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# DNA Sequence of the *phnN* Gene for Benzaldehyde Dehydrogenase from *Pseudomonas* sp. DJ77 and Its Substrate Preference

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**Benzaldehyde dehydrogenase (BZDH), an enzyne involved in the degradation of toluene and xylenes, is encoded by the *phnN* gene of *Pseudomonas* sp. strain DJ77. We determined the nucleotide sequence of a DNA fragment of 1,803 base pairs which included the *phnN* gene. The fragment contained an open reading frame of 1,506 base pairs to accommodate the 55 kDa sized enzyme encoding BZDH. The enzyme efficiently oxidized benzaldehyde, salicylaldehyde, *m*-tolualdehyde and *ps*-tolualdehyde.**

**Key words: Benzaldehyde dehydrogenase, *phnN*, DJ77, DNA sequenee, substrate preference**

In the upper cleavage pathway, toluene or xylenes are transformed into benzoate or toluates by the successive action of xylene monooxygenase, benzyl alcohol dehydrogenase and benzaldehyde dehydrogenase (20). Substituted benzoates or benzoate formed by benzaldehyde dehydrogenase (BZDH, EC 1.2.1.28), the last enzyme of this pathway, are then further metabolized to substrates of the Krebs cycle through the *meta* cleavage pathway (Fig. 1).

*Pseudomonas* sp. strain DJ77 was originally isolated as a biphenyl- and phenanthrene-degrading strain from the contaminated sediment of an industrial complex near Taejon (8,14), Korea. Since this organism is capable of degrading a broad range of aromatic hydrocarbons, such as toluene, *m*-xylene, phenol, salicylate, benzoate and *p*-cresol, molecular analyses, metabolic pathway and enzyme studies on the degradation of these hydnscarbons have been made (6, 11, 12, 13, 19). From these studies, we have localized the structural genes for the *meta* cleavage enzymes to a region approximabely 8 kb in the order *phnDEGHIJKLM* (9). The *phnN* gene encoding BZDH, an upper pathway enzyme, was discovered downstream from the *phnM* gene (10). In this report we present the sequence of the *phnN* gene and the substrate preferences of the *phnN* gene product.

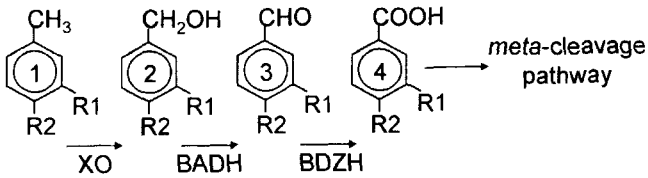


**Fig. 1.** Upper pathway for degradation of toluene and xylenes to benzoate and toluates showing pathway intermediates and enzymes. Enzyme abbreviations: XO, xylene monooxygenase, BADH, benzyl alcohol dehydrogenase; BZDH, benzaldehyde dehydrogenase. Compounds: for R1=H, R2=H, (1) toluene, (2) benzyl alcohol, (3) benzaldehyde, (4) benzoate; for R1=$CH_3$, R2=H, (1) *m*-xylene, (2) *m*-methylbenzyl alcohol, (3) *m*-tolualdehyde (*m*-methylbenzaldehyde), (4) *m*-toluate; for R1=H, R2=$CH_3$, (1) *p*-xylene, (2) *p*-methylbenzyl alcohol, (3) *p*-tolualdehyde (*p*-methylbenzaldehyde), (4) *p*-toluate.

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## Materials and Methods

### Bacterium, plasmids and DNA manipulation

*Escherichia coli* XL1-Blue was used as the host bacterium. Various pBluescript SK II (+)-based plasmids containing the whole or partial sequence of *phnN* were constructed for sequencing and enzyme assay. All techniques for DNA manipulation were carried out as described by Sambrook *et al.* (17).

### Chemicals

Coenzymes were purchased from Sigma (St. Louis, Mo. USA). Other chemicals were purchased from Aldrich (St. Louis, Mo. USA) and were of the highest purity commercially available.

### Sequence determination and analysis

DNA fragments were purified by standard proce-

dures using Qiagen Plasmid Kit (Qiagen Co. Germany). DNA sequencing with double-stranded DNA was performed with an Applied Biosystems 373A automated fluorescent sequencer and the Taq DyeDeoxy terminator cycle system. Searches for specific nucleotide or amino acid sequences from the GenBank database were carried out using the BLAST program (2). Sequences were retrieved from GenBank and compared with sequences obtained in this study.

