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Sequence Characteristics of *xylJQK* Genes Responsible for Catechol Degradation in Benzoate-Catabolizing *Pseudomonas* sp. S-47

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Abstract *Pseudomonas* sp. S-47 is capable of degrading benzoate and 4-chlorobenzoate as well as catechol and 4-chlorocatechol via the *meta*-cleavage pathway. The three enzymes of 2-oxopenta-4-enoate hydratase (OEH), acetaldehyde dehydrogenase (acylating) (ADA), and 2-oxo-4-hydroxypentonate aldolase (HOA) encoded by *xylJQK* genes are responsible for the three steps after the *meta*-cleavage of catechol. The nucleotide sequence of the *xylJQK* genes located in the chromosomal DNA was cloned and analyzed. GC content of *xylJ*, *xylQ*, and *xylK* was 65% and consisted of 786, 924, and 1,041 nucleotides, respectively. The deduced amino acid sequences of *xylJ*, *xylQ*, and *xylK* genes from *Pseudomonas* sp. S-47 showed 93%, 99%, and 99% identity, compared with those of *nahT*, *nahH*, and *nahI* in *Pseudomonas stutzeri* An10. However, there were only about 53% to 85% identity with *xylJQK* of *Pseudomonas putida* mt-2, *dmpEFG* of *P. putida* CF600, *aphEFG* of *Comamonas testosteroni* TA441, and *ipbEGF* of *P. putida* RE204. On the other hand, the *xylLTEGF* genes located upstream of *xylJQK* in the strain S-47 showed high homology with those of TOL plasmid from *Pseudomonas putida* mt-2. These findings suggested that the *xylLTEGFIJQK* of *Pseudomonas* sp. S-47 responsible for complete degradation of benzoate and then catechol via the *meta*-pathway were phylogenetically recombinated from the genes of *Pseudomonas putida* mt-2 and *Pseudomonas stutzeri* An10.

Key words: *xylJQK*, nucleotide and amino acid sequences, catechol degradation, *Pseudomonas* sp. S-47

One of the central metabolic routes for the bacterial degradation of aromatic compounds and chlorine-substituted aromatics is the formation of catechol. A variety of aromatics, including xylene, phenol, toluene, and napthalene, also can be channeled into this pathway via conversion to a catechol by auxiliary enzymes [14, 15, 22]. Catechol can then be oxidized to produce hydroxymuconic semialdehyde via either *ortho*- or *meta*-cleavage, followed by formation of 2-oxopenta-4-enoate, which is a 5-carbon liner organic acid. The resulting organic acid is degraded to produce Krebs cycle intermediates, such as pyruvate and acetaldehyde [4, 9, 11, 26]. This pathway is reported to operate in a number of different organisms, but the best-studied examples are found in *Pseudomonas* spp. [2, 21, 29].

The last three reactions following catechol dioxygenation comprise the conversion of 2-oxopenta-4-enoate to 4-hydroxy-2-oxovalerate (4H-OV) by 2-oxopenta-4-enoate hydratase (OEH), the conversion of 4H-OV to acetaldehyde and pyruvate by 2-oxo-4-hydroxypentonate aldolase (HOA), and the conversion of acetaldehyde to acetyl-CoA by acetaldehyde dehydrogenase (acylating) (ADA). Those three enzymes of OEH, HOA, and ADA are encoded by the *xylJ*, *xylK*, and *xylQ*, respectively, of TOL plasmid pWW0 from benzoate-degrading *Pseudomonas putida* mt-2 [4, 9, 12, 27], the *nahLOM* of napthalene-degrading *P. stutzeri* An10 [3], the *dmpEFG* of phenol-degrading *P. putida* CF600 [17, 26], the *aphEFG* of phenol-degrading *Comamonas testosteroni* TA441 [1], and the *ipbEGF* of isopropylbenzene-degrading *P. putida* RE204 [5, 6]. All these three genes from the strains with same degradative function are found to be in the same order in organization, but there are some differences in the amino acid sequence of the genes.

