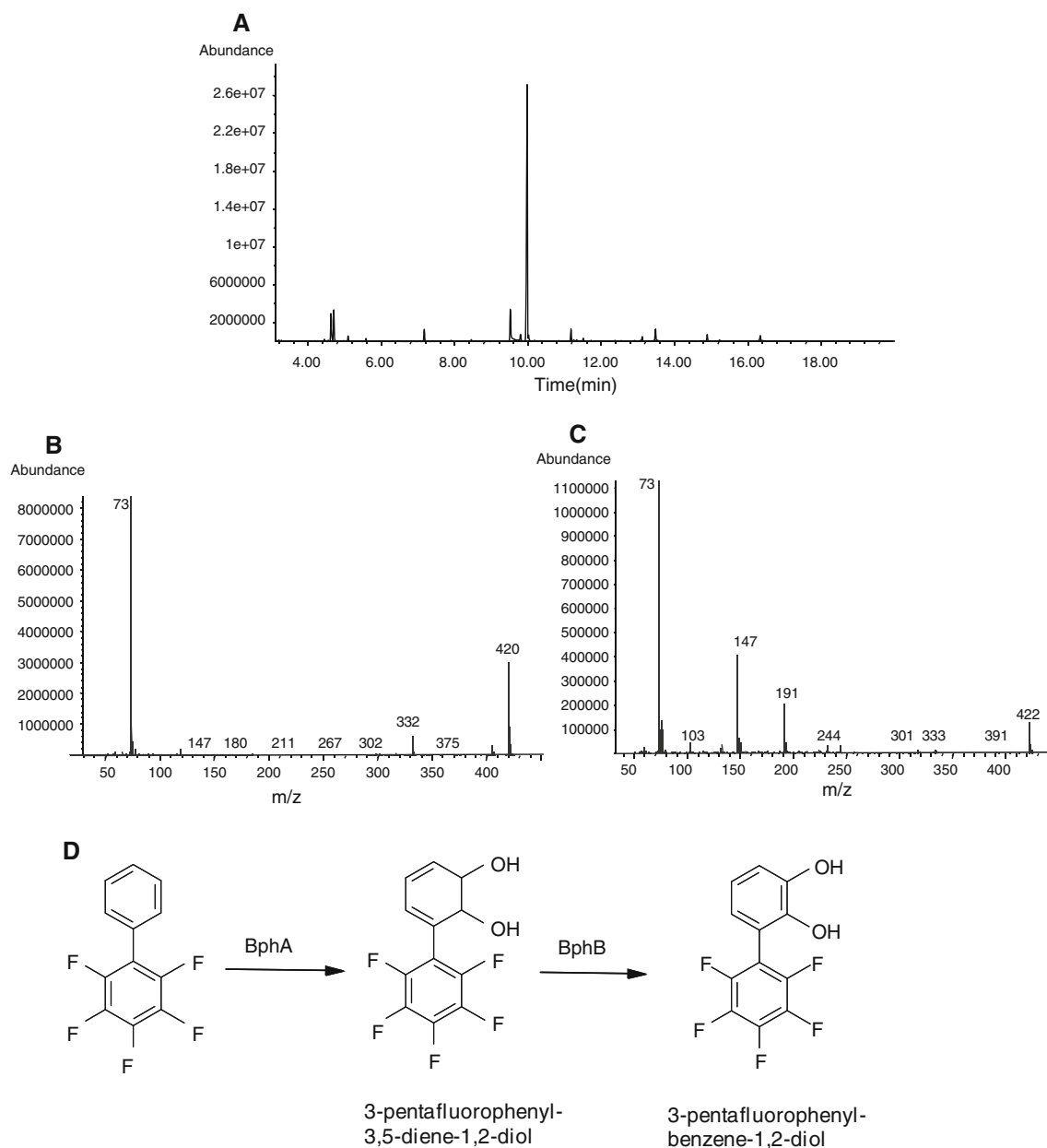


Fig. 4 Total ion count of trimethylsilyl derivatives from *B. xenovorans* LB400 culture supernatant after incubation with pentafluorobiphenyl (a), and mass spectra of the peaks

eluting at 10 min (b) and 9.5 min (c). The biotransformation of the pentafluorobiphenyl by the first two enzymes of the upper pathway is also shown (d)

observed in fluoroaromatic degradation studies. In order to further investigate the origin of this unusual signal, the feeding study was repeated, and the resulting supernatant fractionated by solvent partition to remove non-polar compounds. The resulting aqueous fraction was analysed by both ^{19}F and ^1H NMR. The ^{19}F NMR spectrum revealed only a single

resonance at -136.8 ppm (Fig. 6b). While the ^1H NMR spectrum was complex, proton resonances coupled to fluorine could be readily identified by their coupling constants. In this manner, two ^1H NMR resonances associated with the fluorometabolite of interest were identified at 6.28 (dq, $J = 34.6, 7.0$ Hz, 1H) and 1.73 (dd, $J = 7.0, 2.3$ Hz, 3H) ppm. The