**Enzyme assays**

Cells harvested after culturing for 48 h at 30°C in LB medium were washed and resuspended in of 50 mM of $K^+$-$Na^+$ phosphate buffer (pH 7.0). Crude extract of *E.coli* XL1-Blue containing pYCS505 was prepared by sonication. The assay for BZDH was done as described previously with slight modification (3). BZDH activity was assayed at 25°C in a total volume of 1 ml of 50 mM $K^+$-$Na^+$ phosphate buffer (pH 7.0), containing 100 nmol aromatic alde-

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CTAGTCATCGAAGTTCTATGAAAACCAAGGGAGAGTTGTCATGGCTACGCAGTTGAAAAGTACAGAAAACGACTACGGCA   80
                                    phnN: M  A  T  Q  L  K  S  T  E  N  D  Y  G
TTAAGTCCGAGTACGGCCATTATATCGGCGGCGAGTGGATTGCCGGGGACAGCGGCAAGACCATCGATCTACTCAATCCC  160
 I  K  S  E  Y  G  H  Y  I  G  G  E  W  I  A  G  D  S  G  K  T  I  D  L  L  N  P
TCGACCGGCAAGGTGCTGACCAAGATCCAGGCCGGCAATGCCAAGGACATTGAGCGTGCGATTGCCGCTGCCAAGGCTGC  240
 S  T  G  K  V  L  T  K  I  Q  A  G  N  A  K  D  I  E  R  A  I  A  A  A  K  A  A
TTTTCCCAAGTGGTCGCAAAGCCTGCCCGGCGAGCGCCAGGAGATCCTGATCGAAATTGCGCGCCGGCTGAAGGCACGCC  320
  F  P  K  W  S  Q  S  L  P  G  E  R  Q  E  I  L  I  E  I  A  R  R  L  K  A  R
AGTCGCACTACGCGACGCTCGAAACGTTGAACAACGGCAAGCCGATGCGCGAATCGATGTTCTTCGACATGACCCAGACT  400
 Q  S  H  Y  A  T  L  E  T  L  N  N  G  K  P  M  R  E  S  M  F  F  D  M  T  Q  T
ATCGGTCAGTTCGAGCTGTTCGCTGGCGCGGCCTATGGCCTGCATGGCCAGACGCTCGATTATCCCGATGCGATCGGCAT  480
 I  G  Q  F  E  L  F  A  G  A  A  Y  G  L  H  G  Q  T  L  D  Y  P  D  A  I  G  I
CGTTCACCGCGAACCGCTCGGTGTCTGCGCGCAGATCATCCCGTGGAACGTACCGATGTTGATGATGGCGTGCAAAATCG  560
  V  H  R  E  P  L  G  V  C  A  Q  I  I  P  W  N  V  P  M  L  M  M  A  C  K  I
CACCAGCGCTGGCATCGGGCAACACTGTCGTTCTGAAGCCGGCCGAGACGGTCTGTCTTTCGGTGATCGAATTCTTCGTG  620
 A  P  A  L  A  S  G  N  T  V  V  L  K  P  A  E  T  V  C  L  S  V  I  E  F  F  V
GAAATGGCCGATCTGCTGCCGCCGGGTGTGATCAACGTCGTCACCGGCTATGGTGCGGATGTGGGCGAGGCGCTGGTCAC  700
 E  M  A  D  L  L  P  P  G  V  I  N  V  V  T  G  Y  G  A  D  V  G  E  A  L  V  T
CAGCCCCGATGTCGCCAAGGTAGCATTCACCGGTTCGATCGCCACTGCCCGCCGTATTATCCAGTATGCCTCCGCAAACA  780
  S  P  D  V  A  K  V  A  F  T  G  S  I  A  T  A  R  R  I  I  Q  Y  A  S  A  N
TCATCCCGCAGACGCTCGAGCTGGGCGGCAAGTCGGCGCACATCGTGTGCGGCGATGCCGATATCGACGCGGCGGTGGAA  860
 I  I  P  Q  T  L  E  L  G  G  K  S  A  H  I  V  C  G  D  A  D  I  D  A  A  V  E
AGCGCTACGATGTCGACTGTGCTCAACAAGGGTGAGGTCTGCCTGGCCGGTTCGCGCCTGTTCCTGCACCAGTCGATCCA  940
 S  A  T  M  S  T  V  L  N  K  G  E  V  C  L  A  G  S  R  L  F  L  H  Q  S  I  Q
