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Cloning and Nucleotide Sequence Analysis of *xylE* Encoding Catechol 2,3-Dioxygenase from *Pseudomonas putida* SU10

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Pseudomonas putida **SU10 isolated from Han River carries a TOL plasmid, which has been confirmed to clone *xylE* gene encoding catechol 2,3-dioxygenase enzyme. This plasmid was partially digested with *Sau*3AI, followed by insertion of the resulting DNA fragment into *Bam*HI site of pBluescript SK+vector. The *xylE* gene was screened from *E. coli* DH5α transformed with recombinant plasmids. The resulting clones containing *xylE* gene were able to convert catechol to a yellow hydroxymuconic semialdehyde. In order to isolate the functional area of *xylE* gene, recombinant plasmid DNAs were mapped with restriction endonuclease, *Ava*I. Among the 11 recombinant plasmid DNAs, restriction endonuclease mapping of 2 kb of insert DNA in the recombinant plasmid, pTY1, was carried out with *Pvu*II, *Xho*I, *Ava*I. By means of *Exo*III deletion mutagenesis to both orientation, the functional region of *xylE* was found to be about 1 kb in size including two internal *Pvu*II sites. We determined the nucleotide sequence of 2 kb fragment DNA containing *xylE* gene. One open reading frame of 921 bp was found and specially another ORF is located on reverse orientation. Amino acid sequences of this ORF were highly homologous with other genes encoding catechol 2,3-dioxygenase previously published.**

KEY WORDS □ ***xylE*, catechol 2,3-dioxygenase, *Pseudomonas putida***

Recently, Korea has been developed economically by the industrialization. However, its by-products polluted river, soil as well as ocean, which are severe environmental problems in our country. Specially aromatic hydrocarbons are difficult to be degraded by microorganisms in aquatic environment.

The TOL plasmid of *Pseudomonas putida* mt-2 encodes a pathway for catabolism of toluene and xylene by oxidizing a methyl substituent to a carboxylic acid followed by oxydative ring-cleavage via the *meta* pathway to central metabolites (16). From the induction analysis of degradative enzyme, Worsey *et al.* (17) proposed a model for the regulation of the early enzymes.

The genes for TOL operon are organized into two regulatory units: (1) the *xylABC* operon codes for the genes of toluene oxygenase, benzylalcohol dehydrogenase and bezaldehyde dehydrogenase, and (2) the *xylDEFG* operon codes for the genes of toluate oxygenase, catechol 2,3-dioxygenase, 2-hydroxymuconic semialdehyde dehydrogenase and 2-hydroxymuconic semialdehyde hydrolase. Two regulatory genes, *xylR* and *xylS*, positively control these operons. In the presence of *m*-xylene of *m*-methylbenzyl alcohol, the *xylABC* operon is activated by *xylR*, which the *xylDEFG* operon is activated together with *xylS*. In presence of *m*-toluate, the *xylDEFG* operon is activated by *xylS* alone.

We isolated several bacteria to degrade aromatic hydrocarbon from Han River by selective enrichment culture on *m*-toluate minimal broth medium. *Pseudomonas putida* SU10 has appeared to carry TOL plasmids which could utilize toluene, *m*-xylene, and *p*-xylene as sole carbon and energy sources. In addition to, the strain carrying TOL plasmid was able to produce yellow color using spray of catechol, suggesting that the plasmid contained *xylE* gene encoding catechol 2,3-dioxygenase because this enzyme can catalize catechol to 2-hydroxymuconic semialdehyde (15).

In the present study, we attempted to clone *xylE* gene and determine nucleotide sequence of the gene in *E. coli* on the basis of selection method of yellow color production by spray of catechol.

MATERIALS AND METHODS

Bacterial strain and plasmids

The bacterial strain used was *Pseudomonas putida* SU10 that was isolated from Han River. We confirmed the presence of a TOL plasmid in the strain that was able to utilize *m*-toluate to carboxylic acid via *meta*-cleavage pathway by spray of 0.1 M catechol. The plasmid used was pBluescript SK+. Recipient strain for transformation used was *E. coli* DH5α.

Media and culture conditions

The medium used throughout the experiment was L broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl). Agar was added to 1.5% in the L broth medium to prepare agar plates. Antibiotic concentrations used for selection of transformants were 50 μg of ampicillin per ml. Toluate plates contained 0.1% toluate in minimal plate. Agar plates of L broth were incubated at 37°C for selection of transformants.

