S3-2

PCB degradation pathway enzymes and genes in a Gram-positive *Rhodococcus* degrader.

Masao Fukuda

Department of Bioengineering, Nagaoka University of Technology, Nagaoka, Niigata 940-2188, Japan.

Polychlorinated biphenyls (PCBs) were once widely used in the world for industrial applications because of their high chemical stability and insulating properties, and resistance to burning. Prior to the discontinuation of their use, a large amount of PCBs were released into the environment where they persist as a potential threat to human health. Many PCB-degrading bacteria have been isolated and are expected to be a potential agent for bioremediation of PCB contamination. PCBs are aerobically co-metabolized through a biphenyl degradation pathway illustrated in Fig. 1. In a biphenyl/PCB degradation pathway, biphenyl is converted to dihydrodiol by a multicomponent biphenyl dioxygenase (BphA). Dihydrodiol is oxidized to 2,3-dihydroxybiphenyl (DHBP) by dihydrodiol dehydrogenase (BphB). Then the aromatic ring cleavage of DHBP at 1,2 position is catalyzed by DHBP dioxygenase (BphC). The resulting *meta*-cleavage product (HOPD) is hydrolyzed to benzoate and HPDA by HOPD hydrolase (BphD). Benzoate is metabolized via catechol into a member of TCA cycle. HPDA is decomposed by the sequential actions of HPDA hydratase (BphE), HOVA aldolase (BphF), and acetaldehyde dehydrogenase (BphG) toward TCA cycle. We have isolated gram-positive *Rhodococcus* sp. strain RHA1 from γ–hexachlorocyclohexane (an insecticide named lindane)-contaminated soil. The strain RHA1 efficiently transformed PCBs including mono- to octachlorobiphenyls in three

days (Seto, 1995a). We have characterized the PCB degradation pathway enzymes and genes of RHA1. The findings mentioned below suggested that RHA1 employs multiple enzyme systems for PCB degradation.

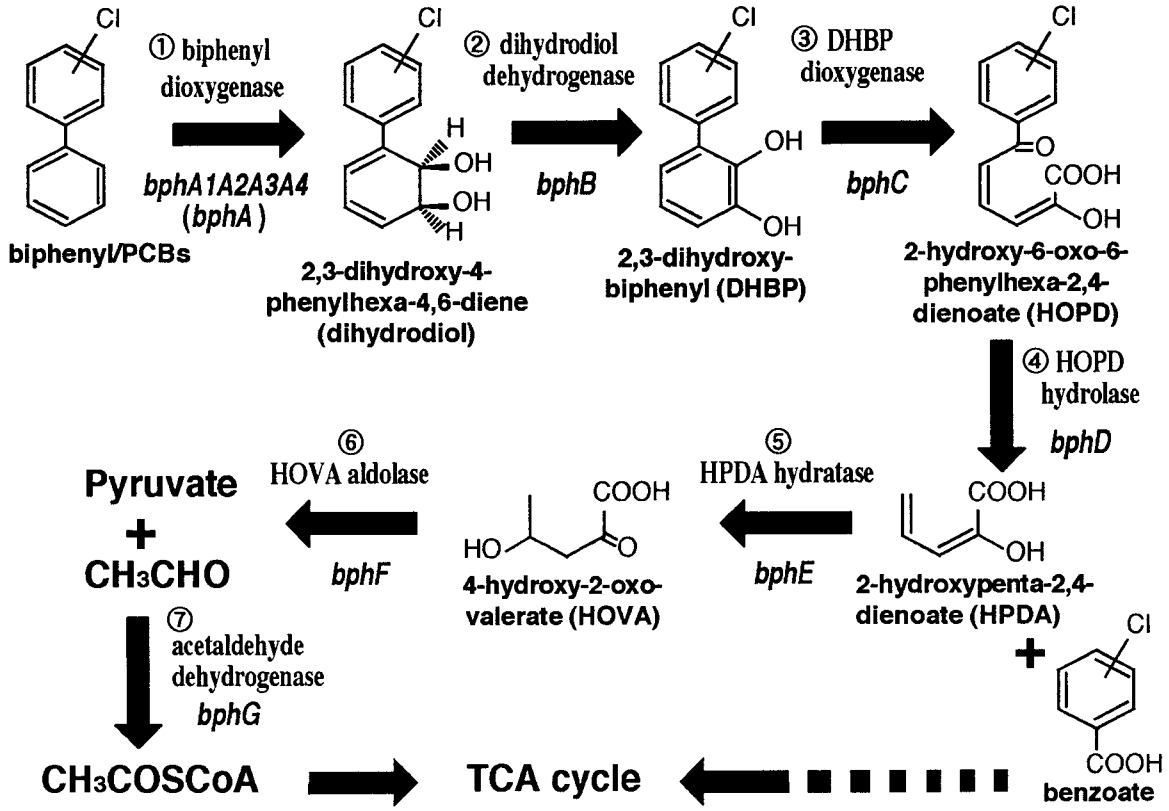

Fig. 1. The aerobic degradation pathway for biphenyl and PCBs.