Pseudomonas sp. S-47 is a bacterial strain that was isolated from contaminated waste by Seo *et al.* [24]. The strain is able to convert benzoate and 4-chlorobenzoate (4CBA) to the corresponding catechols which are then utilized as the sole source of carbon and energy through the *meta*-cleavage pathway. The *xylLTEG* genes encoding these enzymes involved in the degradation of benzoate, such as benzoate dihydrodiol dehydrogenase (BDD), chloroplast-type ferredoxin reductase, catechol 2,3-dioxygenase

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(C 23O), and 2-hydroxymuconic semialdehyde dehydrogenase (2HMSD), respectively, have been previously studied [16, 18, 19, 20]. The genes are homologous with the corresponding genes from TOL plasmid pWW0 of *Pseudomonas putida* mt-2 in the gene order and amino acid sequence.

In this study, the *xylJQK* genes encoding OEH, ADA, and HOA, respectively, which were responsible for degradation of the 2-oxopenta-4-enoate produced from catechol were cloned. The complete nucleotide and amino acid sequences of the genes were analyzed and their phylogenetic relationship was evaluated by comparing it with those of the corresponding genes from other strains.

MATERIALS AND METHODS

Strains and Cultivation

The bacterial strains and plasmids used in this work are listed in Table 1. *Pseudomonas* sp. S-47 is a natural isolate which can degrade benzoate and 4-chlorobenzoates as well as catechol and 4-chlorocatechol [24]. The strain 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 $(NH_4)_2SO_4$, 10 mM potassium phosphate buffer (pH 7.0)] containing 0.5 mM 4CBA or catechol. *E. coli* XL1-Blue was used as a host strain for the transformation and isolation of recombinant plasmids, and grown in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 1% NaCl) at 37°C.

Cloning and Nucleotide Sequencing of *xylJQK* Genes

The genomic and plasmid DNAs were isolated by the alkali lysis method as described by Sambrook *et al.* [23]. The DNA cleavage by restriction endonuclease and ligation of DNA fragments by T4 DNA ligase were performed by standard procedures as recommended by the enzyme supplier (Kosco Co., Seoul, Korea). The *xylJQK* genes were cloned from the chromosomal DNA of *Pseudomonas* sp. S-47 to obtain pCS201, which has the degradation activities of 4CBA and catechol.

From the pCS201 carrying the *xylJQK* genes, the subclones of pCH101, pCH202, pCH402, pCH403, and pCH404 were further constructed by digestion of the pCS201 with various enzymes. Transformation was accomplished by the calcium chloride method [23]. The LB medium supplemented with 50 μM/ml ampicillin was used for the selection of the transformants. pBluescript SK II(+) was used as the cloning and sequencing vector.

Analysis of Nucleotide and Amino Acid Sequences

Nucleotide sequences of *xylJQK* genes were analyzed by the DNASIS software (Hitachi version 7.0, Japan). The deduced amino acid sequences were also analyzed using the DNASIS software. The amino acid sequences were compared with the GenBank database using the programs based on the BLAST algorithm. Multiple alignments were generated using the Clustal X algorithm [28] and used for phylogenetic analysis. The display program of TreeView was used to visualize the phylogenies of tree file of Clustal X.

Nucleotide Sequence Accession No.

The nucleotide sequence of the *xylJQK* genes reported in this study has been deposited in the GenBank under Accession No. AF320981.

RESULTS AND DISCUSSION

Cloning and Nucleotide Sequence of *xylJQK* Genes

The pCS201 carrying the *xylJQK* genes was previously cloned from the chromosomal DNA of *Pseudomonas* sp.

Table 1. Bacterial strains and plasmids used for in this work.