Enzymes and chemicals

Restriction enzymes and bacterial alkaline phosphatase were purchased from Bethesda Research Laboratories and New England Biolabs. Chemicals for nucleotide sequencing were purchased from United States Biochemical.

Purification of plasmid DNA

Isolation of covalently closed circular plasmid DNA has been carried out by the method of Nakazawa *et al.* (11). Crude plsmid DNA was prepared by an alkaline extraction method (2).

Restriction enzyme analysis and gel electrophoresis

Digestion with restriction endonucleases was carried out under the conditions described as recommended by the suppliers. Analysis of restriction fragments and purification of digested DNA fragments by electrophoretic election have been described (13).

Ligation and transformation

Ligation of DNA fragments with T4 DNA ligase and transformation of *E. coli* cells were carried out as method of Sambrook *et al.*(13).

Nucleotide sequence determination

Nucleotide sequence analysis of DNA was performed as method of dideoxynucleotide chain termination (14).

RESULTS AND DISCUSSION

Molecular cloning of *xylE* gene

Strategy : Catechol 2,3-dioxygenase is encoded by *xylE* gene on the TOL plasmid from *Pseudomonas putida* mt-2 (7, 17). We also examined the properties of the TOL plasmid (118 kb) of *P. putida* SU10. We could find the evidence that the gene encoding for catechol 2,3-dioxygenase is located on TOL plasmid, based on the production of yellow color using spray of 0.1 M catechol on

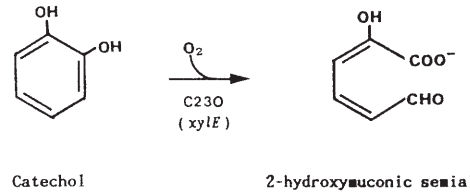

Fig. 1. *The degradative pathway of catechol to 2-hydroxymuconic semialdehyde.*

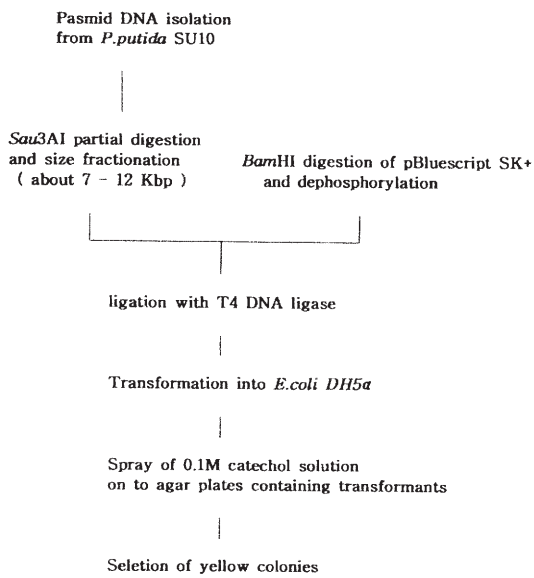

Fig. 2. *Cloning strategy of catechol 2,3-dioxygenase gene from Pseudomonas putida SU10.*

L broth plate. Therefore, we were able to screen the DNA clones encoding the catechol 2,3-dioxygenase gene on the ability of color production of transformants carrying recombinant plasmids of *xylE* gene on L broth plate (Fig. 1, Fig. 2).

***XylE* gene clones**

Recombinant plasmids containing DNA fragments from the TOL plasmid were constructed and screened for clones containing *xylE* genes for catechol 2,3-dioxygenase by testing the ability of color production on LB plate.

Partially restricted TOL plasmid with *Sau*3AI were ligated to the *Bam*HI site of pBluscript SK+ plasmid and the resulting recombinant plasmids were transformed into *E. coli* DH5α. By screening transformants, we obtained several clones of pTY1, pTY3, pTY5, and pTY9 carrying different size fragments containing *xylE* genes.

