The Journal of Microbiology, December 2000, p.275-280 Vol. 38, No. 4
Copyright © 2000, The Microbiological Society of Korea

NOTE

Cloning and Sequence Analysis of the *xylL* Gene Responsible for 4CBA-Dihydrodiol Dehydrogenase from *Pseudomonas* sp. S-47

Dong-Woo Park, Youngsoo Kim[1], Sang-Mahn Lee[2], Jong-Ok Ka[3], and Chi-Kyung Kim*

Department of Microbiology and Biotechnology, and Research Institute for Genetic Engineering, Chungbuk National University, Cheongju 361-763, Korea
[1]Department of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea
[2]Department of Biology, Chongju University, Cheongju 360-764, Korea
[3]Department of Agricultural Biology, Seoul National University, Suwon 441-744, Korea

(Received November 20, 2000 / Accepted December 19, 2000)

***Pseudomonas* sp. S-47 is capable of catabolizing 4-chlorobenzoate (4CBA) as carbon and energy sources under aerobic conditions via the *meta*-cleavage pathway. 4CBA-dioxygenase and 4CBA-dihydrodiol dehydrogenase (4CBA-DD) catalyzed the degradation of 4CBA to produce 4-chlorocatechol in the pathway. In this study, the *xylL* gene encoding 4CBA-DD was cloned from the chromosomal DNA of *Pseudomonas* sp. S-47 and its nucleotide sequence was analyzed. The *xylL* gene was found to be composed of 777 nucleotide pairs and to encode a polypeptide of 28 kDa with 258 amino acid residues. The deduced amino acid sequence of the dehydrogenase (XylL) from strain S-47 exhibited 98% and 60% homologies with those of the corresponding enzymes, *Pseudomonas putida* mt-2 (XylL) and *Acinetobacter calcoaceticus* (BenD), respectively. However, the amino acid sequences show 30% or less homology with those of *Pseudomonas putida* (BnzE), *Pseudomonas putida* F1 (TodD), *Pseudomonas pseudoalcaligenes* KF707 (BphB), and *Pseudomonas* sp. C18 (NahB). Therefore, the 4CBA-dihydrodiol dehydrogenase of strain S-47 belongs to the group I dehydrogenase involved in the degradation of mono-aryls with a carboxyl group.**

Key words: *xylL*, 4CBA-dihydrodiol dehydrogenase (4CBA-DD), nucleotide sequence, 4CBA degradation, *Pseudomonas sp. S-47.*

4-chlorobenzoate (4CBA), a kind of chlorinated aromatic, is a metabolite produced in the biodegradation processes of various chloroaromatics, such as herbicides, polychlorinated biphenyls, polychlorinated benzoates, and bidicin by a variety of microorganisms. In the aerobic degradation of the 4CBA, the metabolic pathway generally leads to the formation of dihydroxy intermediates, such as 4-chlorocatechol (4CC), as shown in Fig. 1. For the formation of the aromatic intermediate, two enzymatic steps must be carried out (5). The initial dihydroxylation step is carried out by the insertion of two atoms of oxygen at carbon positions 1 and 2 by 4CBA dioxygenase complex encoding by *xylXYZ* genes. This is followed by a dehydrogenation reaction to produce 4CC catalyzed by a 4CBA dihydrodiol dehydrogenase (4CBA-DD) encoded by *xylL* (10, 14).

The ring-hydroxylating dioxygenases are multi-component in nature, comprising an electron transport chain and a terminal catalytic component (1, 6, 7, 8, 15, 21). In contrast, almost all of the dihydrodiol dehydrogenase reported are similar with regard to their specificity for *cis*-dihydrodiols and their absolute requirement for NAD^+ as their primary electron acceptor (2, 4, 13, 17). Generally, *cis*-dihydrodiol dehydrogenases are also broad substrate-ranged enzymes, and most dehydrogenases are able to transform several *cis*-dihydrodiol isomers (12, 16, 24). Further catabolism of 4CC involves cleavage of the ring in either an *ortho* (intradiol) or a *meta* (extradiol) manner like the reaction by *xylTE* in Fig. 1 (10, 14).