Strains & Plasmids	Description	References
Strains		
Pseudomonas sp. S-47	$4CBA^+$, $4CC^+$, $4MC^+$, CT^+, Ap^r	21
E. coli XL1-Blue	Tc^r, host cell	Stratagene Ltd
Plasmids		
pBluescript SK(+)II	Ap^r, cloning and sequencing vector	Stratagene Ltd
pCS1	40k b *Sau*3AI insert encoding 4CBA degrading genes from *Pseudomonas* sp. S-47	13
pCS201	15 kb *Bam*HI fragment of pCS1 in pUC18	13
pCH101	0.7 kb *Sal*I fragment of pCH1 in SK II(+)	This work
pCH202	1.0 kb *Xho*I-*Pst*I fragment of pCH2 in SK II(+)	This work
pCH4	8.4 kb *Sac*I fragment of pCS201 in SK II(+)	This work
pCH402	1.5 kb *Sac*II fragment of pCH4 in SK II(+)	This work
pCH403	1.1 kb *Sac*II fragment of pCH4 in SK II(+)	This work
pCH404	0.8 kb *Xho*I fragment of pCH4 in SK II(+)	This work

Abbreviations: 4CBA, 4-chlorobenzoate; 4CC, 4-chlorocatechol, 4MC, 4-methylcatechol; CT, catechol; Ap, ampicillin; Tc, tetracycline.

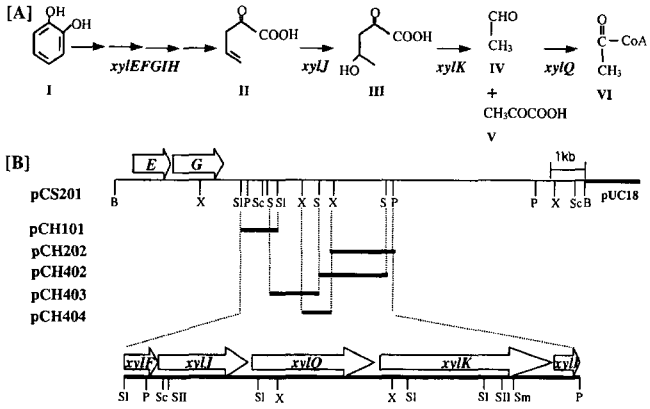

Fig. 1. Pathway for degradation of 2-oxopenta-4-enoate by *Pseudomonas* sp. S-47 [A], and genetic maps of pCS201 and its subclones [B].
I, Catechol; II, 2-Oxopenta-4-enoate; III, 4-Hydroxy-2- oxovalerate; IV, Acetaldehyde; V, Pyruvate; VI, Acetyl-CoA; B, *Bam*HI; C, *Cla*I; P, *Pst*I; Sc, *Sac*I; S, *Sac*II; Sl, *Sal*I; X, *Xho*I.

S-47 [13]. The subclones of pCH101, pCH202, pCH402, pCH403, and pCH404 were further constructed by digesting the pCS201 with various enzymes. The physical maps of those clones are shown in Fig. 1.