GGACGAGTTCCTGGCCAAGTTCAAAACCGCGCTTGAAGGCATCCGCCAGGGCGATCCCCTCGATATGGCAACCCAGCTTG 1020
  D  E  F  L  A  K  F  K  T  A  L  E  G  I  R  Q  G  D  P  L  D  M  A  T  Q  L
GCGCCCAGGCGTCGAAGATGCAGTTCGACAAGGTAGAAAGCTACCTGCAGCTCGCAACCGAGGAAGGGGCCGAAGTTTTG 1100
 G  A  Q  A  S  K  M  Q  F  D  K  V  E  S  Y  L  Q  L  A  T  E  E  G  A  E  V  L
ACCGGCGGCACCCGCGCCGATGCTGCAAATCTGGCGGGTGGCAACTTCATCAAGCCGACGGTGTTCACCAATGTGAAGAA 1180
 T  G  G  T  R  A  D  A  A  N  L  A  G  G  N  F  I  K  P  T  V  F  T  N  V  K  N
CACAATGCGCATCGCGCAGGAGGAGATCTTCCGGCCGGTGACCACTGTAATCAGCTGGAACGACCAAACCCACATGATGC 1260
  T  M  R  I  A  Q  E  E  I  F  R  P  V  T  T  V  I  S  W  N  D  Q  T  H  M  M
AGCAGGCCAATGCCACCACCTACGGCCTGGCCGGCGGCGTGTGGACCAAGGATCTTGCCCGCGCCCACCGGATCGCGCGT 1340
 Q  Q  A  N  A  T  T  Y  G  L  A  G  G  V  W  T  K  D  L  A  R  A  H  R  I  A  R
CAGCTTGAAACCGGCACGGTCTGGATCAACCGCTACTACAACCTTAAGGCCAACATGCCGCTGGGCGGTTACAAACAGAA 1420
 Q  L  E  T  G  T  V  W  I  N  R  Y  Y  N  L  K  A  N  M  P  L  G  G  Y  K  Q  N
CGGCTTCGGGCGTGAATTCAGCCATGAAGTGCTGAACCACTACACCCAGACAAAGTCGGTCGTGGTCAACCTTCAGGAAG 1500
  G  F  G  R  E  F  S  H  E  V  L  N  H  Y  T  Q  T  K  S  V  V  V  N  L  Q  E
GCCGCGTCGGGATGTTCGATCAGTGATCGAGAACCCGCGATCTTCGGGAGGAACGATGTGACTGCAAGGCTCGAAGGACA 1580
 G  R  V  G  M  F  D  Q  *
AGTGGCGCTGCTAACCGGCGGCGCCACAGGGATCGGTGAGGCGGTCGTCGCGCGCTTTATCGAAGAAGGTGCCAAGGTTG 1660
GCGTCCTGGTCAGGGATGCCGCTCAGGCCGACTTGGTGCGTACGCGGCATGGCAACGCAGTAGTCGTGATTGAGGGCGAT 1740
GTCCGCAATTATGTCGACAACGAAAAGGCAGTCGTCGCAACCGTGGCTGCATTCGGCAAGCTT                   1803
                                                                    HindIII
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**Fig. 2.** Nucleotide sequence of the *phnN* gene. The amino acid sequences are also represented in one letter codons beneath the corresponding codons and the stop codon is marked with an asterisk. The putative ribosome binding site is underlined.

hyde, 28.5 nmol $NAD^+$ and 0.1 mg of crude extract protein by measuring absorbance at 340 nm ($A_{340}$) to monitor the formation of NADH. Protein concentration was deter- mined by the Lowry method (16).

### Nucleotide sequence accession number

The nucleotide sequence reported in this paper has been submitted to GenBank under accession number AF073359.

## Results and Discussion

The *phnN* gene, the structural gene for BZDH, has previously been localized by subcloning and partial sequencing (10). In this study, the sequence of the *phnN* gene was determined from plasmid pYCS505, subcloned from pYCS500 (6).

