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Multiple Genes Encoding 2,3-Dihydroxybiphenyl 1,2-Dioxygenase in the Gram-Positive Polychlorinated Biphenyl-Degrading Bacterium *Rhodococcus erythropolis* TA421, Isolated from a Termite Ecosystem

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Rhodococcus erythropolis **TA421 was isolated from a termite ecosystem and is able to degrade a wide range of polychlorinated biphenyl (PCB) congeners. Genetic and biochemical analyses of the PCB catabolic pathway of this organism revealed that there are four different *bphC* genes (*bphC1*, *bphC2*, *bphC3*, and *bphC4*) which encode 2,3-dihydroxybiphenyl dioxygenases. As determined by Southern hybridization, none of the *bphC* genes exhibits homology to any other *bphC* gene. *bphC1*, *bphC2*, and *bphC4* encode enzymes that have narrow substrate specificities and cleave the first aromatic ring in the *meta* position. In contrast, *bphC3* encodes a *meta* cleavage dioxygenase with broad substrate specificity. Asturias et al. have shown that the closely related organism *Rhodococcus globerulus* P6 contains three different *bphC* genes (*bphC1*, *bphC2*, and *bpHC3*) which encode *meta* cleavage dioxygenases. The data suggest that there is a diverse family of *bphC* genes which encode PCB *meta* cleavage dioxygenases in members of the genus *Rhodococcus*.**

Polychlorinated biphenyls (PCBs) are man-made compounds that are composed of biphenyl molecules containing from 1 to 10 chlorine atoms. Since PCB residues have been found in the environment, there has been greater global interest in these widespread and persistent pollutants. It is recognized that PCBs are present in great quantities in the ecosystem, like DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and its metabolites. PCB- and biphenyl-degrading bacteria have been isolated from environments throughout the world and can cometabolize many PCB congeners to chlorobenzoic acids through either oxidative or reductive routes. Most of these bacteria are gram-negative organisms such as *Pseudomonas*, *Alcaligenes*, or *Acinetobacter* strains. There is little information about gram-positive PCB-degrading bacteria other than *Rhodococcus globerulus* P6 (previously designated *Acinetobacter* sp. strain P6, *Arthrobacter* sp. strain M5, and *Corynebacterium* sp. strain MB1) (3, 4). Sequence and biochemical analyses have revealed that the *bphC2* and *bphC3* genes of this organism encode a new type of 2,3-dihydroxybiphenyl dioxygenases (2,3-DHBDs) (3).

Rhodococci are ubiquitous in nature and have frequently been isolated from soil, freshwater, and marine habitats, as well as from the gut contents of blood-sucking arthropods (9, 14). Rhodococci have been implicated in the degradation of lignin-related compounds (10, 11, 22) and have frequently been isolated from soil contaminated with petroleum compounds (20, 21). Biphenyl moieties resulting from the breakdown of lignin within the soil community might represent a carbon-rich resource for biphenyl-degrading bacteria, such as rhodococci. *Rhodococcus erythropolis* is suitable for further genetic analysis as more than 60 genetic markers have been determined in the development of an *R. erythropolis* genetic linkage map and temperate phages are now available for use as cloning vectors.

R. erythropolis TA421 was isolated from the ecosystem of wood-feeding termites and was found to be capable of degrading PCBs (8), suggesting that the termite ecosystem is one possible habitat for PCB- and biphenyl-degrading rhodococci.

The PCB congeners degraded by strain TA421 are different from the PCB congeners degraded by other bacteria, such as *Corynebacterium* sp. strain MB1 (*R. globerulus* P6) (8).

In this study, we cloned and analyzed four different *bphC* genes (*bphC1*, *bphC2*, *bphC3*, and *bphC4*) which encode 2,3-DHBDs in *R. erythropolis* TA421.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. *R. erythropolis* TA421 was grown on C medium, which contained (per liter) 5 g of $(NH_4)_2SO_4$, 2.93 g of KH_2PO_4, 5.87 g of K_2HPO_4, 0.3 g of $MgSO_4 \cdot 7H_2O$, 2 g of NaCl, 0.03 g of $CaCl_2$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.6 mg of $NiSO_4 \cdot 7H_2O$, and 2 ml of a trace elements solution; the pH of this medium was adjusted to pH 7.0. The trace elements solution contained (per liter) 4 mg of MoO_3, 28 mg of $ZnSO_4 \cdot 5H_2O$, 2 mg of $CuSO_4 \cdot 5H_2O$, 4 mg of H_3BO_3, 4 mg of $MnSO_4 \cdot 5H_2O$, and 4 mg of $CoCl_2 \cdot 6H_2O$. Biphenyl was added to C medium to a final concentration of 0.5% (not dissolved) as the sole carbon source, and cultures were incubated on a reciprocal shaker at 120 rpm and 30°C. *Escherichia coli* DH5 (*supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*) and MV1184 [*ara* Δ(*lac-proAB*) *rpsL thi*(ϕ80*lacZ*Δ*M15*), Δ(*srl-recA*)*306*::Tn*10* (*tet*^{r})/F′ (*traD36 proAB*$^+$ *lacI*^{q}, *lacZ*Δ*M15*)] were used as recipient strains for transformation experiments. *E. coli* DH5 and MV1184 were routinely grown at 37°C in Luria-Bertani (LB) broth containing ampicillin (100 μg/ml) or isopropyl-β-D-thiogalactopyranoside (IPTG) (500 μM) when necessary. Solid medium contained 1.5% Bacto Agar (Difco Laboratories). The pUC119 and pUC118 vectors were used to clone the *bphC* gene.