*Pst*I
CTGCAGGCTTCGCTGACCCTCGCGCAGTGGATTCCCAACGCCCAGCTACACGTGTTCGGGCCAGTGCGGCCACTGGACCCA 80
GATCGAACACGCCGAGCGTTTCGCCCCGCTTGGTCGAGAATTTCCTCGCCGAGGCCGACGGCCCTCCATTCCTGAGAGAGAC 160
CGATATGGACAAGACATTGATCAACGAACTCGGCGACGAGCTCTACCAGGCGATGGTCCAGCGCGAGACCGTCACGCCGC 240
xylJ
TGACCAGCCGCGGCTTCGACATCAGCGTCGAGGACGCCTACCACATTTCCCTGCGCATGCTGGAACGCCGCCTGGCCGCC 320
GGCGAGCGGGTGATCGGCAAGAAGATCGGCGTCACCAGCAAGGCCGTGCAGAACATGCTCGGCGTGCACCAGCCGGACTT 400
CGGCTACCTCACCGATGCCATGGTCTACAACAGCGGCGAAGCCATGCCGATCAGCGAGAAGCTGATCCAGCCGCGCGCCG 480
AGGGCGAGATCGCCTTCATCCTCAAGAAGGACCTGATGGGGCCGGGCGTGACCAACGCCGACGTGCTGGCTGCCACCGAA 560
TGCGTGATCCCCTGCTTCGAAGTGGTCGATTCGCGCATCCAGGACTGGAAGATCAAGATCCAGGACACCGTGGCGGACAA 640
CGCCTCCTGCGGGCTGTTCGTGCTCGGCGACCAGGCCGTCTCACCGCGCCAGGTCGATCTGGTCACCTGCGGCATGCTGG 720
TCGAGAAGAACGGCCAGCTGCTCTCCACCGGCGCTGGAGCGGCTGCGCTCGGCTCGCCGGTCAATTGCGTCGCCTGGTTG 800
GCCAACACCCTCGGCCACTTCGGCATCGGCCTGAAGGCCGGCGAAGTGATCCTGTCCGGCTCGCTGGTTCCGCTGGAACC 880
GGTCAAGGCCGGTGATTTCATGCGCGTCGAGATCGGCGGCATCGGCAGCGCCTCCGTGCGCTTCATCTGATCGAGGACAG 960
CCTGATGAACAAGAAACTGAAAGCCGCCATTATCGGCCCGGGCAACATCGGCACAGATCTGGTGATGAAGATGCTGCGTT 1040
xylQ
CCGAGTGGATCGAGCCGGTCTGGATGGTCGGCATCGACCCCGAGTCCGATGGCCTCAAGCGCGCCCGCGAGTTCGGCCTG 1120
AAGACCACCGCCGAGGGCGTCGACGCCCTGCTGCCGCACGTGCTCGAGGACGATATCCGCATCGCCTTCGATGCCACCTC 1200
CGCCTATGTACACGCCGAGAACAGCCGCAAGCTCAACGAGCTGGGCGTGCTGATGGTCGACCTGACCCCGGCCGCCATCG 1280
GCCCGTACTGCGTGCCGCCGGTGAACCTCAAGCAGCATGTCGGCACGCTGGAAATGAACGTCAACATGGTCACCTGTGGT 1360
GGCCAGGCCACCATCCCGATGGTCGCCGCGGTATCGCGCGTGCAGCCGGTGGCCTACGGCGAGATCGTCGCCACCGTGTC 1440
CTCGCGCTCCATCGGCCCGGGCACCCGCAAGAACATCGACGAGTTCACCCGCACCACCGGCGGCGCCATCGAGCAGGTCG 1520
GCGGGGCCAAGGAAGGCAAGGCGATCATCGTCGTCAACCCGGCCGAGCCGCCGCTGATGATGCGCGACACCATCCACTGC 1600
CTGACCGAAACCGAACCGGACCAGGATGCGATCACCGCATCGGTCCACGCGATGATCGCCGAGGTGCAGAAGTACGTGCC 1680
CGGCTACCGGCTGAAGAACGGCCCGGTATTCGACGGCAACCGCGTCTCGATCTTCATGGAAGTCGAGGGCCTGGGCGACT 1760
ACCTGCCCAAGTACGCCGGCAACCTCGACATCATGACCGCCGCCGCCCTGCGCACCGGCGAGATGTTCGCCGAGGAAATC 1840
GCCGCCGGCACCATTCAACTGCCACGTCGTGAAGCCGCACTGGCCTAAAGGAGTCGCACCATGAATCTGCAAGGCAAGAA 1920
xylK
CGTCACCCTGCACGACATGAGCCTGCGCGACGGCATGCACGCCAAGCGCCACCAGATCAGCCTCGAGCAGATGATCGCGG 2000
TCGCCACCGGCCTCGACGCTGCCGGCATGCCACTGATCGAGATCACCCACGGCGACGGCCTCGGCGGTCGCTCGATCAAC 2080
TACGGTTTCCCCGGCGCACAGCGACGAGGAATACCTGCGTGGCGGTGATCCCGCGCCTCAAGCAGGCCAAGGTATCCGCCCT 2160
GCTGCTGCCAGGCATCGGCACGGTCGACCACCTGAAGATGGCGCTCGACTGTGGCGTCTCCACCATTCGAGTGGCCACGC 2240
ATTGCACCGAGGCCGATGTCTCCGAGCAGCACATCGGCATGTCACGCAAGCTGGGCGCCGATACCGTAGGCTTCCTGATG 2320
ATGGCGCACATGATCAGCGCGGAAAAAGTGCTGGAGCAGGCGCGGCTGATGGAAAGCTACGGCGCCAACTGCATCTATTG 2400
CACCGACTCCGCCGGCTACATGCTGCCCGATGAAGTCAGCGAGAAGATCGGCCTGCTGCGCGCCGAGCTGAACCCGGCCA 2480
CCGAGATCGGCTTCCACGGCCACCACAACATGGGCATGGCCATCGCCAATTCGCTGGCCGCCATCGAAGCCGGTGCCTCA 2560
CGCATCGACGGCTCGGTCGCCGGTCTCGGCGCCGGTGCCGGCAACACCCCGCTGGAAGTCTTCGTCGCGGTGTGCAAGCG 2640
CATGGGCGTGGAAACCGGCATCGACCTCTACAAGATCATGGACGTCGCCGAGGACCTGGTGGTGCCGATGATGGATCAGC 2720
CGATCCGCGTCGACCGCGATGCGCTGACCCTGGGCTATGCCGGGGTGTACAGCTCGTTCCTGCTGTTTGCCCAGCGCGCC 2800
GAGAAGAAGTACGGCATACCGGCCCGCGACATTCTGGTCGAGCTGGGGCGCCGCGGCACCGTCGGTGGCCAGGAAGACAT 2880
GATCGAGGACCTCGCCCTGGATATGTCCCGGGCCCGCCAGAACCAGAAGGTGAGCGCATGAACCGTACCCTCAGCCCCGA 2960
*Pst*I
xylI
GCAGGTGCTGGCCCTGGCCGAGCACATCGAGAACGCCGAACTGCAG 3006

