BIODEGRADATION OF CHLORINATED DIOXINS AND DIBENZOFURANS BY THE BIPHENYL-UTILIZING STRAIN JB1

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Introduction

Despite the concern about the environmental fate and effects of PCDDs and PCDFs, relatively little is known about their biodegradation by microorganisms. A number of bacteria are known which are able to degraded dibenzo-*p*-dioxin and dibenzofuran and some of their lightly chlorinated derivatives[e.g. 1,2]. Recently, it has been reported that the more highly chlorinated PCDDs and PCDFs can be dechlorinated by anaerobic microorganisms in sediments[3,4].

The biphenyl-utilizing *Alcaligenes* strain JB1 is able to cometabolize chlorinated biphenyls containing up to six chlorine substituents[5]. In addition, this strain is also able to degrade chlorinated dioxins[6], dibenzofurans[7] and other aromatic compounds[8]. We here report data on the metabolites produced during the degradation by this strain of 2-chlorodibenzo-*p*-dioxin (2-CDD) and 2-chlorodibenzofuran (2-CDF).

Materials and Methods

Cultures of strain JB1 were grown overnight at 25°C on biphenyl (ca. 0.7 g/l) in minimal medium as described elsewhere[5]. These cultures were filtered to remove excess biphenyl and either 2-CDD or 2-CDF were added at about 0.3 mg/l. The cultures were incubated at 25°C. Samples were extracted with pentane, followed by clean-up and analysis using GC-ECD as previously described for experiments with PCBs[5].

Samples were extracted with ethyl acetate for the identification of metabolites. These extracts were evaporated to dryness and redissolved in 50 ml acetone. Potassium carbonate (ca. 2 g) and methyl iodide (ca. 2 ml) were added and the mixtures were refluxed for 8 h. Removal of the potassium carbonate by filtration and evaporation to dryness was followed by dissolution in 1,1,1-trimethylpentane. These samples were analysed on a Hewlett-Packard 5970 GC-MSD.

Results and discussion

As is shown in Figs. 1 and 2, biphenyl-grown cultures of strain JB1 readily degraded both 2-CDD and 2-CDF. The latter compound was degraded much more rapidly than 2-CDD, an initial concentration of about 300 μg/l having decreased to undetectable levels (less than 1 μg/l) after only two hours. However, the different degradation rates may be at least in part the result of different activities of the two cultures used. Further work is required to learn more of the relative degradation rates of such compounds.

Samples from similar experiments were extracted, methylated and analysed using GC-MSD in an attempt to identify the products of the degradation of 2-CDD and 2-CDF. Evidence was found for the presence of two metabolites of each substrate. The methylated derivative of 4-

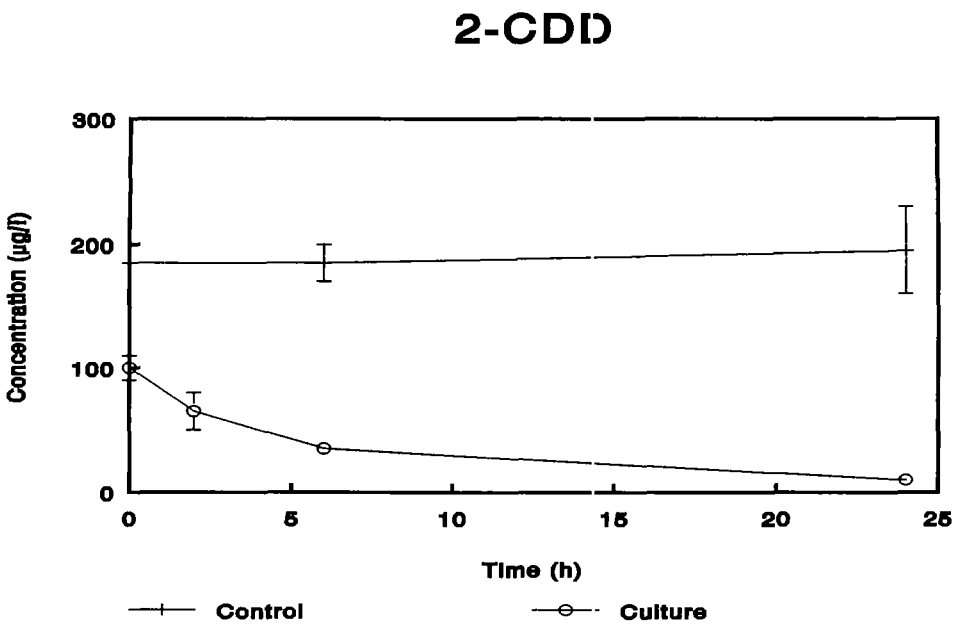

Fig. 1. Degradation of 2-CDD by a biphenyl-grown culture of strain JB1.

