Concentration Effect on the Multiple Transformation Systems for Polychlorinated Biphenyls in a Strong PCB Degrader, *Rhodococcus* sp. Strain RHA1.

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Abstract

The concentration effect of PCBs on the PCB transformation activity and the growth of the strong polychlorinated biphenyls (PCBs) degrader, *Rhodococcus* sp. strain RHA1 was examined. High concentration of PCBs (above 50 μg/ml) inhibited both the PCB transformation activity and the growth on biphenyl and ethylbenzene of the strain RHA1. During the transfer of RHA1 in the minimal salt medium containing ethylbenzene as a carbon source and 100 μg/ml of PCB48, RHA1 derivatives deficient in biphenyl catabolism were accumulated. Some of these derivatives that produced yellow *meta*-cleavage compound during the incubation with biphenyl have *bphD* gene deletion, and the others not produced *meta*-cleavage compound have *bphACB* gene deletion. One of the RHA1 derivatives lacking *bphD* gene, the strain RCD1 was tolerant to the growth inhibition of PCBs and exhibited superior PCB degradation activity to the wild type RHA1. These results suggested that the metabolic intermediates of PCBs, especially those produced after aromatic ring cleavage through *bph* gene coding pathway should be a major cause of the growth inhibition by high concentration PCBs. The introduction of *bphD* gene plasmid into the strain RCD1 restored the sensitivity to the growth inhibition of PCBs. The deletion of *bphD* gene was indicated to confer the tolerance to the growth inhibition by high concentration PCBs and the higher PCB transformation activity.

Introduction

Polychlorinated biphenyls (PCBs) have been used in many industrial applications and caused a serious environmental problem. PCBs are biologically toxic and a source of dioxins. To establish an effective biodegradation system of PCBs, we screened PCB degrading microorganisms from soils and isolated a strong PCB degrader, *Rhodococcus* sp. strain RHA1 from a γ-hexachlorocyclohexane contaminated soil in Japan. The strain

RHA1 efficiently degrade 10 μg/ml of a PCB mixture containing mono- to octa-chlorobiphenyls of Kanechlors 200, 300, 400, and 500 within three days [1]. The biphenyl/PCB degradation genes (*bph* genes) were isolated from RHA1 and characterized [2, 3]. The *bphACB* genes were indicated to encode the initial three degradation steps from biphenyl and chlorobiphenyls to aromatic-ring cleavage compounds at *meta* position (*meta*-ring cleavage compounds), and the *bphD* gene was to be responsible for the conversion of *meta*-ring cleavage compound to benzoic and chlorobenzoic acid and 2-hydroxypenta-2,4-dienoic acid its chlorinated derivatives (Fig. 1). The resulting chlorobenzoic acids seems to be degraded gradually, because RHA1 grew slowly on chlorobenzoic acids. In addition to the *bph* gene encoding PCB degradation system, RHA1 harbors alternate PCB degradation system which is induced by ethylbenzene, but not by biphenyl [4].

In a treatment of PCB contaminated wastes, the higher the concentration of PCBs in a treatment system is, the more it is cost effective. In this study, we examined the concentration effect of PCBs on the PCB transformation activity and the growth of RHA1.

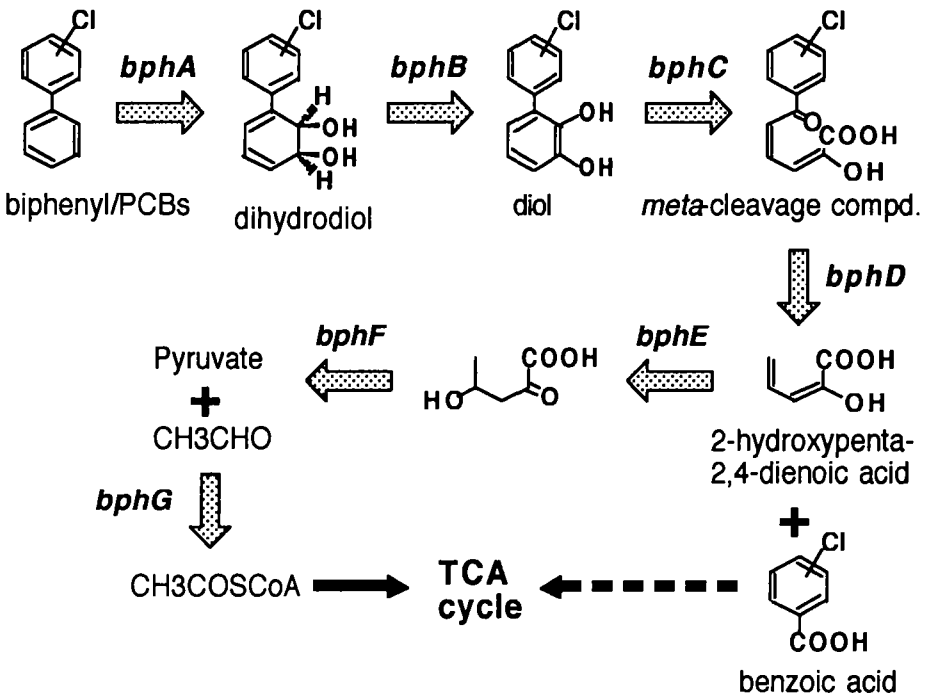

Fig. 1. Proposed major catabolic pathway and responsible genes for the biodegradation of biphenyl and PCBs in *Rhodococcus* sp. RHA1.