Fig. 2. Nucleotide sequences of the *xylJQK* genes from *Pseudomonas* sp. S-47.
Start codons are indicated with arrow and stop codons are underlined.

The nucleotide sequence of the 3-kb fragment between two *Pst*I sites in the 15-kb pCS201 is shown in Fig. 2. Three open reading frames (ORFs) corresponding to *xylJ*, *xylQ*, and *xylK* were identified as being located in the fragment. The *xylJ*, *xylQ*, and *xylK* genes had 65% GC content and consisted of 786, 924, and 1,041 nucleotides, respectively. The *xylJ* gene encodes a polypeptide chain with a molecular mass of 27.8 kDa consisting of 261 amino acid residues. The *xylQ* gene encodes a polypeptide chain with a molecular mass of 32.9 kDa consisting of 307 amino acid residues, and the *xylK* gene encodes a polypeptide chain with a molecular mass of 37.1 kDa consisting of 346 amino acid residues. Databases in the GenBank were searched for proteins having a high degree of similarity to the deduced amino acid sequences of the catechol-catabolic gene products by using the BLAST program. Upstream of *xylQ* is the 3'-end of an ORF of 153 codons, giving 50 amino acids, which showed 100% homology with the C-terminus of *xylF* gene coding for hydroxymuconic semialdehyde hydrolase from TOL plasmid pWW0 [9, 12]. Furthermore, downstream of *xylK* the 5'-end of an ORF was located, the nucleotides of which showed 91% homology with the N-terminus of *xylI* from TOL plasmid pWW0.