Cloning the *bphC* gene. Genomic DNA from *R. erythropolis* TA421 was prepared by a procedure based on the method described by Wilson (27).

R. erythropolis TA421 chromosomal DNA was partially digested with *Sau*3A1, and DNA fragments were fractionated by agarose gel electrophoresis into the following six size fractions: 2 to 3, 3 to 4, 4 to 6, 6 to 8, 8 to 10, and >10 kb. The six fractions containing DNA fragments were separately ligated to pUC118 which was digested with *Bam*HI and treated with alkaline phosphatase. The resulting plasmids were transformed into *E. coli* DH5 and plated onto LB agar plates containing ampicillin and IPTG. Clones that expressed 2,3-DHBD activity

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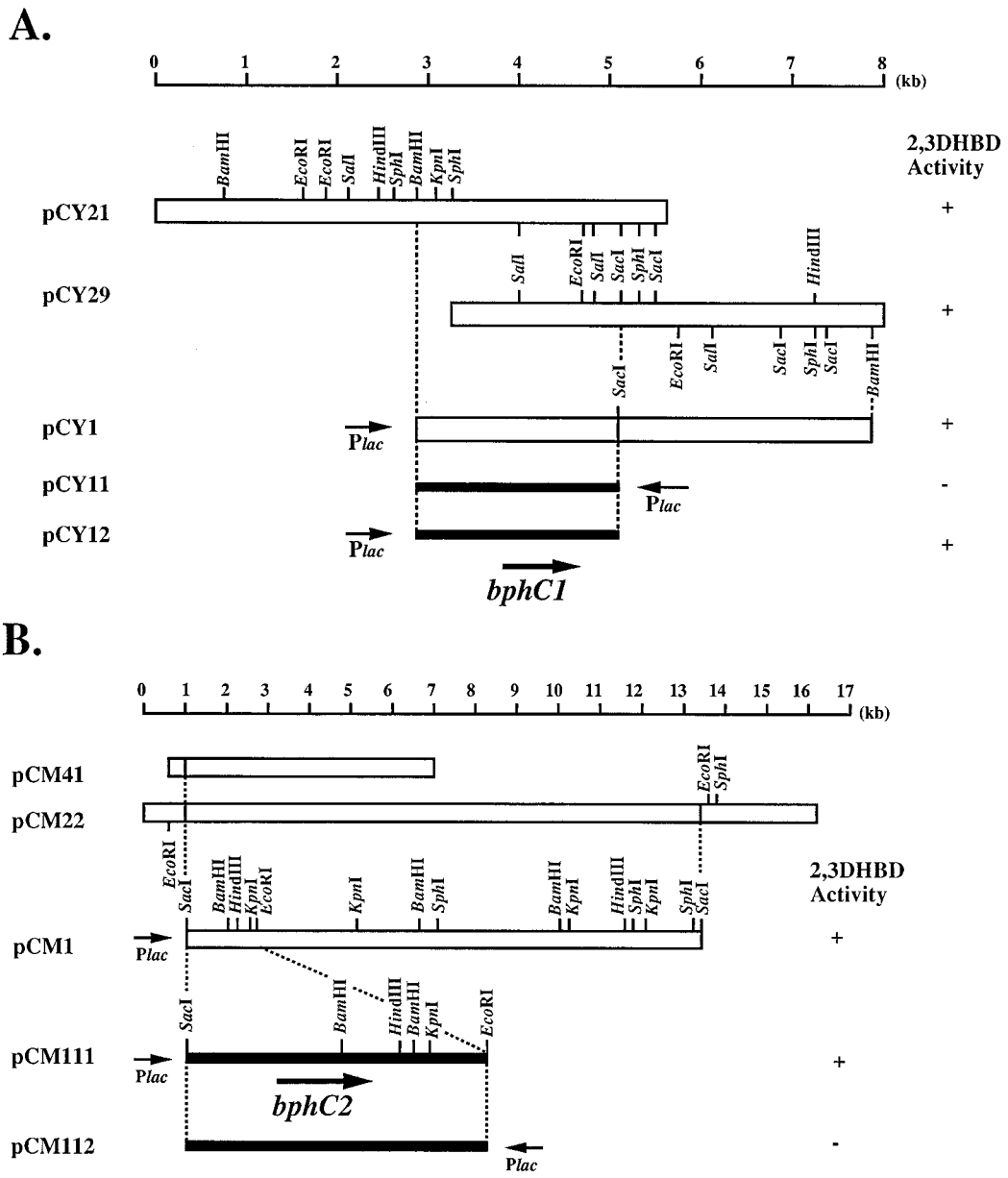