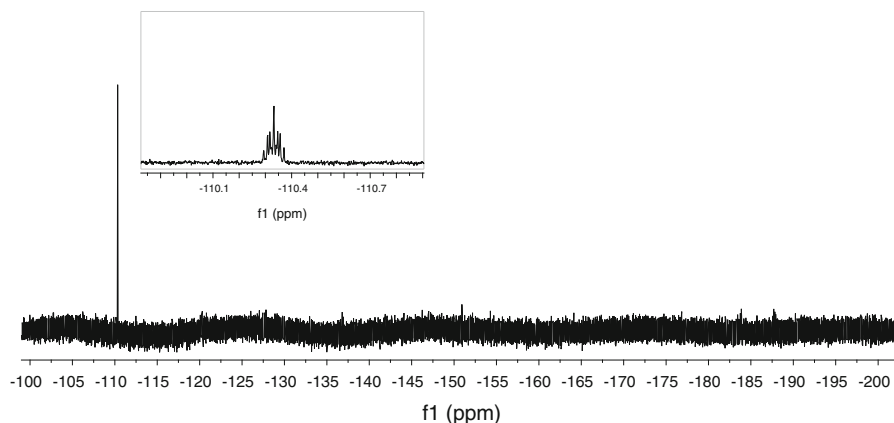


Fig. 5 ^{19}F NMR spectrum of supernatant from cultures of *B. xenovorans* LB400 incubated with difluorobiphenyl, showing the splitting pattern of the signal at -110 ppm

chemical shifts and coupling constants suggested the presence of a (*Z*)-1-fluoro-propen-1-yl moiety in the molecule. The experimental data matched well with literature values for compounds containing this structural fragment (Elkik and Imbeaux-Oudotte 1975; Kawasaki et al. 1998). Based on the spectral data and the proposed degradation pathway, we tentatively propose that the new metabolite is (*Z*)-3-fluoro-2-oxo-pent-3-enoate, which could arise from the transformation of 3-fluoro-2-hydroxy-6-oxo-6-(4-fluorophenyl)-hexa-2,4-dienoate to 3-fluoro-2-hydroxypenta-2,4-dienoate, through the action of the hydrolase BphD (Fig. 7). However, unequivocal identification will require isolation of this compound from culture supernatants and a thorough structural analysis by NMR.