Pseudomonas sp. S-47 is a bacterial strain that was isolated from contaminated waste by Seo *et al.* (19, 20). The strain is able to utilize 4CBA as a sole source of carbon and energy through the *meta*-cleavage pathway. The strain

* To whom correspondence should be addressed.
(Tel) 82-43-261-2300; (Fax) 82-43-264-9600
(E-mail) environ@trut.chungbuk.ac.kr

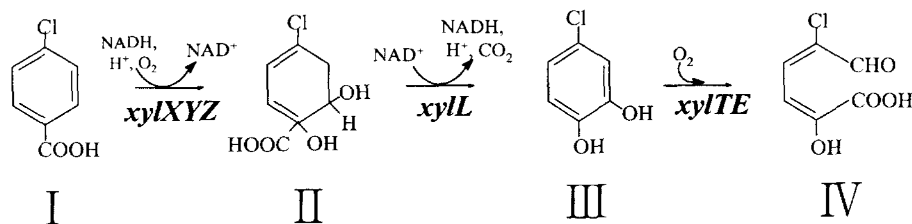

Fig. 1. Degradative pathway of 4-chllorobenzoate by *Pseudomonas* sp. S-47. I, 4-chlorobenzoate; II, 4-chlorobenzoate 1,2-dihydrodiol; III, 4-chlorocatechol; IV, 5-chloro-2-hydroxymuconic semialdehyde; *xylXYZ*, 4CBA 1,2-dioxygenase; *xylL*, 4CBA-dihydrodiol dehydrogenase; *xylT*, plant-type ferredoxin; *xylE*, 4CC 2,3-dioxygenase.

S-47 was reported by Kim *et al.* (9) to transform 4CBA to 4CC by 4CBA 1,2-dioxygenase and 4CBA 1,2-dihydrodiol dehydrogenase encoded by *xylXYZ* and *xylL*, respectively. The 4CC is then degraded via *meta*-cleavage by catechol 2,3-dioxygenase encoded by *xylE*, the nucleotide sequence of which was analyzed previously (14). In this study, the *xylL* gene was cloned from the chromosome of *Pseudomonas* sp. S-47 and the complete nucleotide sequence of the gene was analyzed and compared with those from other strains.

Cloning and nucleotide sequences of xylL

Pseudomonas sp. S-47 was grown at 30°C in MM2 minimal medium [1 μM $FeSO_4 \cdot 7H_2O$, 100 μM $CaCl_2 \cdot 7H_2O$, 1 mM $MgSO_4 \cdot 7H_2O$, 8.5 mM NaCl, 18 mM $(NH_4)SO_4$, 10 mM potassium phosphate buffer (pH 7.0), 1.5% agar] containing 0.5 mM 4CBA. *E. coli* XL1-Blue was used as the host strain for the cloning of the gene encoding 4CBA dihydrodiol dehydrogenase. Plasmid pBluescript SK(+) II was purchased from Stratagene Ltd (La Jolla, CA, USA) for use as the cloning vector. The transformed *E. coli* strains were cultivated in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 1% NaCl) at 37°C. Transformation was accomplished by the calcium chloride method (18). Selection of the transformant was performed on LB medium containing 50 μg/ml ampicillin, 40 μl X-gal, and 4 μl isopropylthio-β-D-galactosidase (IPTG). Plasmid DNA was isolated by the alkali lysis method as described by Sambrook *et al.* (18). DNA cleavage by restriction endonuclease and ligation of DNA fragments by T4 DNA ligase were performed by standard procedures as recommended by the supplier (Posco Co., Seoul, Korea).

The pCS1 and pCSP21 carrying the *xylL* gene were previously cloned from the chromosomal DNA of *Pseudomonas* sp. S-47 (9). In this study, a 3.0 kb fragment of pCSP21 digested with *Cla*I was introduced into the polyclonal region of pBluescript II SK(+) vector to make pRES3. The subclones of pRES301, pRES401, and pRES403 were further constructed by digestion of the pCSP21 with various enzymes. The physical maps of those clones are shown in Fig. 2. Editing and initial analysis of the *xylL* nucleotide sequences were performed using the DNASIS software (Hitachi version 7.0, Japan). Searches for nucleotide and amino acid sequence similarities were done using the FASTA and BLAST programs and the EMBL and GenBank databases.