FIG. 1. Cloning of *R. erythropolis bphC* genes. (A) Cloning of the *bphC1* gene. (B) Cloning of the *bphC2* gene. (C) Cloning of the *bphC3* gene. (D) Cloning of the *bphC4* and *bphD* genes. The restriction sites in parentheses are located in the vector. Only restriction sites of relevant enzymes are shown. The location and orientation of the *lac* promoter of the pUC vector are indicated by an arrow. +, activity; −, no activity. HOPH; 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid hydrolase.

were identified by spraying colonies with a solution containing 20 mM 2,3-dihydroxybiphenyl in acetone. Positive clones turned yellow because they produced the yellow metabolite 2-hydroxy-6-oxo-6-phenyl-hexa-2,4-dienoic acid (the *meta* cleavage product) (13).

E. coli plasmids were prepared by the alkaline sodium dodecyl sulfate (SDS) method of Sambrook et al. (23).

Measurement of enzymatic activity. Strains harboring recombinant plasmids were grown as described above, washed with 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM dithiothreitol, and resuspended in the same buffer. The cells were broken by treatment with a French pressure cell (Ohtake Seisakusho, Tokyo, Japan) at 20,000 lb/in^2. Cell debris was removed by centrifugation at 20,000 × *g* for 30 min at 4°C, and the supernatant (cell extract) was used immediately.

2,3-DHBD activity was determined by measuring the formation of *meta* cleavage reaction products at 434 nm with a Hitachi model 330 spectrophotometer equipped with a water-cooled cell holder and a Haake circulating water bath. Activity assays were performed at 25°C by using 50 mM Tris-HCl buffer (pH 7.5) containing 200 μM 2,3-dihydroxybiphenyl. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of product per min at 25°C. The molar extinction coefficient of the product under assay conditions was taken to be 13,200 cm^{-1} M^{-1} (12). The relative ring cleavage activities were determined from the extinction coefficients of the ring fission products formed from the following substrates: catechol (λ_{max}, 375 nm; ε, 36,000 cm^{-1} M^{-1}), 3-methylcathecol (λ_{max}, 388 nm; ε, 32,000 cm^{-1} M^{-1}), and 4-methylcathecol (λ_{max}, 382 nm; ε, 17,000 cm^{-1} M^{-1}) (4). BphD hydrolase activity was assayed by monitoring the disappearance of the *meta* cleavage compound as determined with the spectrophotometer at 434 nm. Specific activity was defined as the number of enzyme units per milligram of protein. The protein concentration was determined with a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.) by using the method of Bradford (5).

Hybridization experiments. Southern blot experiments (26) were performed with Hybond-N^+ nylon membranes (Amersham, Buckinghamshire, England). Hybridizations were carried out under the conditions described by Anderson and Young (2). Hybridization experiments were performed at 42°C in the presence of 50% formamide–5× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0), and the filters were washed at 68°C in 0.1× SSC