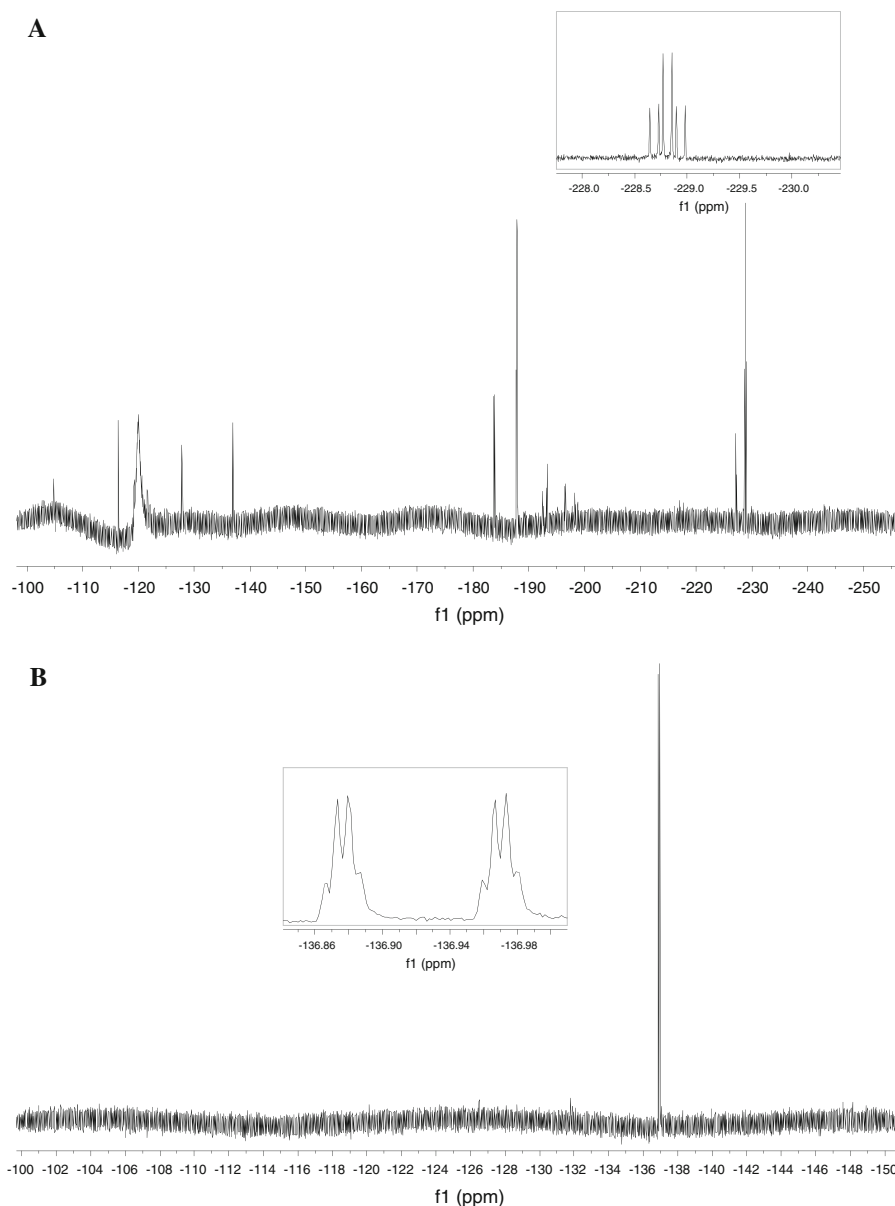
Conclusion

The awareness that polychlorinated biphenyls (PCBs), which have had numerous industrial and commercial uses, are harmful to human and animal health, has resulted in many studies investigating their biodegradation in bacteria (Pieper 2005). In comparison, the biodegradation of fluorinated biphenyls has been largely overlooked, probably because these compounds are not regarded as an environmental threat. However, fluorobiphenyls are used in the synthesis of industrially and commercially important pharmaceuticals, agrochemicals and liquid crystalline dielectrics (Romer et al. 1984; Matsumoto et al. 1996), and thus their environmental fate should be studied. We have

previously shown that 2- and 4-fluorobiphenyl can be used as sole sources of carbon and energy by *P. pseudoalcaligenes* KF707 (Murphy et al. 2008). The initial dioxygenation of the fluorobiphenyl occurs on the non-fluorinated ring, leading to the eventual production of fluorobenzoate and 2-hydroxypenta-2,4-dienoate; the latter compound is the true source of carbon and energy for the bacterium, since KF707 cannot mineralise fluorobenzoate. In this paper we have examined the effect of additional fluorine substitution on the biodegradability of biphenyl by KF707 and another well-studied PCB-degrading strain, *Burkholderia* sp. LB400.

The growth of both strains on pentafluorobiphenyl is analogous to that observed previously with 2- and 4-fluorobiphenyl; thus, the biphenyl dioxygenase (BphA) hydroxylates the non-fluorinated ring, which was confirmed by the detection of 3-pentafluorophenyl-cyclohexa-3,5-diene-1,2-diol and 3-pentafluorophenyl-benzene-1,2-diol in the supernatants of resting cell cultures. The subsequent enzymes of the pathway tolerate the presence of the fluorine atoms in the other ring, eventually yielding pentafluorobenzoate, which was detected in growing cultures, and 2-hydroxypenta-2,4-dienoate. Interestingly, both strains were also able to use 4,4'-difluorobiphenyl as a growth substrate, which requires a greater degree of catabolic flexibility, since the fluorine substitution is not confined to one ring. Potrawfke et al. (1998) observed the growth of LB400 on 2,3'-dichloro- and 2,4'-dichlorobiphenyl, but earlier work by Gibson et al. (1993) demonstrated that biphenyl-grown cells of LB400 only poorly transformed 4,4'-dichlorobiphenyl, whereas