A nucleotide sequence of *xylL* was found to be composed of 777 bp as shown in Fig. 3. The *xylL* gene of strain S-47 is located between *xylZ* and *xylT*, and encodes a polypeptide chain with a molecular mass of 28 kDa consisting of 258 amino acid residues. The nucleotide sequences of corresponding genes from *Pseudomonas putida* mt-2 (*xylL*) and *Acinetobacter calcoaceticus* (*bedD*) have been reported to consist of 774 and 786 bp nucleotides, respectively (13). Those of *Pseudomonas putida* (*bnzE*), *Pseudomonas putida* F1 (*todD*), *Comamonas testosteroni* B-356 (*bphB*) and *Pseudomonas pseudoalcaligenes* KF707 (*bphB*) consist of more than 800 bp nucleotides (8, 22, 23, 25). The GenBank accession number of *xylL* from *Pseudomonas* sp. S-47 is AF320981.

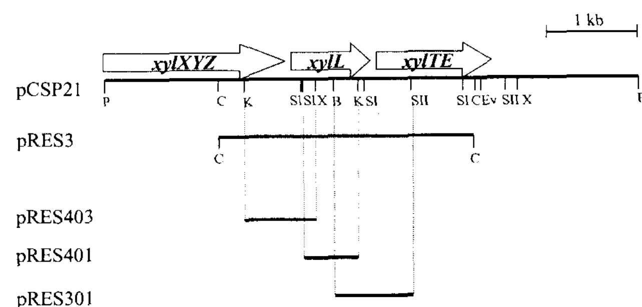

Fig. 2. Physical maps of pCSP21 and its clones carrying *xylL*. Abbreviations: B, *Bam*HI; C, *Cla*I; Ev, *Eco*RV; K, *Kpn*I; P, *Pst*I; SI, *Sal*I; SII, *Sac*II; X, *Xho*I.

Homology of 4CBA dihydrodiol dehydrogenase (4CBA-DD)

The amino acid sequence of the 4CBA-DD deduced from the nucleotide sequence of *xylL* was aligned with six corresponding enzymes reported in other bacterial strains as shown in Fig. 4. Alignments of the amino acid sequences were performed using the ClustalX program. The deduced amino acid sequence of 4CBA-DD produced by *Pseudomonas* sp. S-47 exhibited 97% identity with that of TOL plasmid in *Pseudomonas putida* mt-2 (13), and 60% with that of *Acinetobacter calcoaceticus* (13). However,

```
5' GCCGCCAGCGCCTAGAGGCTCCTTTGGTCCGCTTACTAGGTGAGCGGGCCTGTTTTATTC   60
          xylZ <--┘
   GGGCAGTAGGGCCCAGGTTCTCTACGAACTTTCCCAAATTGCATCCGGGCTACACAACCG  120

   AGGTGGTTCATGAACAAACGTTTCCAGGGCAAGGTTGCCGTTATCACCGGCGCCGCCCAG  180
            └--> xylL                            SalI         SalI
   GGCATCGGTCGCCGCGTGGCCGAACGGATGGCGGCCGAAGGCGGTCGACTGCTGCTGGTC  240

   GACCGTTCCGAGCTAATACATGAGCTGGCCGACGAACTGGTCGGAGTTGCTGAGGTGCTG  300
                                                            XhoI
   ACCCTGACCGCCGACCTTGAGCAGTTCGCCGAGTGCCAACGGGTGATGGCGGCGGCGCTC  360

   GAGCGCTTCGGTCGTCTGGATATTCTGATCAACAACGTTGGCGGCACCATTTGGGCTAAG  420

   CCATTCGAGCATTACCAGGAACGCGAGATCGAGGCCGAAGTGCGGCGTTCACTGTTTCCT  480

   ACCCTATGGTGCTGCCACGCCGCGCTGGCGCCCATGATCGAGCAGGGCAGTGGCGCCATC  540
                                BamHI
   GTAAATGTTTCCTCCGTTGCCACGCGCGGGATCCATCGCGTGCCTTACGGCGCGGCGAAG  600

   GGTGGCGTTAACGCCCTGACCGCCTGTCTGGCCTTTGAAACCGCCGAGCGCGGCATCCGC  660

   GTCAACGCCACCGCACCGGGTGGCACCGAGGCGCCGCCACGGCGGATTCCGCGCAATAGC  720
                                KpnI          SalI
   GCCGAGCCGAGTGAGCAGGAGAAGGTCTGGTACCAGCAGATCGTCGACCAGTCCCTCGAC  780

   AGCAGCCTTATGAAACGCTACGGAAGCATTGACGAGCAGGTCGAGGCAATTCTGTTCCTT  840

   GCTTCTGACGCCGCCTCCTACATCACCGGTATAACTCTTCCGGTGGCAGGGGGAGACCTC  900

   GGCTGACCCTTATGTCTGGTTAAGAAGAGAAATCGACATGCGAAGAAGCAACGTACATAA  960
  xylL --┘
   GACCCCTGAGGCTCATTTTCGGGGTTATGGCGGCATCACCCAGAGCTGTTGGGGGATACT 1020

   TCCGTCATGTTTAGTGTATCTGGGATGAATATGAACAGTGCCGGCTACGAG  3'       1080
                           └--> xylT
```