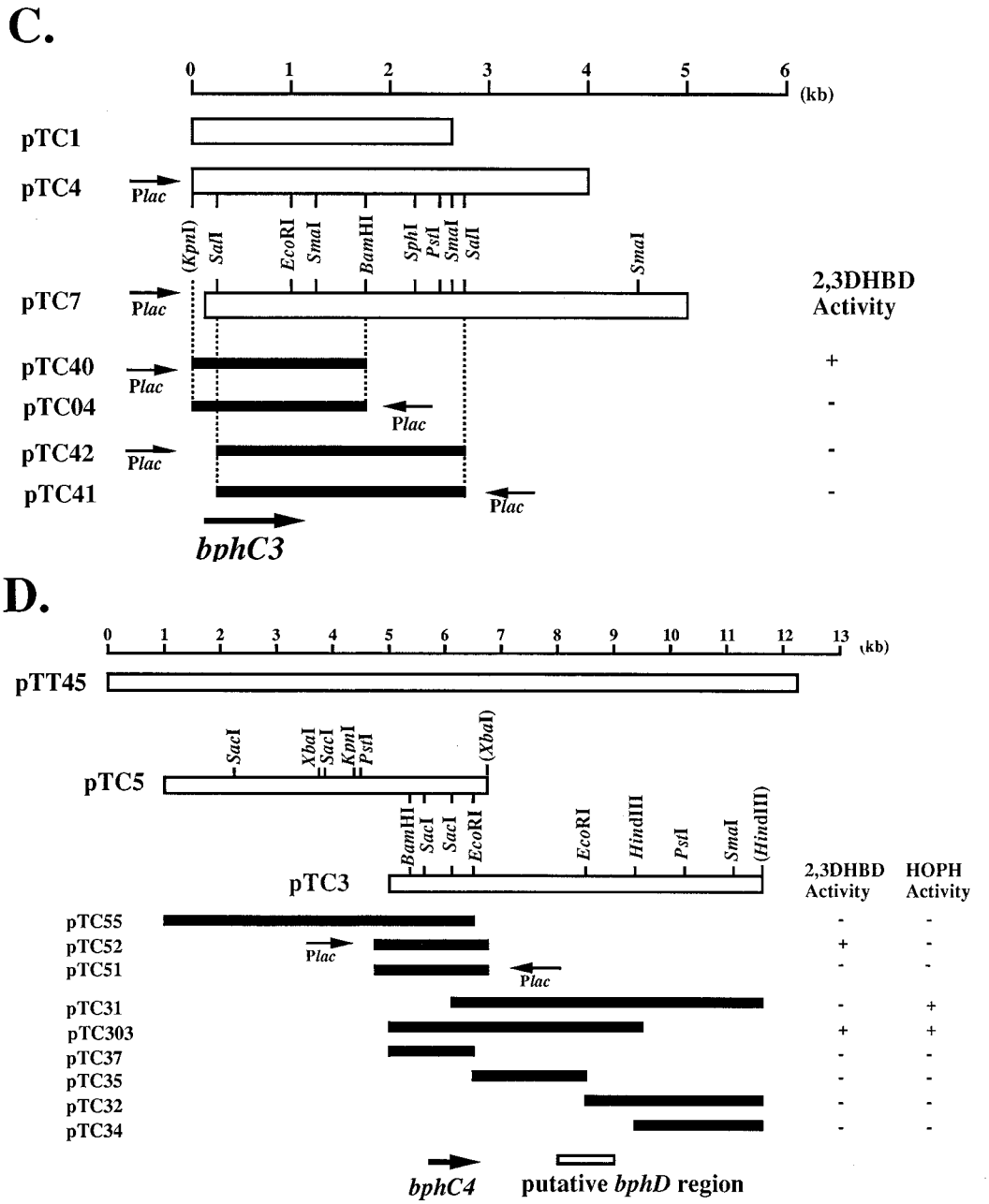

FIG. 1—*Continued.*

containing 0.1% SDS. The DNA fragments used as probes were isolated from agarose. A nonradioactive DIG DNA labeling and detection kit (DIG-ELISA; Boehringer Mannheim Biochemicals, Mannheim, Germany) was used for labeling and to detect probes.

Subcloning procedure and restriction analysis. The DNA fragments that encoded 2,3-DHBD activity were subcloned into pUC118 and pUC119 that had been digested with the appropriate enzymes and treated with alkaline phosphatase. Each ligation mixture was used to transform *E. coli* MV1184 cells. The cells were plated onto LB agar containing ampicillin, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), and IPTG. Clones that expressed 2,3-DHBD activity were identified by the spray method described above. A restriction analysis and recombinant DNA techniques were performed by standard methods (23) or as recommended by the manufacturer.

Deletion analysis of the *bphC2* gene. To determine the location of 2,3-DHBD II activity in the subcloned fragments, we performed a deletion analysis. Plasmids were deleted by using an exonuclease III method and a deletion kit (Takara Shuzo, Kyoto, Japan). The 2,3-DHBD activity of recombinant *E. coli* MV1184 cells harboring the deletion plasmids was determined, and the position of each cloned fragment on the pUC vector was determined by sequencing (24). Deletion mutants of pCM112 were aligned with the orientation of the *lac* promoter on the pUC vector by digesting the preparation with restriction endonuclease *Hin*dIII and ligating the preparation back into the pUC vector.

RESULTS

Screening of the library for 2,3-DHBD expression. Six libraries were constructed as described above. Approximately 20,000 colonies of each library were obtained on LB agar containing 100 μg of ampicillin per ml. All recombinant colonies were tested for 2,3-DHBD activity by spraying the preparations with a solution containing 2,3-dihydroxybiphenyl. More than 100 clones turned brilliant yellow when they were sprayed. All positive clones were isolated, purified, rechecked for 2,3-