An open reading frame was identified with a potential binding site for 16s rRNA (Fig. 2). The molecular mass of the PhnN protein deduced from the nucle-

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PhnN_DJ77          MATQLKSTENDYGIKSEYGHYIGGEWIAGDSGKTIDLLNPSTGKVLTKIQAGNAKD  56
XylC_F199          MATQLRSAENEYGIKSEYGHYIGGEWIAGDSGKTIDLLNPSTGKVLTKIQAGNAKD  56
XylC_pWW0                MRETKEQPIWYGKVFSSNWVEARGG-VANVVDPSNGDILGITGVANGED  48
PhnG_DJ77                      MTILNFIDGSYREGSEGKSFSNVNPATGAEIGVVHEASQAE  41
HumanADH           MSSSGTPDLPVLLTDLKIQYTKIFINNEWHDSVSGKKFPVFNPATEEELCQVEEGDKED  59
*      *

PhnN_DJ77        IERAIAAAKAAFPK---WSQSLPGERQEILIEIARRLKARQSHYATLETLNNGKPMRESM 113
XylC_F199        IERAIAAAKAAFPK---WSQSLPGERQEILIEVARRLKARHSHYATLETLNNGKPMRESM 113
XylC_pWW0        VDAAVNAAKRAQKE---WAAIPFSERAAIVRKAAEKLKEREYEFADWNVRECG-AIRPKG 104
PhnG_DJ77        VEDAVEAAKAALTG--PWGKMTTARTGQADHRVATEIERRADDFLAAEVADTVKPRHVVS  99
HumanADH         VDKAVKAARQAFQIGSPWRTMDASERGRLLYKLADLIERDRLLLATMESMNGGKLYSNAY 119
*  **  *      *            *

PhnN_DJ77        FFDMTQTIGQFELFAGAAYGLHGQTLDYP-----DAIGIVHREPLGVCAQIIPWNVPMLM 168
XylC_F199        YFDMPQTIGQFELFAGAAYGLHGQTLDYP-----DAIGIVHREPLGVCAQIIPWNVPMLM 168
XylC_pWW0        LWEAGIAYEQMHQAAGLASLPNGTLFPSAVP---GRMNLCQRVPVGVVGVIAPWNFPLFL 161
PhnG_DJ77        HIDIPRGAANFRMFADVVSTMPGESFNTPTPDGGQAFIYTVSKPKGVDAAVCPRNFPLLL 159
HumanADH         LNDLAGCIKTLRYCAGWADKIQGRTIPIDG----NFFTYTRHEPIGVCGQIIPWNFPLVM 175
*       *                * **     * * *

PhnN_DJ77        MACKIAPALASGNTVVLKPAETVCLS-VIEFFVEMADL-LPPGVINVVTGYG-ADVGEAL 225
XylC_F199        MACKIAPALASGNTVVLKPAETVCLS-VIEFFVEMADL-LPPGVINVVTGYG-ADVGEAL 225
XylC_pWW0        AMRSVAPALALGNAVILKPDLQTAVTGGALIAEIFSDAGMPDGVLHVLPGGA--DVGESM 219
PhnG_DJ77        MIWKVGPELACGNTAVVKPSEEIART-AALLGDVIDAGVNHHGVFNVAQRFGPASAGEFL 218
HumanADH         LIWKIGPALSCGNTVVVKPAEQTPLT-ALHVASLIKEAGFPPGVVNIVPGYG-PTAGAAI 233
* *  **    **                    **          *

PhnN_DJ77        VTSPDVAKVAFTGSIATARRIIQYAS-ANIIPQTLELGGKSAHIVCGDADIDAAVESATM 284
XylC_F199        VTSPDVAKVAFTGSIATARRIIQYAS-ANIIPQTLELGGKSAHIVCGDADIDAAVESATM 284
XylC_pWW0        VANSGINMISFTGSTQVGRLIGEKCG-RMLKKVALELGGNNVHIVLPDADLEGAVSCAAW 278
PhnG_DJ77        TSNPDVDAITFTGETGTGQAIMQKAA-TGVRDISFELGGKNPAIVFADADLDKAVEGLSR 277
HumanADH         SSHMDIDKVAFTGSTEVGKLIKEAAGKSNLKRVTLELGGKSPCIVLADADLDNAVEFAHH 293
                        ***       *                ****    **  ***   **