Fig. 3. Nucleotide sequence of the *xylL* gene encoding 4CBA 1,2-dihydrodiol dehydrogenase (4CBA-DD) from *Pseudomonas* sp. S-47. It is located between the *xylZ* and *xylT* genes.

the homology was less than 30% when compared to the corresponding enzymes of *Pseudomonas putida* (8), *Pseudomonas putida* F1 (5, 25), *Pseudomonas pseudoalcaligenes* KF707 (5, 23), and *Pseudomonas* sp. C18 (3). In all the six dehydrogenases aligned, 63 amino acids are conserved residues and are shown with asterisks and dots in Fig. 4. The [152]YGAAKGGVNAL sequence of 4CBA-DD from strain S-47 which is indicated with a box fits into the consensus pattern, Y(S, T, A, G, or C) (S, T, A, G, or C) (S, T, A, G, or C) KX (A or G) (L, I, V, M, A, or G) XX (L, I, V, M, A, or G), for short-chain alcohol dehydrogenases, as reported by Neidle *et al.* (13) and Werlen *et al.* (24).

In order to reveal the evolutionary relationship between 4CBA-DD of strain S-47 and corresponding dehydrogenases from other strains, a phylogenetic analysis was carried out by aligning the amino acid sequences of the enzymes using the TreeView program. The dendrogram showing the homology among the amino acid sequences of dehydrogenases is shown in Fig. 5. Earlier alignments of the amino acid sequences of bacterial aryl-dihydrodiol dehydrogenases showed the existence of two groups of these enzymes in the report by Kulakov *et al.* (11). Group I dehydrogenase comprises enzymes involved in the degradation of mono-aryls with a carboxyl group, such as benzoate. In this case, the initial reaction of degradation is catalyzed by class I dioxygenase. Group II dehydrogenase includes the dehydrogenases involved in the degradation of compounds without a carboxyl group, for which the initial reaction is catalyzed by class II dioxygenases, such as biphenyl 2,3-dioxygenase or by class III dioxygenases, such as naphthalene 1,2-dioxygenase (3, 5, 23). Therefore, the 4CBA-dihydrodiol dehydrogenase (XylL) of *Pseudomonas* sp. S-47, together with the dehydrogenases of *Pseudomonas putida* mt-2 (XylL) and *Acinetobacter calcoaceticus* (BenD), are classified in group I dehydro-

```
                                                                          60
XylL_S-47     --MNKRFQGKVAVITGAAQGIGRRVAERMAAEGGRLLLVDRSELIHELADELVGV-AEVL
XylL_mt-2     --MNKRFQGKVAVITGAAQGIGRRVAERMAAEGGRLLLVDRSELIHELADELVGV-AEVL
BenD_ACICA    MNSTQRFEHKVVIVTGAAQGIGRGVALRIAQEGGCLILADRSDLIQAVLAEIKALGALAI
BnzE_PSEPU    ----MRLEGEVALVTGGGAGLGRAIVDRYVAEGARVAVLDKSAAGLEALRKLHG--DAIV
TodD_F1       ----MRLEGEVALVTGGGAGLGRAIVDRYVAEGARVAVLDKSAAGLEALRKLHG--DAIV
BphB_KF707    ----MKLKGEAVLITGGASGLGRALVDRFVAEGAKVAVLDKSAERLAELETDHG--DNVL