Experimental Methods

(1) Bacterial strains and culture conditions. A gram-positive strong PCB degrader, *Rhodococcus* sp. strain RHA1 was used in this study. Growth on biphenyl or ethylbenzene and PCB transformation activity were examined using W medium [1] as a minimal salt medium.

(2) Determination of PCB transformation activity. The method employed was essentially the same as that described previously. The strains were grown on biphenyl or ethylbenzene in the W minimal salt medium in the presence of PCBs for three days. PCB congeners remained were extracted and subjected to congener specific PCB analysis using gas chromatography-mass spectrometry. The congener-specific ions were collected to provide the selected ion chromatograms of PCBs.

Results And Discussion

(1) Concentration effect on the activities of multiple PCB transformation systems in the strain RHA1. The PCB transformation activity of the strain RHA1 at the different concentration of PCB48 (equivalent to Aroclor 1248) were determined in the presence of either biphenyl or ethylbenzene, which were added as co-substrates. At 30 μg/ml of PCB48, RHA1 exhibited superior transformation activity to those at 10 μg/ml. The transformation activities of RHA1 diminished at 50 μg/ml especially in the presence of biphenyl. At 100 μg/ml, the transformation activities suppressed seriously. Not only the PCB transformation activities, but also the growth of RHA1 was inhibited by the higher concentration of PCB48. Any significant growth of RHA1 was observed at 100 μg/ml of PCB48 neither in the presence of biphenyl nor ethylbenzene. Thus, high concentration of PCBs inhibited both the PCB transformation activity and the growth on biphenyl and ethylbenzene of the strain RHA1.

(2) Accumulation of RHA1 derivatives deficient in biphenyl catabolism after the growth on ethylbenzene in the presence of 100 μg/ml PCB48. To overcome the inhibitory effect of high concentration PCBs on the growth of RHA1, we transferred RHA1 several times in the minimal salt medium containing ethylbenzene as a carbon source and 100 μg/ml of PCB48. RHA1 derivatives adapted to the growth inhibition by high concentration PCBs occurred after the growth on ethylbenzene in the presence of 100 μg/ml PCB48. All these derivatives did not grow on biphenyl, and some of them accumulated yellow colored substances (*meta*-cleavage compound) during the incubation with biphenyl, indicating the lack of aromatic-ring cleavage enzyme activity. After four times of transfer, there detected only the derivatives which accumulated yellow colored substances from biphenyl. Southern hybridization experiments using *bphACB* and *bphD* probes indicated that the RHA1 derivatives accumulated yellow colored meta-cleavage compounds have *bphD* gene deletion, and that those deficient in growth on biphenyl without production of yellow colored *meta*-cleavage compounds have *bphACB* gene

deletion.

(3) The RHA1 derivatives deficient in biphenyl catabolism. One of the RHA1 derivatives lacking *bphD* gene, the strain RCD1 and that lacking *bphACB* gene, the strain RCA1 were characterized further. The strain RCD1 grew on ethylbenzene as well in the presence of 100 μg/ml PCB48 as in the absence of PCB48. RCD1 exhibited superior PCB degradation activity on 10 μg/ml PCB congener mixture to the wild type RHA1, probably because it is almost completely saved from the inhibitory effect of PCBs. The growth of the strain RCA1 on ethylbenzene was partially inhibited in the presence of 100 μg/ml PCB48. The PCB degradation activity of RCA1 on 10 μg/ml PCB congener mixture was inferior to RHA1. Because *bphACB* genes conferring biphenyl and PCB degradation activity were deleted in RCA1, only the alternate PCB degradation system seems to be responsible for PCB transformation. These results suggested that the metabolic intermediates of PCBs, especially those produced after aromatic ring cleavage through *bph* gene coding pathway should be a major cause of the growth inhibition by high concentration PCBs. This notion was confirmed by the introduction of *bphD* gene plasmid into the strain RCD1. In RCD1 containing a *bphD* gene plasmid, the accumulation of yellow meta-cleavage compounds was suppressed , and the growth was as seriously inhibition by 100 μg/ml PCB48 as the wild type RHA1.

Thus the deletion of *bphD* gene was indicated to essentially confer the tolerance to the growth inhibition by high concentration PCBs and the higher PCB transformation activity.

Acknowledgments

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