Metabolism of Dibenzofuran and Dibenzodioxin as Model for 2, 3, 7, 8-Tetrachlorodibenzodioxin Degradation

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Dibenzofuran (DBF) has been employed in some recent studies as a model compound for studying the microbial degradation of cyclic biaryl ethers (2,3,5). Public attention has focused on this class of compounds since it comprises some of the most pernicious and at same time persistent molecules, such as TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). For dibenzofuran a novel angular dioxygenation mechanism is described and a degradation sequence via a biphenyl related pathway outlined (2).

RESULTS

Dibenzofuran-degrading bacteria were enriched from various environmental sources. One of these isolates, Brevibacterium strain DPO 1361 showed limited growth on dibenzofuran (OD₅₄₆nm 4) as sole source of carbon and energy, when the substrate (10 mM) was added in a suspended form. The use of a two-liquid-phase system substantially improved the growth rate and increased the optical density (546 nm) up to 10. This two-liquid-phase system consisted of phosphate buffer and an organic phase of 2,2,4,4,6,8,8-heptamethylnonane (HMN) (10% [vol/vol]), in which DBF (10 mM referred to the whole culture volume) was completely dissolved. Turnover of substrate was monitored by following its concentration in the organic phase using HPLC (see Fig. 1).

![Graph showing the relationship between dibenzofuran concentration and OD(546nm) over time.]

Fig.1: Growth of strain DPO 1361 in a two liquid-phase system with dibenzofuran as sole source of carbon and energy. (Explanation see text.)
Fig. 2: Turnover of cyclic biarelether and related compounds by strain DPO 220 (Explanation see text). The percentage of substrate degraded after 60 minutes is given in brackets (DBF = 100%).

Fig. 3: Legend see next side
The DBF degrading bacteria transformed a broad range of anellated aromatics and heteroaromatics. For co-metabolism experiments cells of the strain DPO 220 (DBF-grown) were resuspended in phosphate buffer to an optical density (546nm) and substrate (1 mM) added from a DMSO stock solution (100 mM). Two flasks were used respectively for each substrate. After an incubation period of 0 and 60 min the substrate was totally solubilized by adding dioxane and the concentrations were determined using HPLC (see Fig. 2)(5).

In resting cells experiments, fluorene was used as substrate analogue of DBF in order to show the mode of initial attack. After cooxidation of fluorene by cells of strain DPO 1361 (DBF-grown), one metabolite was isolated and unambiguously characterized by high-resolution MS, $^1$H-NMR and $^{13}$C-H-NMR as 1,10-dihydro-1,10-dihydroxyfluoren-9-one (see figure 3). This indicated the presence of a novel dioxygenase, which attacks aromatic ethers in angular position (2).

Applying the concept of angular dioxygenation likewise to DBF, the extremely stable aryl ether bond is transformed into a labile hemiacetal structure. Spontaneous cleavage of the hemiacetal and subsequent rearomatization affords 3-(2-hydroxyphenyl)catechol (HPC). HPC was transformed by a partially purified metapyrocatechase to an unstable yellow ring cleavage product, 2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid (2'-Hydroxy-HOPDA). This compound showed spontaneous cyclization by straightforward intramolecular Michael addition to a dead end metabolite, which was unequivocally characterized as 3-(chroman-4-on-2-yl)pyruvate. When HPC was incubated together with partially purified metapyrocatechase and HOPDA-hydrolase enzymes, it was converted to salicylate and 2-oxo-4-pentenoate (see Fig. 4).

Fig. 3: Partial 300 MHz $^1$H-NMR spectrum of 1,10-dihydro 1,10-dihydroxyfluoren-9-one (20 mg in 1 ml CDCL$_3$, 330K, TMS as internal standard, FID manipulated by Gaussian multiplication for improved resolution)

Fig. 4: Pathway proposed for the degradation of dibenzofuran by strain DPO 1361.
DISCUSSION

The poor water solubility of biaryl ethers appears one of the factors which limits the biodegradation of this substances. By the use of a two-liquid-phase system the growth rates were improved and strain DPO 1361 reached twice of the optical density, compared to a culture fed by adding the substrate in a suspended form.

Bacterial strains, enriched on DBF as sole carbon source, showed high cometabolic potential towards anellated aromatics and heteroaromatics, which is correlated by the accumulation of metabolites. The ability of these strains can be used for production of optical active diendiois, for example 1,2-dihydro-1,2-dihydroxynaphthaline from naphthaline.

We demonstrated that fluorene and DBF are attacked by strain DPO 1361 via an angular dioxygenation (2). A pathway for DBF degradation is presented, which indicates an analogy to the pathway established for diphenyl (1,4). Further investigations will have to show whether the same angular dioxygenation mechanism is involved also in the degradation of dibenzodioxin.

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REFERENCES