NahB_C18      ----MGNQ-QVVSITGAGSGIGLELVRSFKSAGYYVSALVRNEEQEALLCKEFK--DALE
                      • •   •**   *•*  •      *  •   •

                                                                         120
XylL_S-47     TLTADLEQFAECQRVMAAALERFGRLDILINNVGGTIWAKPFE-HYQEREIEAEVRRSLF
XylL_mt-2     TLTADLEQFAECQRVMAAALERFGRLDILINNVGGTIWAKPFE-HYQEREIEAEVRRSLF
BenD_ACICA    AVETDLETYAGAELVVSHAIAEYGRIDVLINNVGGAIWMKPFQ-EFSEEEIIQEVHRSLF
BnzE_PSEPU    GVEGDVRSLDSHREAVARCVEAFGKLDCLVGNAG--VWDYLTQLVDIPDDLISEAFEEMF
TodD_F1       GVEGDVRSLDSHREAVARCVEAFGKLDCLVGNAG--VWDYLTQLVDIPDDLISEAFEEMF
BphB_KF707    GIVGDVRSLEDQKQAASRCVARFGKIDTLIPNAG--IWDYSTALVDLPEESLDAAFDEVF
NahB_C18      IVVGDVRDHATNEKLIKQTIDRFGHLDCFIANAG--IWDYMLS-IEEPWEKISSSFDEIF
                •  *•           •  •*••* •• * *  •*             •      •*

                                                                         180
XylL_S-47     PT----LWCCHAALAPMIEQGSGAIVNVSSVA--TRGIHRVPYGAAKGGVNALTACLAFE
XylL_mt-2     PT----LWCCHAALAPMIEQGSGAIVNVSSVA--TRGIHRVPYGAAKGGVNALTACLAFE
BenD_ACICA    PA----LWCCRAVLPEMLKHQQGTIVNVSSIA--TRGIHRIPYSACKGGVNALTASLAFE
BnzE_PSEPU    EVNVKGYILAAKAALPALYQSKGSAIFTVSNAGFYPGGGGVLYTAGKHAVIGLIKQLAHE
TodD_F1       EVNVKGYILAAKAALPALYQSKGSAIFTVSNAGFYPGGGGVLYTAGKHAVIGLIKQLAHE
BphB_KF707    HINVKGYIHAVKACLPALVASRGNVIFTISNAGFYPNGGGPLYTAAKHAIVGLVRELAFE
NahB_C18      DINVKSYFSGISAALPELKKTNGSVVMTASVSSHAVGGGGSGYIASKHAVLGMVKALAYE
                                •     *   •    * •       * * *  •  •   ** *

                                                                         240
XylL_S-47     TAERGIRVNATAPGGTEAPPRRIPRNSAEPSEQEKVWYQQIVDQSLDSSLMKRYGSIDEQ
XylL_mt-2     TAERGIRVNATAPGGTEA-RHGGFRNSAEPSEQEKVWYQQIVDQSLDSSLMKRYGSIDEQ
BenD_ACICA    HAQHGIRVNAVATGGTKAPPRKIPRNAQPLSKSEQVWMQQVVDQTIDRSFLGRYGSIDEQ
BnzE_PSEPU    WGPR-IRVNGIAPGGILG-SDLRGLKSLDLQDKSISTFP-LDDMLKSVLPTGRAATAEEY
TodD_F1       WGPR-IRVNGIAPGGILG-SDLRGLKSLDLQDKSISTFP-LDDMLKSVLPTGRAATAEEY
BphB_KF707    LAPY-VRVNGVGPGGIN--SDMRGPSSLGMGSKAISTVP-LADMLKSVLPIGRMPEVEEY
NahB_C18      LAPE-VRVNAVSPGGTV--TSLCGSASAGFDKMHMKDMPGIDDMIKGLTPLGFAAKPEDV
                  •***     **             •              • *              ••

XylL_S-47     VEAILFLAS-DAASYITGITLPVAGGD---------LG-----------    258
XylL_mt-2     VEAILFLAS-DAASYITGITLPVAGGD---------LG-----------    257
BenD_ACICA    VNAITFLAS-DESSYITGSVLPVGGGD---------QG-----------    261
BnzE_PSEPU    AGAYVFFATRGDTVPLTGSVLNFDGGMGVRGLFEASLGAQLDKHFG---    275
TodD_F1       AGAYVFFATRGDTVPLTGSVLNFDGGMGVRGLFEASLGAQLDKHFG---    275
BphB_KF707    TGAYVFFATRGDAAPATGALLNYDGGLGVRGFFSGAGGNDLLEQLNIHP    277
NahB_C18      VAPYLLLASRKQGKFITGTVISIDGGM--------ALGRK---------    259
                  •••*     **  •  **         *
```