Restriction map and nucleotide sequence of *xylE* gene

The recombinant plasmids, pTY1, pTY3, pTY5,

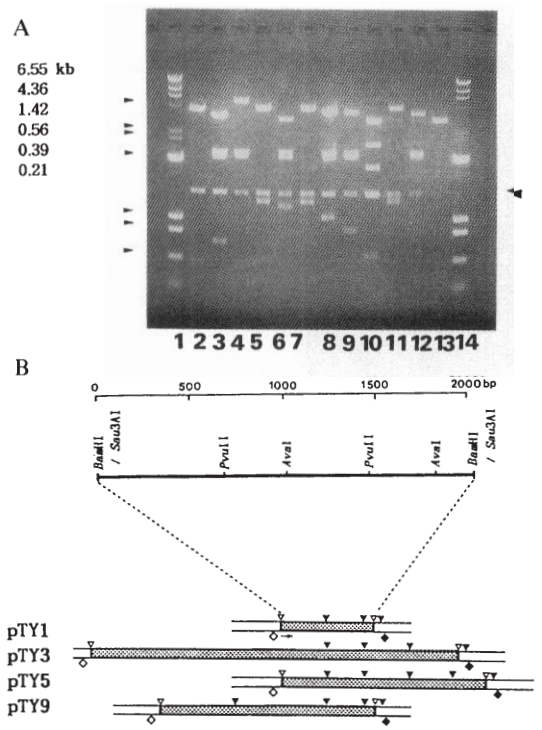

Fig. 3. *Restriction mapping of the recombinant DNAs, pTY1, pTY3, pTY5, and pTY9.*
A. Agarose gel electrophoresis of recombinant DNAs from pTY1 to pTY11. lane 1 and 14, DNA size markers of λ DNA digested with *Hin*dIII and pUC19 with *Hin*fI; lane 2 to 12, recombinant DNAs of pTY1 to pTY11 digested with *Ava*I, respectively; lane 13, pBluescript SK+ digested with *Ava*I.
B. Restriction endonuclease map of the recombinant DNAs including *xylE* gene. Open boxes represent vector (pBluescript SK+) DNA, and hatched boxes pTY DNA. ▽, *Bam*HI/*Sau*3AI; ▼, *Ava*I; ◇, *Xba*I; ◆, *Eco*RI.

and pTY9, were digested with various restriction enzymes to construct restriction maps. Fig. 3 summarizes the DNA fragment sizes generated from the plasmid treated with various enzymes.

To obtain a minimal sized fragment of the recombinant DNA neccessary for catechol 2,3-dioxygenase, we choose the recombinant DNA, pTY1, because this was the minimal sized fragment DNA among 4 clones.

For the more subcloning from pTY1, the recombinant plasmid pTY1 was deleted using unidirectional exonuclease III as well as testing color production by spray of catechol on colonies of L broth plates (Fig. 5). From the experiment of Exo III deletion, we obtained a minimal sized DNA carrying *xylE* gene as shown in Fig. 4.

Fig. 6 shows the partial restriction map containing pertinent restriction sites and nucleotide sequencing strategy. Twenty one clones were obtained after subcloning the restricted fragments into M13 mp18 and M13 mp19. The nucleotide sequence were determined in both direction by chain termination method (14). The sequences were then analyzed by PC/GENE transl program. As shown in Fig. 7, one open reading frames within the cloned *xylE* gene were found. The start and stop codons of the open reading frames of *xylE* gene are located at 1 and 922, respectively (Fig. 7). The ribosome recognition site, Shine-Dalgarno sequence was found at -10 position of the nucleotide sequence on upstream of *xylE* gene.

As shown in Fig. 8, when the amino acid sequence of the *xylE* gene products was compared to that of *P. putida* mt-2 (pWWO) (10), they exhibited 99% homology. When compared with that of *P. putida* HS1 (pDK1) (4) and *Pseudomonas* sp. (NAH7 plasmid) (12), they were 81.8%, 83.7% homologous, respectively. In addition, the nucleotide sequence of the *xylE* gene also shows 99.1% homology compared to that of *P. putida* mt-2, 81.4% to that of *Pseudonomas* sp. (NAH7