PhnN_DJ77        STVLNKGEVCLAGSRLFLHQSIQDEFLAKFKTALEGIRQGDPLDMATQLGAQASKMQFDK 344
XylC_F199        STVLNKGEVCLAGSRLFLHQSIQDEFLAKFKTALEGIRQGDPLDMATQLGAQASKMQFDK 344
XylC_pWW0        GTFLHQGQVCMAAGRHLVHRDVAQQYAEKLALRAKNLVVGDPNSDQVHLGPLINEKQVVR 338
PhnG_DJ77        SVFLNTGQVCLGTERVYVERPIFDAFVARMAAAAQDFKPGVTGDRAY-LGPLISAEHREK 336
HumanADH         GVFYHQGQCCIAASRIFVEESIYDEFVRRSVERAKKYILGNPLTPGVTQGPQIDKEQYDK 353
                       * *    *                          *         *

PhnN_DJ77        VESYLQLATEEGAEVLTGGTRADAANLA-GGNFIKPTVFTNVKNTMRIAQEEIFRPVTTV 403
XylC_F199        VQSYLRLATEEGAEVLTGGSRSDAADLA-DGNFIKPTVFTNVNNSMRIAQEEIFGPVTSV 403
XylC_pWW0        VHALVESAQRAGAQVLAGG----TYQD--R--YYQATVIMDVKPEMEVFKSEIFGPVAPI 390
PhnG_DJ77        VLAYYPRAVEDGPTVFTGGGVPEISGAETGGFFVEPTLWIDVAHGDTVMREEIFGPCCDI 396
HumanADH         ILDLIESGKKEGAKLECGG--GPWGN---KGYFVQPTVFSNVTDEMRIAKEEIFGPVQQI 408
*     **              *    *         *** *

PhnN_DJ77        ISWNDQTHMMQQANATTYGLAGGVWTKDLARAHRIARQLETGTVWIN-RYYNLKANMPLG 462
XylC_F199        ITWSDEDDMMKQANNTTYGLAGGVWTKDIARAHRIARKLETGTVWIN-RYYNLKANMPLG 462
XylC_pWW0        TVFDSIEEAIELANCSEYGLAASIHTRALATGLDIAKRLNTGMVHINDQPINCEPHVPFG 450
PhnG_DJ77        LPFDSEDEVIALANDTVYGLCASVWTENLSRGHRVAAAMEVGVCWVN-SWFLRDLRTAFG 455
HumanADH         MKFKSLDDVIKRANNTFYGLSAGVFTKDIDKAITISSALQAGTVWVN-CYGVVSAQCPFG 467
**   ***     *            *   *          *

PhnN_DJ77        GYKQNGFG-REFSHEVLNHYTQTKSVVVNLQEGRVGMFDQ 501
XylC_F199        GYKQSGFG-REFSHEVLNHYTQTKSVVVNLQEGRTGMFDQ 501
XylC_pWW0        GMGASGSGGRFGGPASIEEFTQSQWISMVEKPANYPF--- 487
PhnG_DJ77        GSGHSGIG-REGGVHSLEFYTEITNICVKL---------- 484
HumanADH         GFKMSGNG-RELGEYGFHEYTEVKTVTVKISQKNS----- 501
*    * * *          *
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**Fig. 3.** Amino acid sequence comparison of PhnN with other dehydrogenases from various sources. Amino acids identical in all proteins are indicated by asterisks. Boxes indicate the location of conserved glutamate and cysteine residues believed to be important for the catalytic activity of aldehyde dehydrogenase.

otide sequence was 54,400 Da, which corresponds to the result of SDS-PAGE (data not shown). The homology search between the *phnN* sequence and other sequences in GenBank indicated that the PhnN protein is closely related to BZDH from *Sphingomonas aromaticivorans* F199 (XylC, 94%) and salicylaldehyde dehydrogenase from *Sphingomonas* sp. BN6 (NsaF, 89%). This enzyme also displayed homology to BZDH (XylC) from TOL plasmid pWW0 of *Pseudomonas putida*, the mammalian cytosolic aldehyde dehydrogenase and the 2-hydroxymuconic semialdehyde dehydrogenase (PhnG) with 31%, 37% and 32% sequence identity, respectively.