1. Multiple PCB transformation systems in RHA1.

The strong PCB degradation activity of RHA1 during the growth on biphenyl indicates that the biphenyl/PCB degradation enzymes are induced in the presence of biphenyl. The mutant strain RDA1 lost PCB degradation activity that is induced during the growth on biphenyl. RDA1 had a deletion of the region including the *bphA1A2A3A4* genes encoding BphA. These results indicated that the *bphA1A2A3A4* genes are primarily responsible for PCB degradation activity induced by biphenyl

(Masai, 1995). The strain RDA1, however, showed PCB degradation activity during the growth on ethylbenzene (Seto, 1995b). An isozyme of BphA is presumed to be responsible for this secondary PCB degradation activity induced preferentially by ethylbenzene. Two sets of BphA isozyme genes, namely *bphA* homologs were cloned and characterized (Fig. 2). They are candidates for the determinant of the secondary PCB degradation activity.

2. Diversity of DHBP dioxygenase genes in RHA1.

Seven isozyme genes for BphC dioxygenase were cloned from RHA1 (Fig. 2). Each of them exhibited BphC activity in *E. coli*. These isozymes include six class 2 and one class 1 extradiol (*meta*-) ring-cleavage dioxygenases (Senda, 1996). The mutant strain RDC1 lacking *bphC* gene region did not grow on biphenyl suggesting that the gene *bphC* is primarily responsible for biphenyl/PCB degradation in RHA1 (Masai, 1995). A BphC isozyme encoded by *etbC* gene was found in the crude cell extract prepared from the cells grown on biphenyl and ethylbenzene (Hauschild, 1996). Northern blot analysis indicated that the transcription of at least three BphC isozyme genes including *bphC* and *etbC* were induced by biphenyl.

3. Diversity of HOPD hydrolase genes in RHA1.

Two kinds of HOPD hydrolase isozymes were purified from the crude cell extract prepared from the cells grown on biphenyl. One of them was specific to HOPD and was encoded by *bphD* gene (Yamada, 1998). This isozyme is thought to be primarily responsible for biphenyl/PCB degradation. The other one was specific to 2-hydroxy-6-oxohepta-2,4-dienoate, a *meta*-cleavage product of 3- methylcatechol