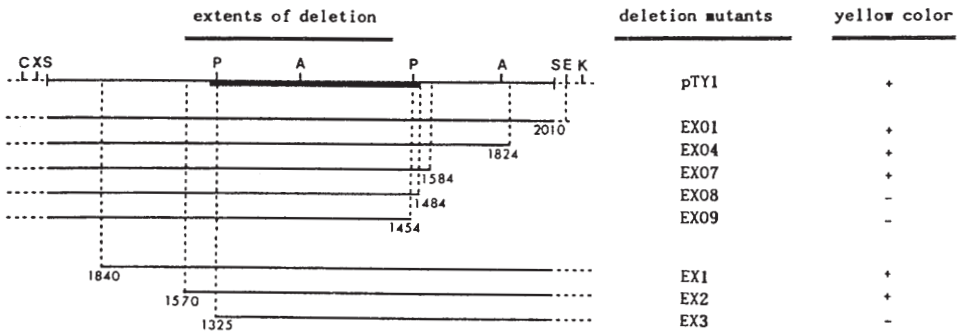

Fig. 4. *Localization of xylE gene on pTY1.*
The bold line designates the minimum region contained *xylE* gene. Numbers denote the size of inserts.
C, *Sac*I; X, *Xba*I; S, *Sau*3AI; P, *Pvu*II; A, *Ava*I; E, *Eco*RI; K, *Kpn*I.

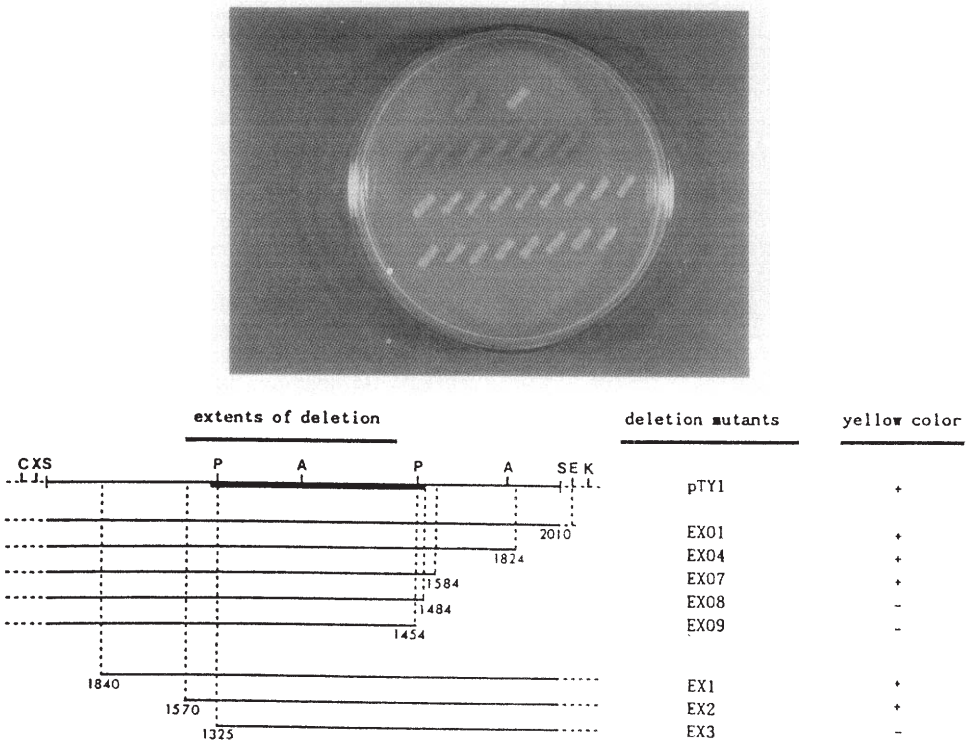

Fig. 5. *Catechol 2,3-dioxygenase activity of deletant subcloned cell of E. coli.*

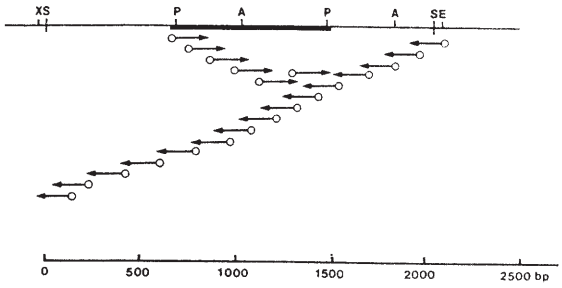

Fig. 6. *Sequencing strategies of pTY1.*
The bold line region represents *xylE* gene. The horizontal arrows indicate the extent and direction of sequencing from each site.

plasmid) (Table 1).