From the amino acid sequence of BZDH, it has been deduced that the PhnN enzyme is a member of the aldehyde dehydrogenase family. The highly conserved residues of the aldehyde dehydrogenase family, Cys-302 and Glu-268 (the number system refers to the human cytosolic aldehyde dehydrogenase), which have been implicated as essential residues for $NAD^+$ binding and a charge relay network (1,4,5,7) were also conserved in the PhnN sequence (Fig. 3).

Enzyme assay was performed using subclone pYCS505 containing the *phnN* gone. The activity of PhnN for the oxidation of benzaldehyde and its methyl, nitro and chloro substituents were determined. The highest value of substrate preference of BZDH was for salicylaldehyde, although that of *p*-tolualdehyde was almost as high. Benzaldehyde and *m*-tolualdehyde were good substrates for BZDH (Fig. 4). The PhnN enzyme displayed relatively low activity toward *o*-tolualdehyde, chlorobenzaldehyde and did not display any activity toward nitrobenzaldehyde.

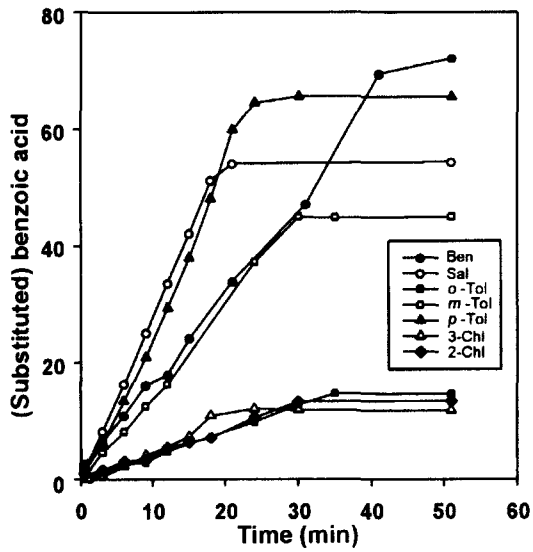


**Fig. 4.** Conversion of aromatic aldehyde to substituted benzoic acid by cell extracts of *E.coli* XL1-Blue (pYCS505) at 25°C. Ben, benzoic acid; Sal, salicylic acid; *o*-Tol, *o*-toluic acid; *m*-Tol, *m*-toluic acid; *p*-Tol, *p*-toluic acid; 2-Chl, 2-chlorobenzoic acid; 3-Chl, 3-chlorobenzoic acid.

In this study we determined the whole sequence of the *phnN* gene and assayed the substrate preference of its gene product. The substrate preference profile of strain DJ77 is different from those of other previously characterized BZDHs. BZDH from TOL plasmid pWW0 (18) had the highest specificity for 3- or 4-nitrobenzaldehyde, which is not the case for the PhnN enzyme. BZDH from DJ77 had a relatively higher substrate preference for *p*-tolualdehyde rather than *m*-tolualdehyde, whereas the opposite is true for BZDH I from *A. calcoaceticus* NCIB 8250 and pWW0-encoded BZDH (15). This result is contradictary to the property of DJ77 being capable of growing more efficiently on *m*-xylene than on *p*-xylene. It suggests that the specificity of a pathway does not necessarily correspond to the preferences of the component enzymes.

## Acknowledgment

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## References

1. **Abriola, D.P., R. Fields, S. Stein, A.D. MacKerell, Jr., and R. Pietruszko.** 1987. Active site of human liver aldehyde dehydrogenase. *Biochemistry* **26**, 5679-5684.
2. **Altschul, S.R., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman.** 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410.
3. **Eaton, R.W. and P.J. Chapman.** 1992. Bacterial metabolism of naphthalene: construction and use of recombinant bacteria to study ring cleavage of 1,2-dihydroxynaphthalene and subsequent reactions. *J. Bacteriol.* **174**, 7542-7554.
4. **Hempel, J., H. Nicholas, and R. Lindahl.** 1993. Aldehyde dehydrogenases: widespread structural and functional diversity within a shared framework. *Protein Sci.* **2**, 1890-1900.
5. **Horn, J.M., S. Harayama, and K.N. Timmis.** 1991. DNA sequence determination of the TOL plasmid (pWW0) *xylGFJ* genes of *Pseudomonas putida*-implications for the evolution of aromatic catabolism.*Mol. Microbiol.* **5**, 2459-2474.
6. **Hwang, S., S.-J. Kim, C.K. Kim, Y. Kim, and Y.C. Kim.** 1999. The *phnIJ* genes encoding acetaldehyde dehydrogenase (acylating) and 4-hydroxy-2-oxovalerate aldolase in *Pseudomonas* sp. DJ77 and their evolutionary implications. *Biochem. Biophys. Res. Commun.* **256**, 469-473.
7. **Inoue, J., J.P. Shaw, M. Rekik, and S. Harayama.** 1995. Overlapping substrate specificities of benzaldehyde dehydrogenase (the *xylC* gene product) and 2-hydroxymuconic semialdehyde dehydrogenase (the *xylG* gene product) encoded by TOL plasmid pWW0 of *Pseudomonas putida*. *J. Bacteriol.* **177**, 1196-1201.
8. **Kim, C.K., J.W. Kim, Y.C. Kim, and T.I. Mheen.** 1986.

