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Biodegradation of Dibenzo-*p*-dioxins and Related Compounds by Bacteria

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Pseudomonas sp. CA10, which can grow on carbazole (CAR) as the sole source of carbon, nitrogen and energy, was isolated from an activated sludge sample. The matabolic intermediates were detected in the culture broth ofstrain CA10, and the degradation pathway of CAR by strain CA10 was proposed. The degradation pathway consisted of the initial oxidation of an angular position of CAR, a *meta*-cleavage pathway of 2?aminobiphenyl-2,3-diol, and the further degradation of anthranilic acid via *ortho*-cleavage of catechol(1).

The DNA fragment encoding mata-cleavage enzymes and the *meta*-cleavage compound hydrolase, involved in carbazole degradation, was cloned from the carbazole-utilizing bacterium *Pseudomonas* sp. strain CA10. DNA sequence analysis of this 2.6-kb SmaI-SphI fragment revealed that there were three open reading frames (ORF1, ORF2, and ORF3, in this gene order). ORF1 and ORF2 were indispensable for *meta*-cleavage activity for 2'-aminobiphenyl-2,3-diol and its easily available analog, 2,3-dihydroxybiphenyl, and were designated *carBa* and *carBb*, respectively(2).

Nucleotide sequence analysis of the flanking regions of the *carBC* genes of *Pseudomonas* sp. strain CA10 revealed that there were two open reading frames (ORFs) ORF4 and ORF5, in the upstream region of *carBC*. Similarly, there ORFs, ORF6 to ORF8, were found in the downstream region of *carBC*. The deduced amino acid sequences of ORF6 and ORF8 showed homologies with ferredoxin and ferredoxin reductase components of bacterial multicomponent dioxygenase systems, respectively. ORF4 and ORF5 had the same sequence and were tandemly linked. Their deduced amino acid sequences showed about 30% homology with large (a) subunits of other terminal oxygenase components. Functional analysis using resting cells harboring the deleted plasmids revealed that the products of ORF4 and -5, ORF6, and ORF8 were terminal dioxygenase, ferredoxin, and ferredoxin reductase, respectively, of carbazole, and that the product of ORF7 is not indispensable for CARDO activity. Based on the results, ORF4, ORF5, ORF6, and ORF8 were designated *carAa*, *carAa*, *carAc*, and *carAd*, respectively(3).

CARDO has the ability to oxidize a wide variety of polyaromatic compounds, including dibenzo-p-dioxin, dibenzofuran, biphenyl, and polycyclic aromatic hydrocarbons such as naphthalene and phenanthrene. Since 2,2',3-trihydroxydiphenyl ether and 2,2',3-trihydroxybiphenyl were identified as metabolites of dibenzo-*p*-dioxin and dibenzofuran, respectively, it was considered that CARDO attacked at the angular position adjacent to the oxygen atom of dibenzo-*p*-dioxin and dibenzofuran as In the case with carbazole(4).

Carbazole 1,9a-dioxygenase (CARDO) from *Pseudomonas* sp. strain CA10 is a

multicomponent enzyme that catalyzes the angular dioxygenation of carbazole, dibenzofuran, and dibenzo-p-dioxin. It was revealed by gas chromatography-mass spectrometry and 1H and 13C nuclear magnetic resonance analyses that xanthene and phenoxathiin were converted to 2,2',3-trihydroxydiphenylmethane and 2,2',3-trihydroxydiphenyl sulfide, respectively. Thus, for xanthene and phenoxathiin, angular dioxygenation by CARDO occurred at the angular position adjacent to the oxygen atom to yield hetero ring-cleaved compounds. In addition to the angular dioxygenation, CARDO catalyzed the cis dihydroxylation of polycyclic aromatic hydrocarbons and biphenyl. Naphthalene and biphenyl were converted by CARDO to cis-1,2-dihydroxy-1,2-dihydronaphthalene and cis-2,3-dihydroxy-2, 3-dihydrobiphenyl, respectively. On the other hand, CARDO also catalyzed the monooxygenation of sulfur heteroatoms in dibenzothiophene and of the benzylic methylenic group in fluorene to yield dibenzothiophene-5-oxide and 9-hydroxyfluorene, respectively. These results indicate that CARDO has a broad substrate range and can catalyze diverse oxygenation: angular dioxygenation, cis dihydroxylation, and monooxygenation. The diverse oxygenation catalyzed by CARDO for several aromatic compounds might reflect the differences in the binding of the substrates to the reaction center of CARDO(5).

Bioaugmentation was performed by using strain CA10, and the degradation of chlorinated dibenzo-p-dioxin and the number of bacteria in soils were monitored.

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