According to the RNA sequence analysis of 31 nucleotides upstream from AUG start codon, we found 4 UGA stop codons in two reading frames. It was found that AAGAGGTG sequence at −10 of *xylE* is a purine-rich region, that has also been shown at the 3′ terminal sequence of 16S rRNA in *E. coli* and *P. aeruginosa*. We assume that the 3′ terminal sequence of 16S rRNA in *P. putida* could be complementary to the nucleotide sequence of *P. aeruginosa*. Therefore, the sequence AAGAGGTG at −10 in *E. coli* and *P. putida* is thought to be the Shine-Dalgarno sequence at which ribosomes attach.

Pseudomonas sp. can grow on the minimal medium containing salicylate, camphor, octane, and other short-chain alkanes, and naphthalene as carbon sources. These chemicals are commonly degraded to catechol, benzene compounds, before becoming a linear structure. Catechols are degraded through two cleavage pathways by using two enzymes. One of them is catechol 2,3-dioxygenase (C230), extradiol-type dioxygenase, which is composed of 4 subunits containing ferrous form ion (10). It starts *meta*-cleavage pathways by dividing extradiol ring of catechol. The other enzyme is catechol 1,2-dioxygenase which is intradiol type dioxygenase, and is composed of α and β subunits.

We identified that C230 is composed of 307 amino acids, and its molecular weight is calculated to be 33.8 kd (307X110). Nakai *et al.* (10) reported that C230 of plasmid pWWO has 35 kDa molecular weight, comparably. The expecting second structure of catechol 2,3-dioxygenase has 29% polypeptide in α-helix region which was derived from computer analysis. The G+C content in the structural gene of catechol 2,3-dioxygenase is generally high and it provides

gatcgtcgaccagtccctcgacagcagccttatgaaacgctacggaagcattgacgagcaggtcgaggca

aattctgttccttgcttctgacgccgcctcctacatcaccggtataactcttccaatggcaggggggagac

tgccagagctgttccgtcatgtttagtgtatctggg (pWW0)

↓

ctcggctgacccttatgtctggttaataatataaatcgacatgcgaagaagcaacgtacataagacccct

gaggctcattttcggggttatggcggcatcacccagagctgttgggggatacttccgtcatgtttagtgt

atctgggatgaatatgaacagtgccggctacgaggtgttcgaagtgctaagcggccagtcattccgctgt

gcggagggccagtcggtactgcgcgcgccatggaagcccagggcaagcgctgcataccggtgggctgtcg

cggtggcggttgcggcctttgtagagtgcgggtgctcagcggagcctaccggagcggacgcatgagccgc

ggtcacgtgccggccaaggccgccgccgaaggcctggccctggcctgtcaagtgtttccgcaaaccgact

-10

tgaccatcgagtactttcgccacgttggcggaaacaaacctgacaacatgaactatgaaagaggtgacgtc

1 ATG AAC AAA GGT GTA ATG CGA CCG GGC CAT GTG CAG CTG CGT GTA CTG

met asn lys gly val met arg pro gly his val gln leu arg val leu

49 GAC ATG AGC AAG GCC CTG GAA CAC TAC GTC GAG TTG CTG GGC CTG ATC
asp met ser lys ala leu glu his tyr val glu leu leu gly leu ile

97 GAG ATG GAC CGT GAC GAC CAG GGC CGT GTC TAT CTG AAG GCT TGG ACC
glu met asp arg asp asp gln gly arg val tyr leu lys ala trp thr

145 GAA GTG GAT AAG TTT TCC CTG GTG CTA CGC GAG GCT GAC GAG CCG GGC
glu val asp lys phe ser leu val leu arg glu ala asp glu pro gly

193 ATG GAT TTT ATG GGT TTC AAG GTT GTG GAT GAG GAT GCT CTC CGG CAA
met asp phe met gly phe lys val val asp glu asp ala leu arg gln

241 CTG GAG CGG GAT CTG ATG GCA TAT GGC TGT GCC GTT GAG CAG CTA CCC
leu glu arg asp leu met ala tyr gly cys ala val glu gln leu pro

289 GCA GGT GAA CTG AAC AGT TGT GGC CGG CGC GTG CGC TTC CAG GCC CCC
ala gly glu leu asn ser cys gly arg arg val arg phe gln ala pro

337 TCC GGG CAT CAC TTC GAG TTG TAT GCA GAC AAG GAA TAT ACT GGA AAG
ser gly his his phe glu leu tyr ala asp lys glu tyr thr gly lys