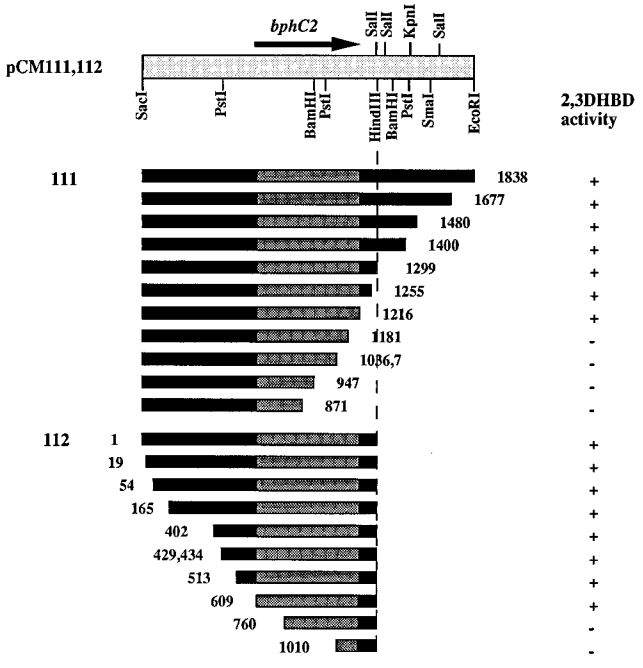

FIG. 2. Determination of the DNA region for 2,3-DHBD II activity. The numbers next to the bars indicate the positions of the 5′ (left) and 3′ (right) ends. The shaded portions of the bars represents the putative open reading frame of *bphC2* as deduced from the nucleotide sequence. Deletion mutants of pCM112 were aligned with the orientation of the *lac* promoter on the pUC vector by digesting preparations with restriction endonuclease *Hin*dIII and ligating them back into the pUC vector.

DHBD activity, and tested for 3-methylcatechol dioxygenase activity by spraying them with a solution containing 3-methylcatechol. A large number of the positive clones turned yellow quickly, but a few clones did not turn yellow. We selected four of the 3-methylcatechol dioxygenase-negative clones and five of the 3-methylcatechol dioxygenase-positive clones for further study. The positions of cloned fragments in plasmid DNAs that exhibited dioxygenase activity were determined by restriction mapping before subcloning. Southern blot analyses were performed with other clones by using each subcloned fragment as a probe. On the basis of differences in their restriction patterns and the results of Southern blot analyses, these clones were classified into the following four groups: group I (pCY21 and pCY29), group II (pCM22 and pCM41), group III (pTC1, pTC4, pTC7, and 13 additional clones), and group IV (pTC3, pTC5, and 12 additional clones, including pTT45). To confirm that clones which belonged to groups I and II were derived from TA421 and had the correct arrangement, we cloned new fragments designed on the basis of restriction maps and Southern blot analysis results. The resulting group I and II plasmids (which were 2,3-DHBD positive) were pCY1 and pCM1, respectively. Plasmid pCY1 contains an approximately 4.5-kb *Bam*HI fragment while pCM1 contains an approximately 12.0-kb *Sac*I fragment (Fig. 1A and B). Plasmids pCY1 and pCM1 were used for all further analyses. Below, the four 2,3-DHBDs are referred to as follows: 2,3-DHBD I is the enzyme encoded by the *bphC1* gene found in the group I library; 2,3-DHBD II is the enzyme encoded by the *bphC2* gene found in the group II library; 2,3-DHBD III is the enzyme encoded by the *bphC3* gene found in the group III library; and 2,3-DHBD IV is the enzyme encoded by the *bphC4* gene found in the group IV library.

Subcloning of the genes that encode 2,3-DHBDs. Plasmid DNAs from *E. coli* DH5(pCY1), DH5(pCM1), DH5(pTC4), DH5(pTC3), and DH5(pTC5) were isolated and characterized by restriction endonuclease digestion and agarose gel electrophoresis. The sizes of the inserts in pCY1, pCM1, pTC4, pTC3, and pTC5 were approximately 4.5, 12.0, 4.1, 7.3, and 6.0 kb, respectively. Restriction maps for the five inserts (Fig. 1) were determined by performing single and double digestions with restriction endonucleases and Southern hybridization experiments. The *bphC1* gene was subcloned from pCY1 as a 2.1-kb *Bam*HI-*Sac*I fragment into pUC118 and pUC119, yielding plasmids pCY11 and pCY12, respectively (Fig. 1A).

The gene that encodes the 2,3-DHBD II activity of pCM1 was subcloned as a 1.9-kb *Eco*RI fragment (the upstream site was derived from the pUC vector) into pUC118, yielding plasmids pCM111 (2,3-DHBD positive) and pCM112 (Fig. 1B). A deletion analysis revealed that the *bphC2* gene was located in a 0.6-kb DNA region (Fig. 2). The amino acid sequence of 2,3-DHBD II contains approximately 200 residues, as deduced from the size of the region which encodes 2,3-DHBD activity.

The *bphC3* gene was subcloned from pTC4 as a 2.2-kb *Bam*HI fragment into pUC119, giving rise to plasmid pTC40.

The *bphC4* gene was subcloned from pTC5 as a 2.1-kb *Xba*I fragment into pUC119, giving rise to plasmids pTC51 and pTC52 (2,3-DHBD positive) and was also subcloned from pTC3 as a *Hin*dIII fragment, resulting in plasmid pTC303.

All *bphC* genes expressed 2,3-DHBD activity under control of the vector *lac* promoter.