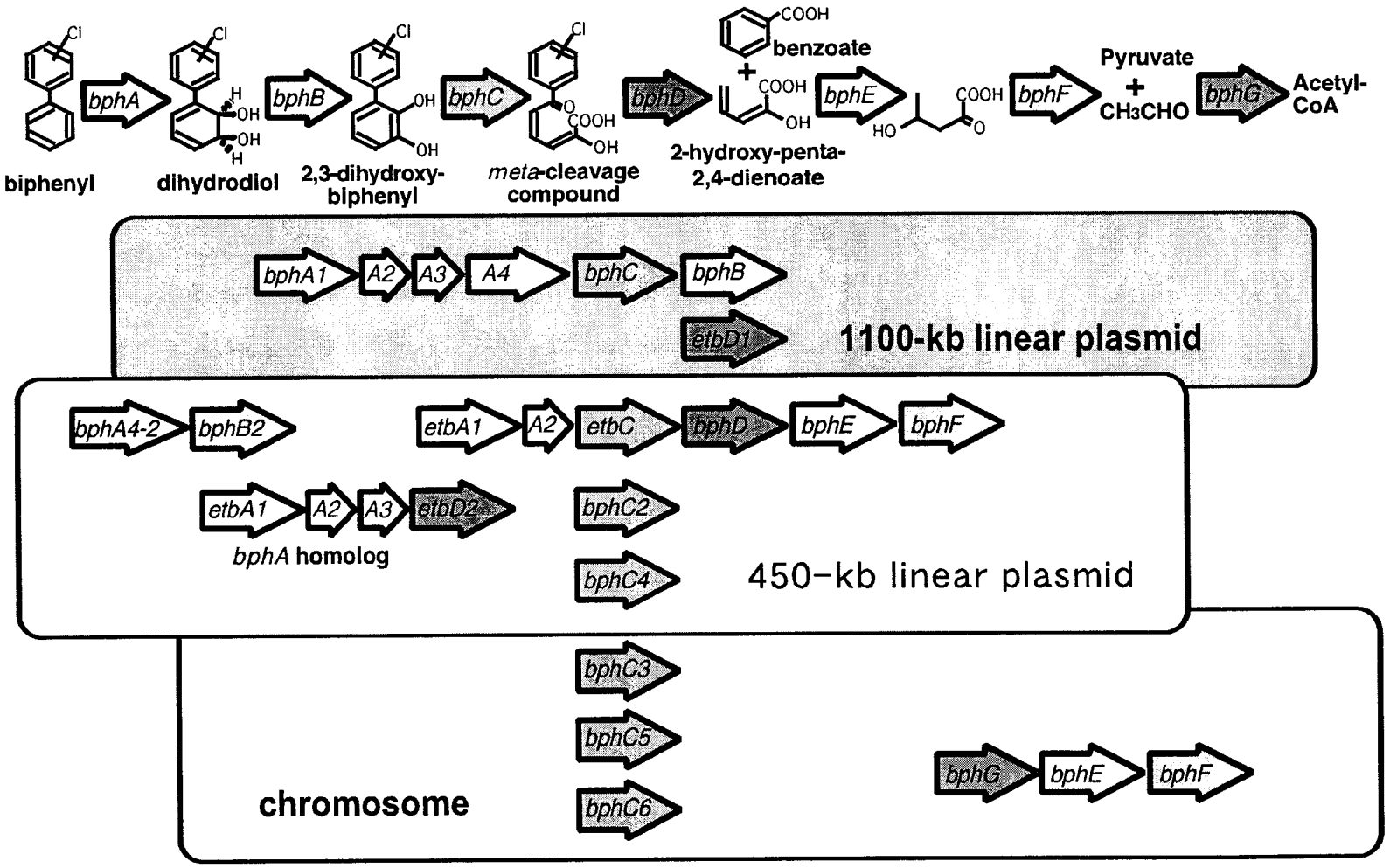

Fig. 2. The organization and distribution of isozyme genes related to PCB degradation.

and was encoded by *etbD1*. An *etbD2* gene which is highly homologous (97%) to *etbD1* was also cloned. These three genes were similarly induced by a variety of aromatic compounds, suggesting that they are controlled under the same regulatory system.

Thus, RHA1 employs multiple enzyme systems that consist of isozymes of each step encoded by homologous genes. Such multiple enzyme systems seem to be of benefit to PCB degradation by expanding the substrate spectrum of each degradation step. Because PCBs are mixture of chlorinated biphenyl congeners which are different from each other in the number and the position of chlorine substitutions. Most of these isozyme genes are located on two linear plasmids of 1,100 kb and 450 kb in size as illustrated in Fig. 2 (Masai, 1997). We have obtained a variety of deletion or insertion derivatives of these plasmids, and these plasmids seem to be susceptible to DNA rearrangement. Frequent DNA rearrangement on these linear plasmids might have participated in the evolution of multiple isozyme genes in the strain RHA1.

References:

Hauschild JE, Masai E, Sugiyama K, Hatta T, Kimbara K, Fukuda M, Yano K, 1996, Appl. Environ. Microbiol., 62: 2940-46.

Masai E, Yamada A, Healy JM, Hatta T, Kimbara K, Fukuda M, Yano K, 1995, Appl. Environ. Microbiol., 61: 2079-85.

Masai E, Sugiyama K, Iwashita N, Shimizu S, Hauschild JE, Hatta T, Kimbara K, Yano K, Fukuda M, 1997, Gene, 187: 141-9.

Senda T, Sugiyama K, Narita H, Yamamoto T, Kimbara K, Fukuda M, Sato M, Yano K,

Mitsui Y, 1996, J. Molec. Biol., 255: 735-752

Seto M, Kimbara K, Shimura M, Hatta T, Fukuda M, Yano K, 1995a, Appl. Environ. Microbiol., 61: 3353-8.

Seto M, Masai E, Ida M, Hatta T, Kimbara K, Fukuda M, Yano K, 1995b, Appl. Environ. Microbiol., 61: 4510-13.

Yamada A, Kishi H, Sugiyama K, Hatta T, Nakamura K, Masai E, Fukuda M, 1998, Appl. Environ. Microbiol., 64: 2006-12.