385 TGG GGT TTG AAT GAC GTC AAT CCC GAG ACA TGG CCG CGC GAT TTG AAA
trp gly leu asn asp val asn pro glu thr trp pro arg asp leu lys

433 GGT ATG GCG GCT GTG CGT TTC GAC CAC GCC CTC ATG TAT GGC GAC GAA
gly met ala ala val arg phe asp his ala leu met tyr gly asp glu

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481 TTG CCG GCG ACC TAT GAC CTG TTC ACC AAG GTG CTC GGT TTC TAT CTG
    leu pro ala thr tyr asp leu phe thr lys val leu gly phe tyr leu

529 GCC GAA CAG GTG CTG GAC GAA AAT GGC ACG CGC GTC GCC CAG TTT CTC
    ala glu gln val leu asp glu asn gly thr arg val ala gln phe leu

577 AGT CTG TCG ACC AAG GCC CAC GAC GTG GCC TTC ATT CAC CAT CCG GAA
    ser leu ser thr lys ala his asp val ala phe ile his his pro glu

625 AAA GGC CGC CTC CAT CAT GTG TCC TTC CAC CTC GAA ACC TGG GAA GAT
    lys gly arg leu his his val ser phe his leu glu thr trp glu asp

673 CTT CTT CGC GCC GCC GAC CTG ATC TCC ATG ACC GAA ACA TCT ATC GAT
    leu leu arg ala ala asp leu ile ser met thr glu thr ser ile asp

721 ATC GGC CCA ACC CGC CAC GGC CTT ACT CAC GGC AAG ACC ATC TAC TTC
    ile gly pro thr arg his gly leu thr his gly lys thr ile tyr phe

769 TTC GAC CCG TCC GGT AAC CGC AAC GAA GTG TTC TGC GGG GGA GAT TAC
    phe asp pro ser gly asn arg asn glu val phe cys gly gly asp tyr

817 AAC TAC CCG GAC CAA AAA CCG GTG ACC TGG ACC ACC GAC CAG CTG GGC
    asn tyr pro asp gln lys pro val thr trp thr thr asp gln leu gly

865 AAG GCG ATC TTT TAC CAC GAC CGC ATT CTC AAC GAA CGA TTC ATG ACC
    lys ala ile phe tyr his asp arg ile leu asn glu arg phe met thr

913 GTG CTG ACC TGA tggtccggtaccacttattgcagagattgtgcagattaaaaaaatcaag
    val leu thr OPA

catttcattagcggtgcctccgtcggttcggccagcggcaagctgttcgacaatgtcagcccgccaaacg

gccaggtgatcggccgcgtccacgaggccggccgcgccgaggtcgacgccgcggtcagggcggcacgccc

tgcgctgaagggaccctgggggaagatgacggtggccgagcgcgctgagattctgcatcgcgtggccgat

ggcatcacggcgcgcttcggcgagtttctcgaggcccgaatgcctggacaccggcaagccgaagtcgctg

gccagccacatcgacattccgcgcggcgcgccaatttcaaggtgttcgccgacctggtcaagaatgttgc

caatgaagccttcgagatggccaccccggacggcggcggtgcacctcaactagcctgcgccggcccaagg

gggtgatc
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Fig. 7. *Nucleotide sequence and deduced amino acid sequence of xylE gene in pTY1.*
TGA codons located immediate upstream of ORF is indicated as open letters. The open reading frame region and flanking regions are designated by upper and lower case respectively. Putative ribosome binding site (Shine-Dalgarno sequence) at −10 is underlined.

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pTY1     MNKGVMRPGHVQLRVLDMSKALEHYVELLGLIEMDRDDQGRVYLKAWTEV    50
XylE I   MNKGVMRPGHVQLRVLDMSKALEHYVELLGLIEMDRDDQGRVYLKAWTEV    50
XylE II  MNKGIMRPGHVQLRVLDMSKALEHYVELLGLIEMDRDDQGRVYLKAWTEV    50
NahH     MNKGVMRPGHVQLRVLDMGKALEHYVELLGLIEMDRDDQGRVYLKAWTEV    50
DmpB     MKKGVMRPGHVQLRVLNLESALAHYRDLLGLIEMDRDEQGRVYLKAWTEV    50
pDK1     MKKGVMRPGHVQLRVLNLEAALTHYRDLLGLIEMDRDEQGRVYLKAWSEV    50
         * ** ***********    ** **  ********** ********* **