Southern hybridization experiments in which we used the *bphC* gene probes with the clonal fragments and the TA421 genome were performed in order to determine the levels of similarity among the four *bphC* genes and their arrangement on the TA421 genome. A 2.1-kb *Bam*HI-*Sac*I fragment containing *bphC1* hybridized to pCY1 DNA (positive control) that was double digested with *Bam*HI and *Sac*I and to *R. erythropolis* TA421 total DNA, but did not hybridize to pCM1, pTC4, or pTT45 (Fig. 3A). A faint band was observed at approximately 4 kb, but this band did not contain either *bphC* regions or the pUC vector. Similarly, the 1.9-kb *bphC2* gene-containing *Sac*I-*Eco*RI fragment from pCM1 hybridized to positive control pCM1, which was double digested with *Sac*I and *Eco*RI to liberate a fragment of the same size containing the *bphC2* gene, and to the same size of *R. erythropolis* TA421 DNA which was treated in the same way (Fig. 3B). The 2.2-kb *bphC3* gene-containing *Sal*I fragment from pTC4 hybridized to a 2.2-kb *Sal*I fragment of positive control pTC4 and of *R. erythropolis* TA421. No other bands were revealed by the probe (Fig. 3C). The 2.0-kb *bphC4* gene-containing *Xba*I fragment (the downstream *Xba*I site was derived from pUC118) from pTC5 hybridized to a 4.8-kb *Xba*I-*Hin*dIII fragment of positive control pTT45 and of *R. erythropolis* TA421 (Fig. 3D).

2,3-DHBD activities in bacteria carrying recombinant plasmids. Recombinant plasmids pCY12, pCM111, pTC4, and pTC303, representing groups I, II, III, and IV, respectively, were transformed into *E. coli* MV1184. 2,3-DHBD activity was analyzed by using cell extracts from recombinant *E. coli* cultures grown in LB broth supplemented with ampicillin and IPTG. The levels of enzyme activity were 0.30 U/mg in extracts from *E. coli* MV1184(pCY12), 1.48 U/mg in extracts from MV1184(pCM111), 4.88 U/mg in extracts from MV1184(pTC4), and 3.55 U/mg in extracts from MV1184(pTC4). No activity was observed with extracts from pUC-carrying *E. coli* MV1184 cells. All four cloned 2,3-DHBDs exhibited narrow substrate specificities. Only 2,3-DHBD I exhibited activity for 4-methylcatechol, while only 2,3-DHBD III exhibited activity for