Isolation of aromatic hydrocarbon-degrading bacteria and genetic characterization of their plasmid genes. *Kor. J. Microbiol.* **24,** 67-72.

9. **Kim, S-J.** 1999. Ph.D. Thesis. Chungbuk National University, Cheongju, Republic of Korea.
10. **Kim, S.-J. and Y. C. Kim.** 1998. Genetic structure of the *phnM* gene encoding Plant-type Ferredoxin from *Pseudomonas* sp. strain DJ77. *Kor. J. Microbiol.* **34,** 115-119.
11. **Kim, S., O.K. Kweon, Y. Kim, C.-K. Kim, K.-S. Lee, and Y. C. Kim.** 1997. Localization and sequence analysis of the *phnH* gene encoding 2-hydroxypent-2,4-dienoate hydratase in *Pseudomonas* sp. strain DJ77. *Biochem. Biophys. Res. Commun.* **238,** 56-60.
12. **Kim, S., H.-J. Shin, Y.S. Kim, S.J. Kim, and Y.C. Kim.** 1997. Nucleotide sequence of the *Pseudomonas* sp. DJ77 *phnG* gene encoding 2-hydroxymuconic semialdehyde dehydrogenase. *Biochem. Biophys. Res. Commun.* **240,** 41-45.
13. **Kim, S.-J., H.-J. Shin, Y.-C. Park, Y. Kim, K.-H. Min, and Y.C. Kim.** 1999. The 2,3-dihydroxybiphenyl 1,2-dioxygenase gene (*phnQ*) of *Pseudomonas* sp. DJ77: nucleotide sequence, enzyme assay and comparison with isofunctional dioxygenases. *J. Biochem. Mol. Biol.* **32,** 399-404.
14. **Kim, Y.C., K.S. Youn, M.S. Shin, H.S. Kim, M.S. Park, and H.J. Park.** 1992. *Pseudomonas* sp. DJ77 and its expression in *Escherichia coli*. *Kor. J. Microbiol.* **30,** 1-7.
15. **Kitson, T.M., J.P. Hill, and G.G. Midwinter.** 1991. Identification of a catalytically essential nucleophilic residue in sheep liver cytoplasmic aldehyde dehydrogenase. *Biochem. J.* **275,** 207-210.
16. **Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R. J. Randall.** 1951. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **193,** 265-275.
17. **Sambrook, J., E.F. Fritsch, and T. Maniatis.** 1990. Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, New York.
18. **Shaw, J.P., F. Schwager, and S. Harayama.** 1992. Substrate-specificity of benzyl alcohol dehydrogenase and benzaldehyde dehydrogenase encoded by TOL plasmid pWW0. Metabolic and mechanistic implications. *Biochem. J.* **283,** 789-794.
19. **Shin, H.J., S. Kim, and Y.C. Kim.** 1997. Sequence analysis of the *phnD* gene encoding 2-hydroxymuconic semialdehyde hydrolase in *Pseudomonas* sp. strain DJ77. *Biochem. Biophys. Res. Commun.* **232,** 288-291.
20. **Worsey, M.J. and P.A. Williams.** 1975. Metabolism of toluene and xylenes by *Pseudomonas* (*putida* (*arvilla*) mt-2: evidence for a new function of the TOL plasmid. *J. Bacteriol.* **124,** 7-13.