pTY1     DKFSLVLREADEPGMDFMGFKVVDEDALRQLERDLMAYGCAVEQLPAGEL   100
XylE I   DKFSLVLREADEPGMDFMGFKVVDEDALRQLERDLMAYGCAVEQLPAGEL   100
XylE II  DKFSVVLRERDEPGMEFMGFKVVDEDALRQLERDLTAYGCAVEQLPAGEL   100
NahH     DKFSVVLREADEPGMDFMGFKVVDEDSLNRLTDDLLNFGCLIENVAAGEL   100
DmpB     DKFSVVLREADQPGMDFMGFKVIDEDCLNRLTQKLLNYGCLIETIPAGEL   100
pDK1     DKFSVVLREADQPGMDFMGFKVTDDACLTRLERELLEFGCQVEEIPAGEL   100
         **** ****** ********** * * *  *   *   **  *   ****

pTY1     NSCGRRVRFQAPSGHHFELYADKEYTGKWGLNDVNPETWPRDLKGMAAVR   100
XylE I   NSCGRRVRFQAPSGHHFELYADKEYTGKWGLNDVNPEAWPRDLKGMAAVR   150
XylE II  NSCGRRVRFQAPSGHHFELYADKEYTGKWGVNEVNPEAWPRDLKGMAAVR   150
NahH     KGCGRRVRFQAPSGHHFELYADKEYTGKWGVNEVNPEAWPRDLKGMAAVR   150
DmpB     KGCGRRVRFQTPSGHFFELYADKEYTGKWGLEEINPEAWPRNLKGMRAVR   150
pDK1     KHCGRRVRFLAPSGHFFELYAEKEYTGKWGIEDVNPEAWPRELKGMRAVR   150
           *******  **** ***** ********    *** *** **** ***

pTY1     FDHALMYGDELPATYDLFTKVLGFYLAEQVLDENGTRVAQFLSLSTKAHD   200
XylE I   FDHALMYGDELPATYDLFTKVLGFYLAEQVLDENGTRVAQFLSLSTKAHD   200
XylE II  FDHCLLYGDELPATYDLFTKVLGFYLAEQVLDENGTRVAQFLSLSTKAHD   200
NahH     FDHCLLYGDELQATYELFTEVLGFYLAEQVVDADGIRLAQFLSLSTKAHD   200
DmpB     FDHCLLYGDELQATYALFTEVLGFYLAEQVIDDDGTRVAQFLSLSTKAHD   200
pDK1     FDHCLMYGDELQATYELFTEVLGFYLAEQVIEDNGTRISQFLSLSTKAHD   200
         *** * ***** *** *** **********    * *  ***********

pTY1     VAFIHHPEKGRLHHVSFHLETWEDLLRAADLISMTETSIDIGPTFHGLTH   250
XylE I   VAFIHHPEKGRLHHVSFHLETWEDLLRAADLISMTDTSIDIGPTRHGLTH   250
XylE II  VAFIHHPEKGRLHHVSFHLETWEDVLRAADLISMTDTSIDIGPTRHGLTH   250
NahH     VAFIHHAEKGKFHHASFFLDTWEDVLRAADLISMTDTSIDIGPTRHGLTQ   250
DmpB     VAFIHCPEKGKFHHVSFFLETWEDVLRAADLISMTDTSEDIGPTRHGLTH   250
pDK1     VAFIQHAEKGKFHHVSFFLETREDVLRAADLISMTDTSIDIGPTRHGLTH   250
         ****   ***  ** ** * * * ** ********** ** ***** *****

pTY1     GKTIYFFDPSGNRNEVFCGGKYNYPDGKPVTWTTDQLGKAIFYHDRILNE   300
XylE I   GKTIYFFDPSGNRNEVFCFFDYNYPDHKPVTWTTDQLGKAIFYHDRILNE   300
XylE II  GKTIYFFDPSGNRSEVFCGGNYSYPDHKPVTWLAKDLGKAIFYHDRVLNE   300
NahH     GKTIYFFDPSGNRCEVFCGGNYNYPDHKPVTWLAKDVGKAIFYHDRVLNE   300
DmpB     GKTIYFFDPSGNRNEVFCGGCYCYQDHKPVTWLAKDLGKAIFYHDRVLNE   300
pDK1     GKTIYFFDPSGNRNEVFCGGDYNYPDHKPVTWTADQLGKAIFYHDRILNE   300
         ************** ****   * * * *****     ********* ***