Fig. 4. Alignments of amino acid sequences of XylL dehydrogenase from *Pseudomonas* sp. S-47 and those from corresponding strains. The conserved amino acid residues identical in all enzymes are indicated by asterisks. Multiple alignments were carried out by using the Clustal X software. The GenBank accession numbers of the genes are shown in parenthesis. S-47, *Pseudomonas* sp. S-47 (AF320981); mt-2, *Pseudomonas putida* mt-2 (S23485); ACICA, *Acinetobacter calcoaceticus* (P07772); PSEPU, *Pseudomonas putida* (P08088); F1, *Pseudomonas putida* F1(P13859); KF707, *Pseudomonas pseudoalcaligenes* KF707 (F42409); C18, *Pseudomonas* sp. C18 (Q52459). The nucleotide sequences in the box indicate the consensus pattern for short-chain alcohol dehydrogenases.

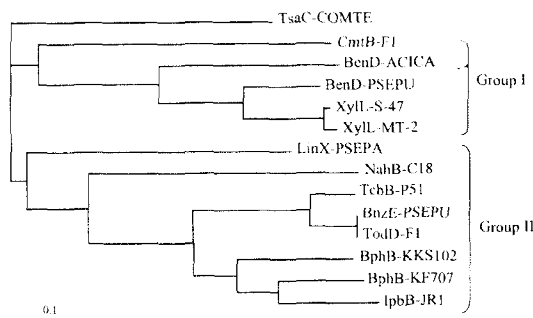

Fig. 5. Dendrogram showing the level of homology among the amino acid sequences of XylL dehydrogenases. The GenBank accession numbers of the genes are shown in parentheses: COMTE, *Comamonas testosteroni* (U32622.1); F1-CmtB, *Pseudomonas putida* F1 (U24215.1); ACICA, *Acinetobacter calcoaceticus* (P07772); PSEPU-BenD, *Pseudomonas putida* (AF218267.1); S-47, *Pseudomonas* sp. S-47 (AF320981); mt-2, *Pseudomonas putida* mt-2 (S23485); PSEPA, *Sphingomonas paucimobilis* (P50198); C18, *Pseudomonas* sp. C18 (Q52459); P51, *Pseudomonas* sp. strain P51 (U15298.1); PSEPU-BnzE, *Pseudomonas putida* (P08088); F1-TodD, *Pseudomonas putida* F1 (P13859); KKS102, *Pseudomonas* sp. KKS102 (P50206); KF707, *Pseudomonas pseudoalcaligenes* KF707 (F42409); JR1, *Pseudomonas* sp. JR1 (U53507.1).

genase on the basis of amino acid sequence of the enzyme.