Fig. 6 ^{19}F NMR spectra of (a) supernatant from cultures of *P. pseudoalcaligenes* KF707 incubated with difluorobiphenyl and (b) after the fluorometabolite with a resonance at -136 ppm was semi-purified. *Insets* show the splitting patterns of resonances at -229 and -136 ppm



biphenyl-grown cells of KF707 could degrade this congener relatively well. In the present study 4-fluorobenzoate was detected in resting cell culture supernatants, indicating that the enzymes of the upper pathway (BphABCD) could degrade the difluorinated intermediates. No other fluorometabolites were detected in LB400 cultures, but several were observed in the KF707 cultures by ^{19}F NMR which have not been observed previously. One of these ($\delta_{\text{F}} -229$ ppm) was identified as fluorolactate, which arises from fluoropyruvate, while another ($\delta_{\text{F}} -136.8$) was tentatively

identified as (Z)-3-fluoro-2-oxo-pent-3-enoate. LB400 does not grow on 4-chlorobenzoate (Billingsley et al. 1997), and is probably unlikely to grow on 4-fluorobenzoate, so for both strains the carbon and energy must come from the further catabolism of (Z)-3-fluoro-2-oxo-pent-3-enoate, eventually yielding acetaldehyde and fluoropyruvate (Fig. 7). The former can be converted to acetyl CoA and introduced to intermediary metabolism, and the latter can be transformed to fluorolactate via lactate dehydrogenase or defluorinated by pyruvate dehydrogenase (Leung and Frey, 1978),

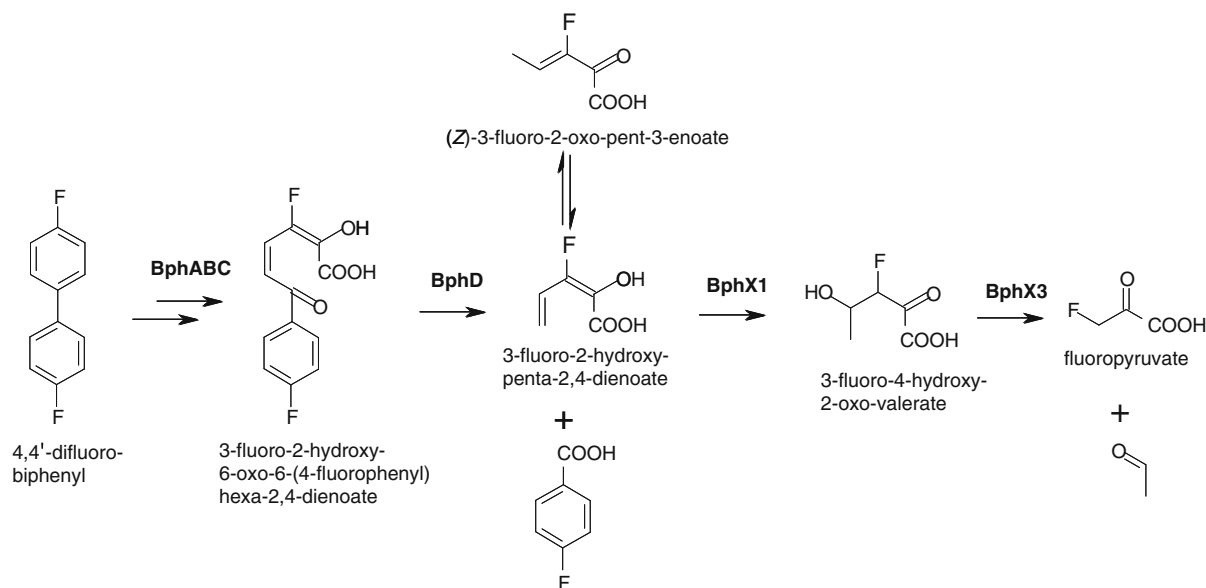


Fig. 7 Proposed catabolism of 4,4'-difluorobiphenyl along the upper and lower pathways

which would account for the fluoride ion present in the KF707 resting cultures (Fig. 6). Further investigations on the degradation of fluorinated biphenyls is required to fully characterise bacterial degradation of this class of compound, by examining the effect of fluorine substitution in other positions on growth and catabolism.

Acknowledgments The authors acknowledge financial support from Enterprise Ireland. We thank Jennifer Power for assistance with the resting cell experiments.