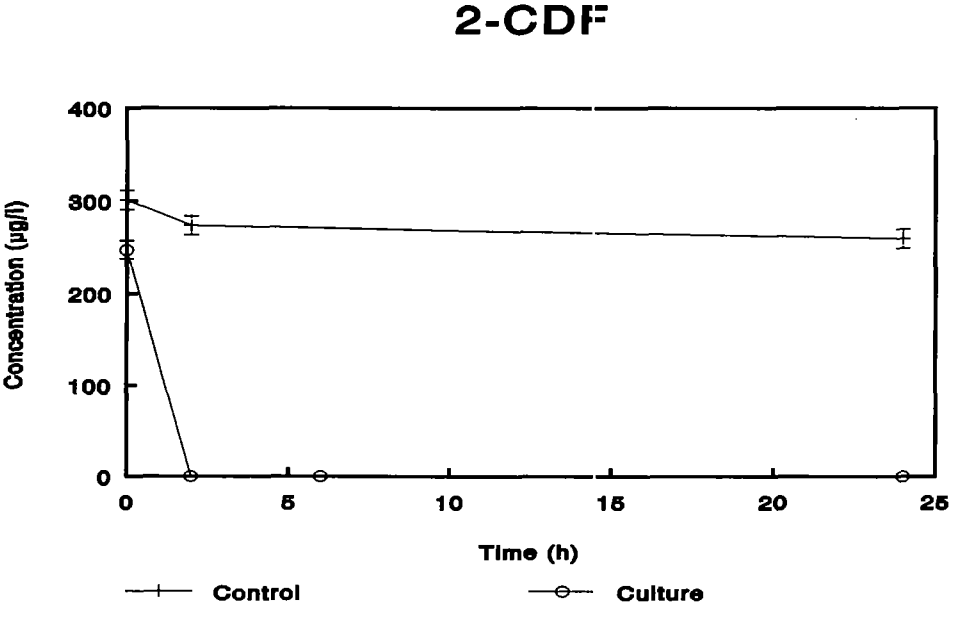

Fig. 2. Degradation of 2-CDF by a biphenyl-grown culture of strain JB1.

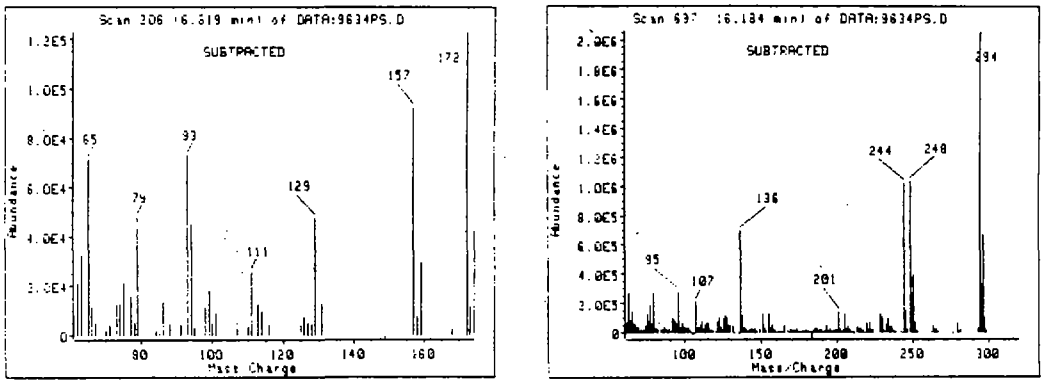

Fig. 3. Identification of 4-chlorocatechol (left) and a chlorotrihydroxydiphenyl ether (right) as metabolites of 2-CDD.

chlorocatechol was positively identified in samples from the experiment with 2-CDD by comparison of its retention time and mass spectrum with those of an authentic sample of the compound (Fig. 3). These samples also contained a chlorinated compound with *m/z* of 294 (^{35}Cl). This corresponds to that expected for a methylated chlorotrihydroxydiphenyl ether, which is one of the metabolites identified as products of the degradation of chlorinated dioxins by *Sphingomonas* sp. strain RW1[2].