Acknowledgment

This work was supported by a grant (1999-2-202-004-3) from the Korea Science and Engineering Foundation.

References

1. Axcell, B.C., and P.J. Geary. 1975. Purification and some properties of a soluble benzene-oxidizing system from a strain of *Pseudomonas*. *Biochem. J.* 146, 173-183.
2. Barriault, D., M. Vedadi, J. Powlowski, and M. Sylvestre. 1999. *Cis*-2,3-dihydro-2,3-dihydroxybiphenyl dehydrogenase and *cis*-1, 2-dihydro-1,2-dihydroxynaphthalene dehydrogenase catalyze dehydrogenation of the same range of substrates. *Biochem. Biophys. Res. Comm. 260*, 181-187.
3. Denome, S.A., D.C. Stanley, E.S. Olson, and K.D. Young. 1993. Metabolism of dibenzothiophene and naphthalene in *Pseudomonas* strains: complete DNA sequence of an upper naphthalene catabolic pathway. *J. Bacteriol.* 175, 6890-6901.
4. Fong, K.P.Y., C.B.H. Goh, and H-M. Tan. 1996. Characterization and expression of the plasmid-borne *benD* gene from *Pseudomonas putida* ML2, which codes for a NAD$^+$-dependent *cis*-benzene dihydrodiol dehydrogenase. *J. Bacteriol.* 178, 5592-5601.
5. Furukawa, K., J. Hirose, A. Suyama, T. Zaiki, and S. Hayashida. 1993. Gene components responsible for discrete substrate specificity in the metabolism of biphenyl (*bph* operon) and toluene (*tod* operon). *J. Bacteriol.* 175, 5224-5232.
6. Harayama, S., M. Rekik, A. Bairoch, E.L. Neidle, and N. Ornston. 1991. Potential DNA slippage structures acquired during evolutionary divergence of *Acinetobacter calcoaceticus* chromosomal *benABC* and *Pseudomonas putida* TOL pWW0 plasmid *xylXYZ*, genes encoding benzoate dioxygenase. *J. Bacteriol.* 173, 7540-7548.
7. Irie, S., S. Doi, T. Yorifuji, M. Takagi, and K. Yano. 1987. Nucleotide sequencing and characterization of the genes encoding benzene oxidation enzymes of *Pseudomonas putida*. *J. Bacteriol.* 169, 5174-5179.
8. Karegoudar, T.B., and C.K. Kim. 2000. Microbial degradation of monohydroxybenzoic acid. *J. Microbiol.* 38, 53-61.
9. Kim, K.P., D.I. Seo, D.H. Lee, Y. Kim, and C.K. Kim. 1998. Cloning and expression in *E. coli* of the genes responsible for degradation of 4-chlorobenzoate and 4-chlorocatechol from *Pseudomonas* sp. S-47. *J. Microbiol.* 36, 99-105.
10. Kim, K.P., D.I. Seo, K.H. Min, J.O. Ka, Y.K. Park, and C.K. Kim. 1997. Characteristics of catechol 2,3-dioxygenase produced by 4-chlorobenzoate by *Pseudomonas* sp. S-47. *J. Microbiol.* 35, 295-299.
11. Kulakov, L.A., C.C.R. Allen, D.A. Lipscomb, and M.J. Larkin. 2000. Cloning and characterization of a novel *cis*-naphthalene dihydrodiol dehydrogenase gene (*narB*) from *Rhodococcus* sp. NCIMB12038. *FEMS Microbiol. Lett.* 182, 327-331.
12. Morawski, B., G. Casy, C. Illaszewicz, H. Griengl, and D.W. Ribbons. 1997. Stereochemical course of two arene-*cis*-diol dehydrogenases specifically induced in *Pseudomonas putida*. *J. Bacteriol.* 179, 4023-4029.
13. Neidle, E., C. Hartnett, L.N. Ornston, A. Bairoch, M. Rekik, and S. Harayama. 1992. *Cis*-diol dehydrogenase encoded by the TOL pWW0 plasmid *xylL* gene and the *Acinetobacter calcoaceticus* chromosomal *benD* gene are members of the short-chain alcohol dehydrogenase superfamily. *Eur. J. Biochem.* 204, 113-120.
14. Noh, S-J., Y. Kim, K-H. Min, T.B. Karegoudar, and C-K. Kim. 2000. Cloning and nucleotide sequence analysis of *xylE* gene responsible for *meta*-cleavage of 4-chlorocatechol from *Pseudomonas* sp. S-47. *Mol. Cells* 10, 475-479.
15. Pflugmacher, U., B. Averhoff, and G. Gottschalk. 1996. Cloning, sequencing, and expression of isopropylbenzene degradation genes from *Pseudomonas* sp. strain JR1: Identification of isopropylbenzene dioxygenase that mediates trichloroethene oxidation. *Appl. Environ. Microbiol.* 62, 3967-3977.
16. Raschke, H., T. Fleischmann, J.R. van der Meer, and H-P. E. Kohler. 1999. *Cis*-chlorobenzene dihydrodiol dehydrogenase (TcbB) from *Pseudomonas* sp. strain P51, expressed in *Escherichia coli* DH5α (pTCB149), catalyzes enantioselective dehydrogenase reactions. *Appl. Environ. Microbiol.* 65, 5242-5246.
17. Rogers, J.E., and D.T. Gibson. 1977. Purification and properties of *cis*-toluene dihydrodiol dehydrogenase from *Pseudomonas putida*. *J. Bacteriol.* 130, 1117-1124.
18. Sambrook, J., E.F. Fritisch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. 2nd ed., Cold Spring Harbor, New York.
19. Seo, D.I., J.C. Chae, K.P. Kim, Y.S. Kim, G.S. Lee, and C.K. Kim. 1998. A pathway for 4-chlorobenzoate degradation by *Pseudomonas* sp. S-47. *J. Microbiol. Biotech.* 8, 96-100.
20. Seo, D.I., J.Y. Lim, Y.C. Kim, K.H. Min, and C.K. Kim. 1997. Isolation of *Pseudomonas* sp. S-47 and its degradation of 4-chlorobenzoic acid. *J. Microbiol.* 35, 188-192.
21. Subramanian, V., T.N. Liu, W.K. Yeh, and D.T. Gibson. 1979. Toluene dioxygenase: purification of an ion-sulfur protein by affinity chromatography. *Biochem. Biophys. Res. Commun.*