Amino Acid Homology of XylJQK

The deduced amino acid sequence of the *xylJ* gene from *Pseudomonas* sp. S-47 exhibited 93% identity with that of the NahL from *Pseudomonas stutzeri* An10 reported by

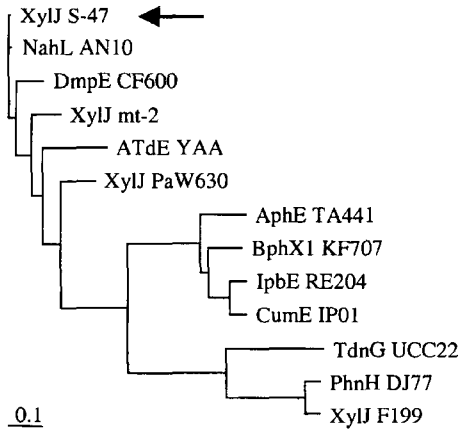

Fig. 3. Phylogenic relationship of XylJ from *Pseudomonas* sp. S-47 with 12 corresponding proteins.
The GenBank accession numbers of the genes are shown in parenthesis. AN10, *Pseudomonas stutzeri* AN10 (AF039534); CF600, *Pseudomonas putida* CF600 (X60835); mt-2, *Pseudomonas putida* mt-2 (AJ344068); YAA, *Acinetobacter* sp. YAA (AB008831); PaW630, *Pseudomonas putida* PaW630 (AF134348); TA441, *Comamonas testosteroni* TA441 (AB029044); KF707, *Pseudomonas pseudoalcaligenes* KF707 (D85853); RE204, *Pseudomonas putida* RE204 (AF006691); IP01, *Pseudomonas fluorescens* IP01 (D63377); UCC22, *Pseudomonas putida* UCC22 (D85415); DJ77, *Sphingomonas chungbukensis* DJ77 (U97697); F199, *Novosphingobium aromaticivorans* F199 (AF079317). The scale bar denotes the number of amino acid substitutions per site.

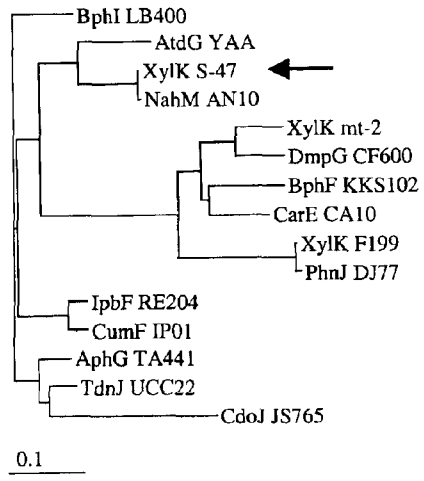

Fig. 4. Phylogenic relationship of XylK from *Pseudomonas* sp. S-47 with 14 corresponding proteins.
The GenBank accession numbers of the genes are shown in parenthesis. LB400, *Pseudomonas* sp. LB400 (X76500); YAA, *Acinetobacter* sp. YAA (AB008831); AN10, *Pseudomonas stutzeri* AN10 (AF039534); mt-2, *Pseudomonas putida* mt-2 (AJ344068); CF600, *Pseudomonas putida* CF600 (X60835); KKS102, *Pseudomonas* sp. KKS102 (D16407); CA10, *Pseudomonas* sp. CA10 (AB047548); F199, *Novosphingobium aromaticivorans* F199 (AF079317); DJ77, *Sphingomonas chungbukensis* DJ77 (AF061803); RE204, *Pseudomonas putida* RE204 (AF006691); IP01, *Pseudomonas fluorescens* IP01 (D63377); TA441, *Comamonas testosteroni* TA441 (AB029044); UCC22, *Pseudomonas putida* UCC22 (D85415); JS765, *Comamonas* sp. JS765 (AF190463). The scale bar denotes the number of amino acid substitutions per site.