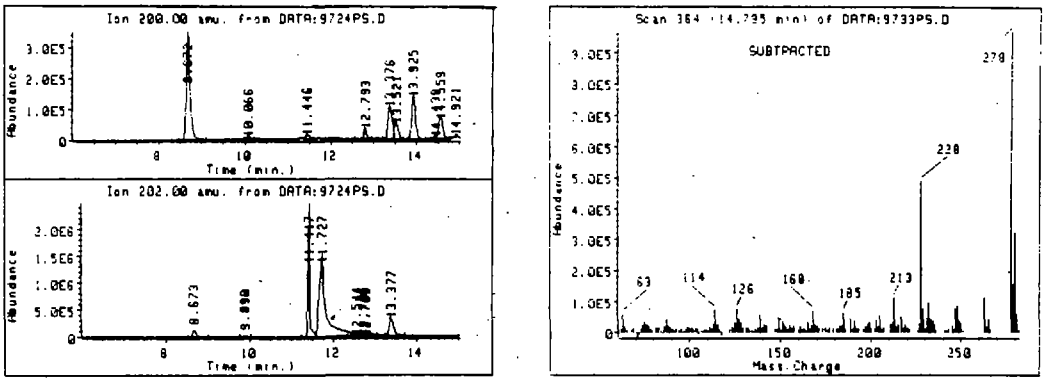

Fig. 4. Identification of 5-chlorosalicylate (left) and a chlorotrihydroxybiphenyl (right) as metabolites of 2-chlorodibenzofuran.

Similarly, the samples from the experiments with 2-CDF also appeared to contain two chlorinated metabolites (Fig. 4). The methylated derivative of 5-chlorosalicylic acid was identified by selected ion monitoring and comparison with an authentic sample. A compound with a molecular ion of *m/z* 278 was also present. The compound is probably a methylated chlorotrihydroxybiphenyl, which has been reported as product of the degradation of chlorinated dibenzofurans[2].

These results indicate that *Alcaligenes* strain JB1 degrades 2-CDD and 2-CDF by pathways similar to those reported for *Sphingomonas* sp. strain RW1 and similar strains[1,2]. These pathways involve initial angular dioxygenation adjacent to an ether bridge, followed by spontaneous cleavage of the ether bridge to generate either a trihydroxydiphenyl ether or a trihydroxy-

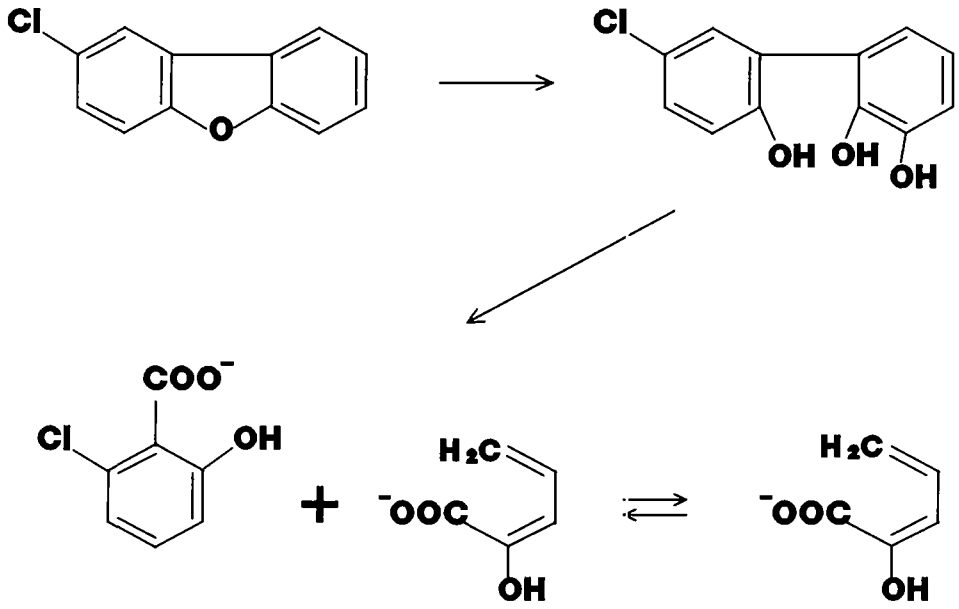

Fig. 5. Proposed biodegradation pathway for 2-CDF.

biphenyl[2]. Further degradation proceeds either via salicylate or catechol derivatives, respectively. An example is shown in Fig. 5 for 2-chlorodibenzofuran.

The fact that strain JB1 is also able to degrade biphenyl and some of its chlorinated derivatives suggests that the degradation of these three groups of compounds may be related in this strain. In fact, preliminary indications are that the biphenyl-degrading enzymes of *Alcaligenes* strain JB1 are indeed also involved in the degradation of chlorinated dioxins, dibenzofurans and other aromatic compounds[8]. Work is currently in progress to further investigate the relationships between the degradation pathways of PCBs, PCDDs, PCDFs and other chlorinated aromatic compounds in this and similar strains.

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