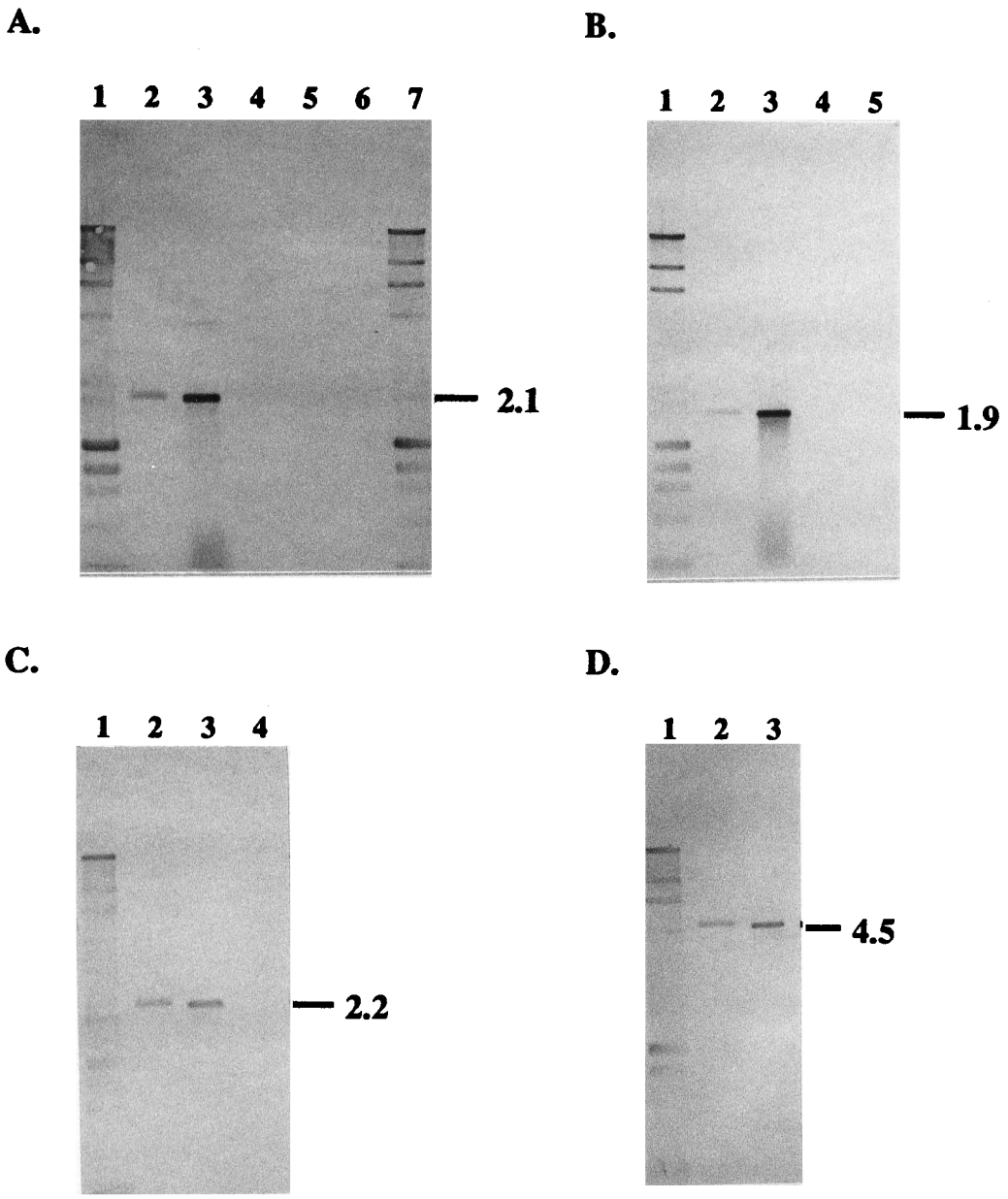

FIG. 3. Hybridization of the *bphC* genes with one another and with genomic DNA from *R. erythropolis* TA421. (A) Lanes 1 and 7, molecular weight marker (*Hin*dIII-digested λ and *Hae*III-digested ϕ X 1.74); lanes 2 and 3, genomic DNA and pCY1 double digested with *Bam*HI and *Sac*I, respectively; lane 4, pCM1 double digested with *Eco*RI and *Sac*I; lane 5, pTC4 digested with *Sal*I; lane 6, pTT45 double digested with *Xba*I and *Hin*dIII. The probe used was the 2.1-kb *Bam*HI-*Sac*I fragment from pCY1 containing the *bphC1* gene. (B) Lane 1, marker; lanes 2 and 3, genomic DNA and pCM1 double digested with *Eco*RI and *Sac*I, respectively; lane 4, pTC4 digested with *Sal*I; lane 5, pTT45 double digested with *Xba*I and *Hin*dIII. The probe used was the 1.9-kb *Sac*I-*Eco*RI fragment from pCY1 containing the *bphC2* gene. (C) Lane 1, marker; lanes 2 and 3, genomic DNA and pTC4 digested with *Sal*I, respectively; lane 4, pTT45 double digested with *Xba*I and *Hin*dIII. The probe used was the 2.2-kb *Sal*I fragment from pTC4 containing the *bphC3* gene. (D) Lane 1 marker; lanes 2 and 3, genomic DNA and pTT45 double digested with *Xba*I and *Hin*dIII, respectively. The probe used was the 2.0-kb *Xba*I fragment from pTC5 containing the *bphC4* gene. The slightly different molecular weights observed in lanes 2 and 3 in all panels were probably due to different amounts of sample DNA.

3-methylcatechol (Table 1). These results indicate that each of the ring cleavage enzymes is a bona fide 2,3-DHBD.

The kinetic parameters of the four 2,3-DHBDs were calculated from Lineweaver-Burk plots. These four enzymes had significantly different kinetic parameters (Table 2). 2,3-DHBD III had the greatest affinity for 2,3-dihydroxybiphenyl; its K_m value was between 4 and 26 times lower than the K_m values of the other three enzymes. The K_m value of 2,3-DHBD III for 3-methylcatechol was approximately 100 times higher than the corresponding value for 2,3-dihydroxybiphenyl. The maximum rate of metabolism (V_{max}) of 2,3-DHBD I was much lower than the V_{max} values of the other 2,3-DHBDs. The low V_{max} of 2,3-DHBD I may have been due to the oxygen sensitivity of this enzyme.

Localization of other genes involved in biphenyl degradation. To analyze whether the four *bphC* genes occur alone or are associated with genes that encode other enzymatic activities in the biphenyl degradation pathway, the activity of 2-hy-

TABLE 1. Substrate specificities of the different 2,3-DHBDs from *R. erythropolis* TA421

Substrate	Substrate concn (μM)	Activity with[a]:			
		2,3-DHBD I	2,3-DHBD II	2,3-DHBD III	2,3-DHBD IV
2,3-Dihydroxybiphenyl	300	100	100	100	100
Catechol	1,000	0	0	8.3	1.5
3-Methylcatechol	1,000	1.6	0.6	57	3.4
4-Methylcatechol	1,000	1.9	0	0	0

[a] Activities are expressed as percentages of the activity observed when 2,3-dihydroxybiphenyl was the substrate, which was defined as 100%.