pTY1     RFMTVLT   307
XylE I   RFMTVLT   307
XylE II  RFMTVLT   307
NahH     RFMTVMT   307
DmpB     RFLTVLT   307
pDK1     RFMTVLT   307
         ** ** *
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Fig. 8. *Comparison of homology of amino acids in xylE gene products from several strains.*
Asterisks indicate conserved amino acid in all *meta*-cleavage enzymes found so far. XylE I (10), catechol 2,3-dioxygenase by TOL plasmid derived from *P. putida*; XylEII (9), catechol 2,3-digoxygenase from *P. aeruginosa*; NahH (4), C23O encoded by the NAH7 plasmid; DmpB (1), C23O from *Pseudomonas* sp. CF600; pDK1 (12), C23O from *P. putida* HS-1.

Table 1. *Homologies of nucleotide of xylE gene and amino acids of C23O with those of pWWO, pDK1, and NAH7 plasmids.*

xylE gene	Homology Nucleotide sequence of *xylE* gene with *P. putida* SU10	Amino acid sequence of C230 with *P. putida* SU10
$xylE_{PWWO}$		
P. putida mt-2 (10)	99.1%	99.0%
$xylE_{pDK1}$		
P. putida HS1 (12)	80.9%	81.8%
$nahH_{NAH7}$		
Pseudomonas sp. (4)	81.4%	83.7%

strong evidence that this organism belongs to *Pseudomonas* genus (10). The identity of nucleotide sequence in NAH7, pWWO, and pDK1 supplies clues to suspect them coming from the same origin (12). Harayama reported that *xylE* is expressed as a polycistron of *xylXYZLTEGFJQKIH* operon in TOL plasmid.

Inouye *et al.* (7) reported that promoter of *xylE* gene was located at upstream of *xylDEFG* operon. Keil *et al.* found that there is internal promoter sequence upstream of *xylL* and *xylE* in *xyl* operon of pWW53-4. Therefore, it is expected that there is a promoter upstream of 5′ flanking region of *xylE*. We also found that *xylE* uses its own promoter instead of vector promoter for producing proteins. We found that promoter of *xylE* gene is located at upstream of *xylE* gene itself, because the inverted orientation of *xylE* gene was able to express catechol 2,3-dioxygenase in *E. coli* (unpublished data). Currently, we are determining the correct position of promoter on upstream of *xylE* gene.

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초 록: *Pseudomonas putida* SU10으로부터 Catechol 2,3-Dioxygenase를 암호화하는 *xylE* 유전자의 클로닝과 염기서열 분석

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서울 근교의 하천에서 분리한 *Pseudmonas putida* SU10에 TOL 플라스미드 내에 catecol 2,3-dioxygenase를 합성하는 *xylE* 유전자가 있음을 확인하였다. 이 TOL plasmid를 순수 분리한 후 *Sau*3A1으로 부분절제하여 pBluescript SK$^+$ vector의 *Bam*H1 site에 삽입시켜 *E. coli* DH5α에 형질전환시킨 다음 *xylE* gene을 포함하는 여러가지 재조합 DNA를 제한효소인 *Ava*1으로 처리하여 간단한 제한효소지도를 작성하였다. 그 결과, *xylE* gene은 PTY1의 약 2.01 kb 크기의 insert DNA 내에 위치해 있었으며, 이것을 *Ava*I, *Pvu*II로 다시 제한효소지도를 작성하였다. Insert DNA를 양방향으로 ExoIII deletion mutagenesis 방법을 수행하여 *xylE* gene의 위치를 1 kb 내로 subcloning하였다. *xylE* gene을 포함한 2.01 kb 크기의 pTY1의 DNA 절편의 염기서열을 결정한 결과, 이 절편은 catechol 2,3-dioxygenase에 해당하는 하나의 open reading frame을 포함하고 있었다. 307 잔기로 구성된 아미노산 서열과 그것의 amino 말단과 carboxyl 말단 서열은 이미 보고된 동일 종류의 DNA의 염기서열과 매우 유사하였다.