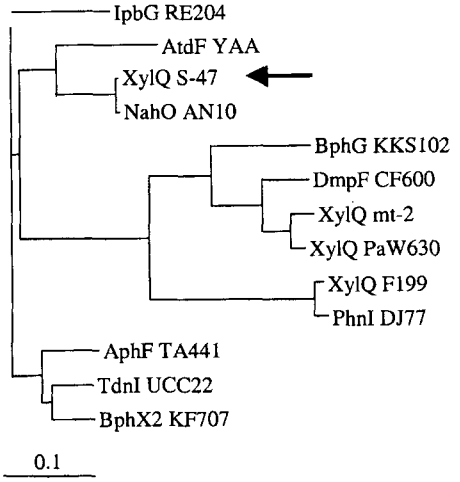

Fig. 5. Phylogenic relationship of XylQ from *Pseudomonas* sp. S-47 with 12 corresponding proteins.
The GenBank accession numbers of the genes are shown in parenthesis. RE204, *Pseudomonas putida* RE204 (AF006691); YAA, *Acinetobacter* sp. YAA (AB008831); AN10, *Pseudomonas stutzeri* AN10 (AF039534); KKS102, *Pseudomonas* sp. KKS102 (D16407); CF600, *Pseudomonas putida* CF600 (X60835); mt-2, *Pseudomonas putida* mt-2 (AJ344068); PaW630, *Pseudomonas putida* PaW630 (AF134348); F199, *Novosphingobium aromaticivorans* F199 (AF079317); DJ77, *Sphingomonas chungbukensis* DJ77 (AF061803); TA441, *Comamonas testosteroni* TA441 (AB024335); UCC22, *Pseudomonas putida* UCC22 (D85415); KF707, *Pseudomonas pseudoalcaligenes* KF707 (D85853). The scale bar denotes the number of amino acid substitutions per site.

Bosch *et al.* [3] as can be seen in Fig. 3. However, there was only about 64% to 85% identity with those of *Pseudomonas putida* CF600 [17, 26], *Pseudomonas putida* mt-2 [4, 9, 12, 27], *Comamonas testosteroni* TA441 [1], *Pseudomonas putida* RE204 [5, 6], and *Acinetobacter* sp. YAA [8]. Recent study on the crystal structure of enoyl-CoA hydratase suggests that a glutamate serves as the catalytic acid for providing the alpha-protein and that another glutamate serves as the catalytic base for the activation of a water molecule in the hydratase reaction [7]. The *xylJ* gene of strain S-47 has two glutamates (E106 and E108) and four aspartates (D78, D154, D158, and D 78) which might represent potential active site residues.

The amino acid sequence of the XylK gene from the strain S-47 exhibited 99% identity with that of the NahM of the *P. stutzeri* An10 [3], as shown in Fig. 4. However, there was only about 53% to 76% identity with those of *C. testosteroni* TA441 [1], *P. putida* RE204 [5, 6], *Pseudomonas* sp LB400 [11], *Comamonas* sp. JS765 [10], *P. putida* mt-2 [4 9, 12, 27], and *P. putida* CF600 [17, 26].

The XylQ amino acid sequence from the S-47 strain exhibited 99% identity with that of the NahO of the *P. stutzeri* An10 [3], as shown in Fig. 5. However, there was only about 56% to 79% identity with those of *C. testosteroni* TA441 [1], *P. putida* RE204 [5, 6], *P. putida* CF600 [17, 26], *P. putida* mt-2 [4, 9, 12, 27], and *P. putida* sp. KKS102 [25]. The deduced amino acid sequence of the *xylQ* gene exhibited a short region that is homologous with a number of corresponding dehydrogenases (data not shown). The homologous region coincides with a fold fingerprint which was identified as the ADP binding site [26]. The degree of agreement with the fingerprint (10 to 11 amino acid residues) and the requirement for the cofactor NAD^+ for enzymatic activity strongly suggest that this region participates in NAD^+ binding.