204, 912-917.

22. Sylvestre, Y., Y. Hurtubise, D. Barriault, J. Bergeron, and D. Ahmad. 1996. Characterization of active recombinant 2,3-dihydro-2,3-dihydroxybiphenyl dehydrogenase from *Comamonas testosteroni* B-356 and sequence of the encoding gene (*bphB*). *Appl. Environ. Microbiol.* 62, 2710-2715.
23. Taira, K., J. Hirose, S. Hayashida, and K. Furukawa. 1992. Analysis of *bph* operon from the polychlorinated biphenyl-degrading strain of *Pseudomonas pseudoalcaligenes* KF707. *J. Biol. Chem.* 267, 4844-4853.
24. Werlen, C., H-P. E. Kohler, and J.R. van der Meer. 1996. The broad substrate chlorobenzoate dioxygenase and *cis*-chlorobenzene dihydrodiol dehydrogenase of *Pseudomonas* sp. strain P51 are linked evolutionarily to the enzymes for benzene and toluene degradation. *J. Biol. Chem.* 271, 4009-4016.
25. Zylstra, G.J., and D.T. Gibson. 1989. Toluene degradation by *Pseudomonas putida* F1. Nucleotide sequence of the *todC1C2BADE* genes and their expression in *Escherichia coli*. *J. Biol. Chem.* 264, 14940-14946.