droxy-6-oxo-6-phenylhexa-2,4-dienoic acid hydrolase, which converts the *meta* cleavage compound to benzoic acid and is encoded by the *bphD* gene, was assayed spectrophotometrically. We examined cell extracts derived from *E. coli* MV1184(pCY1), MV1184(pCM1), MV1184(pTC7), MV1184(pTC5), and MV1184 (pTC3), and only the *E. coli* MV1184(pTC3) extract exhibited a significant decrease in the amount of *meta* cleavage compound. This implies that 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid may be converted to benzoic acid. To determine the location of the *bphD* gene, pTC3 was subcloned (Fig. 1D). The results suggested that *bphD* is located more than 1-kb downstream of the *bphC4* gene.

DISCUSSION

R. erythropolis TA421 was isolated from the ecosystem of wood-feeding termites and was found to be capable of degrading PCBs. PCB- and biphenyl-degrading bacteria are usually gram-negative organisms, such as *Pseudomonas*, *Alcaligenes*, or *Acinetobacter* strains, and there is little information about gram-positive PCB-degrading bacteria except *R. globerulus* P6 (previously designated *Acinetobacter* sp. strain P6, *Arthrobacter* sp. strain M5, and *Corynebacterium* sp. strain MB1) (3, 4).

Asturias et al. described three different *bphC* genes (*bphC1*, *bphC2*, and *bphC3*) in *R. globerulus* P6 (3, 4). These three *bphC* genes encode enzymes that have narrow substrate specificities and *meta* cleave the first aromatic rings. Sequence and biochemical analyses revealed that *bphC2* and *bphC3* encode new types of 2,3-DHBDs. A phylogenetic analysis revealed that *bphC1*-encoded 2,3-DHBD has a different evolutionary origin than the previously described enzymes encoded by *bphC* genes.

To obtain more genetic information about gram-positive bacteria, we cloned and analyzed the four *bphC* genes (*bphC1*, *bphC2*, *bphC3*, and *bphC4*) which encode 2,3-DHBDs in *R. erythropolis* TA421. Our results suggest that there is diverse family of *bphC* genes which encode PCB *meta* cleavage enzymes in members of the genus *Rhodococcus*. The restriction map and the substrate specificities of the four *bphC* genes suggested that these four genes were different from each other. The previously characterized extradiol dioxygenases usually contain approximately 300 amino acid residues (1, 6, 7, 13, 15–19, 25, 28). Subcloning experiments and a partial DNA sequence analysis (unpublished data) revealed that our 2,3-DHBD II is an extremely small extradiol dioxygenase like 2,3-DHBD II in strain P6, suggesting that the 2,3-DHBD II in strain TA421 belongs to the new class of extradiol dioxygenases proposed by Asturias et al. (3). The enzymatic properties and restriction map of the strain TA421 2,3-DHBD III were similar to those of the strain P6 2,3-DHBD I. These results suggest that the *bphC3* gene in strain TA421 may be located with *bphB* and *bphD*. However, no hydrolase activity was found in the recombinant strain containing these regions, suggesting that the gene organization in strain TA421 is different from the organization of the *bphC1* gene cluster in strain P6. The *bphC4* gene in strain TA421 may be located with the *bphD* gene, suggesting that there is a *bph* gene cluster. Further DNA sequence analyses should provide more information about the diverse family of *bphC* genes that encode PCB *meta* cleavage dioxygenases in members of the genus *Rhodococcus.*

The PCB congeners degraded by strain TA421 are different from the PCB congeners degraded by previously characterized bacteria, including *Corynebacterium* sp. strain MB1 (*R. globerulus* P6). This difference probably reflects differences in the types and activities of the enzymes in the PCB and biphenyl *meta* degradation pathways. The role of multiple genes is not clear, but multiple independent genes may be important for broad PCB congener specificity.

TABLE 2. Kinetic parameters of the different 2,3-DHBDs from *R. erythropolis* TA421[a]

Enzyme	Substrate	K_m (μM)	V_{max} (nmol/min/mg)
2,3-DHBD I	2,3-DHBP	141.8	178
2,3-DHBD II	2,3-DHBP	51.1	9,964
2,3-DHBD III	2,3-DHBP	5.4	9,438
2,3-DHBD III	3-MC	540.5	7,750
2,3-DHBD III	PC	4,764	4,731
2,3-DHBD IV	2,3-DHBP	21.0	3,797
2,3-DHBD IV	3-MC	419.8	162

[a] Activities were measured in cell extracts of *E. coli* recombinant strains carrying plasmids pCY12, pCM111, pTC4, and pTC303 for detection of 2,3-DHBD I, 2,3-DHBD II, 2,3-DHBD III, 2,3-DHBD IV activities, respectively. 2,3-DHBP, 2,3-dihydroxybiphenyl; 3-MC, 3-methylcatechol; PC, pyrocatechol.

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