References

- Amadio J, Murphy CD (2010) Biotransformation of fluorobiphenyl by *Cunninghamella elegans*. *Appl Microbiol Biotechnol* 86:345–351
- Arnett CM, Parales JV, Haddock JD (2000) Influence of chlorine substituents on rates of oxidation of chlorinated biphenyls by the biphenyl dioxygenase of *Burkholderia* sp strain LB400. *Appl Environ Microbiol* 66:2928–2933
- Billingsley KA, Backus SM, Juneson C, Ward OP (1997) Comparison of the degradation patterns of polychlorinated biphenyl congeners in Aroclors by *Pseudomonas* strain LB400 after growth on various carbon sources. *Can J Microbiol* 43:1172–1179
- Boersma MG, Solyanikova IP, Van Berkel WJH, Vervoort J, Golovleva LA, Rietjens I (2001) F-19 NMR metabolomics for the elucidation of microbial degradation pathways of fluorophenols. *J Ind Microbiol Biotechnol* 26:22–34
- Boersma FGH, McRoberts WC, Cobb SL, Murphy CD (2004) A F-19 NMR study of fluorobenzoate biodegradation by *Sphingomonas* sp HB-1. *FEMS Microbiol Lett* 237:355–361
- Brooks SJ, Doyle EM, Hewage C, Malthouse JPG, Duetz W, O'Connor KE (2004) Biotransformation of halophenols using crude cell extracts of *Pseudomonas putida* F6. *Appl Microbiol Biotechnol* 64:486–492
- Cobb SL, Murphy CD (2009) F-19 NMR applications in chemical biology. *J Fluorine Chem* 130:132–143
- Elkik E, Imbeaux-Oudotte M (1975) Formyl fluoro cétones et esters. II. hydroxyalcoylation: stéréochimie et déshydratation des fluorhydrines obtenues. *B Soc Chim Fr* 7–8:1633–1638
- Ferreira MIM, Marchesi JR, Janssen DB (2008) Degradation of 4-fluorophenol by *Arthrobacter* sp. strain IF1. *Appl Microbiol Biotechnol* 78:709–717
- Gibson DT, Cruden DL, Haddock JD, Zylstra GJ, Brand JM (1993) Oxidation of polychlorinated biphenyls by *Pseudomonas pseudoalcaligenes* KF707. *J Bacteriol* 175:4561–4564
- Green NA, Meharg AA, Till C, Troke J, Nicholson JK (1999) Degradation of 4-fluorobiphenyl by mycorrhizal fungi as determined by F-19 nuclear magnetic resonance spectroscopy and C-14 radiolabelling analysis. *Appl Environ Microbiol* 65:4021–4027
- Kawasaki T, Ichige T, Kitazume T (1998) An efficient stereoselective synthesis of fluorinated trisubstituted alkenes. *J Org Chem* 63:7525–7528
- Key BD, Howell RD, Criddle CS (1997) Fluorinated organics in the biosphere. *Environ Sci Technol* 31:2445–2454
- Kirsten E, Sharma ML, Kun E (1978) Molecular toxicology of (–)-erythro-fluorocitrate — selective-inhibition of citrate transport in mitochondria and binding of fluorocitrate to mitochondrial proteins. *Mol Pharmacol* 14:172–184

- Leung LS, Frey PA (1978) Fluoropyruvate —unusual substrate for *Escherichia coli* pyruvate dehydrogenase. *Biochem Biophys Res Commun* 81:274–279
- Matsumoto M, Watanabe N, Mori E, Ishihara M, Yamaura T, Aoyama M, Ikawa H, Kobayashi H (1996) 4-Fluorobiphenyl derivatives. US Patent 5,460,523
- Meyer JJM, O'Hagan D (1992) Conversion of fluoropyruvate to fluoroacetate by *Dichapetalum-cymosum*. *Phytochemistry* 31:499–501
- Murphy CD, Quirke S, Balogun O (2008) Degradation of fluorobiphenyl by *Pseudomonas pseudoalcaligenes* KF707. *FEMS Microbiol Lett* 286:45–49
- Murphy CD, Clark BR, Amadio J (2009) Metabolism of fluoroorganic compounds in microorganisms: impacts for the environment and the production of fine chemicals. *Appl Microbiol Biotechnol* 84:617–629
- Pieper DH (2005) Aerobic degradation of polychlorinated biphenyls. *Appl Microbiol Biotechnol* 67:170–191
- Potrawfke T, Lohnert TH, Timmis KN, Wittich RM (1998) Mineralization of low-chlorinated biphenyls by *Burkholderia* sp. strain LB400 and by a two membered consortium upon directed interspecies transfer of chlorocatechol pathway genes. *Appl Microbiol Biotechnol* 50: 440–446
- Romer M, Krause J, Weber G (1984) 4-fluorophenyl derivatives, their preparation, and dielectrics and electro-optical display element containing them. US Patent 4,473,487