Phylogenetic Characteristics of *xylJQK*

The order of *xylJQK* genes was found to be the same as that of the corresponding genes which are responsible for

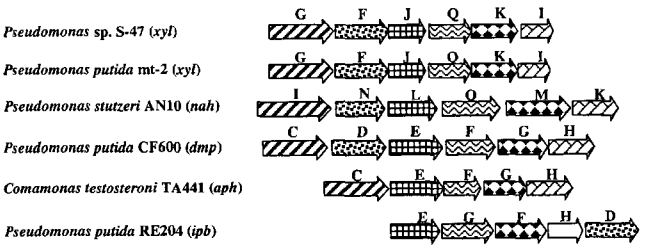

Fig. 6. Genetic organization of *xylJQK* and corresponding genes from several strains degrading catechol via the *meta*-cleavage pathway.

Table 2. *xyl* genes products from *Pseudomonas* sp. S-47 and their identity with other corresponding gene products.

Gene	Gene products	Identity with other gene products (%)*				References
		Xyl	Nah	Dmp	Aph	
xylL	Benzoate *cis*-dihydrodiol dehydrogenase	XylL (97)	-	-	-	16
xylT	Chloroplast-type ferredoxin	XylT (**98**)	NahT (55)	DmpQ (50)	AphQ (19)	17
xylE	Catechol 2,3-dioxygenase	XylE (**100**)	NahH (94)	DmpB (83)	AphB (43)	14
xylG	Hydroxymuconic semialdehyde dehydrogenase	XylG (**90**)	NahI (83)	DmpC (77)	AphC (61)	18
xylJ	2-Oxopent-4-enoate hydrotase	XylJ (79)	NahL (**93**)	DmpE (85)	AphE (64)	This study
xylQ	Acetaldehyde dehydrogenase	XylQ (57)	NahO (**99**)	DmpF (57)	AphF (79)	This study
xylK	2-Oxo-4-hydroxy pentanoate aldolase	XylK (55)	NahM (**99**)	DmpG (53)	AphG (76)	This study

*Percentage identity is given at the amino acid level: Xyl is from TOL plasmid pWW0 of *Pseudomonas putida* mt-2; Nah, *Pseudomonas stutzeri* AN10; Dmp, *Pseudomonas putida* CF600; Aph. *Comamona testosteroni* TA441.

the *meta*-cleavage *xyl* operon of TOL plasmid from *P. putida* mt-2 [4, 9, 12, 27], the *nah* operon of *P. stutzeri* An10 [3], and the *dmp* operon of *P. putida* CF600 [17, 26], as can be seen in Fig. 6. In those strains, the genes are organized as a conserved gene cluster (homologous to *xylGFJQKI*) encoding the conversion of catechol to central metabolites which feed into the tricarboxylic acid cycle. The same group of three genes was also found to exhibit the same order as the terminal three genes in the *aph* operon of *Comamonas testosteroni* TA441 [1] and the *ipb* operon of *P. putida* RE204 [5, 6]. But these strains do not have the conserved gene cluster (homologous to *xylGFJQKI*) that was observed in the *xyl* operon of the TOL plasmid from *P. putida* mt-2 [4, 9, 12, 27], *nah* operon of *P. stutzeri* An10 [3], and the *dmp* operon of *P. putida* CF600 [17, 26].

The amino acid sequences of XylL, XylT, XylE, and XylG from the strain S-47 showed 97, 98, 100, 98, and 90% identity, respectively, as compared with those in TOL plasmid from *Pseudomonas putida* mt-2 as shown in Table 2. But the XylJ, XylQ, and XylK showed 79%, 57%, and 55% identity with those of the mt-2 strain, respectively. On the other hand, the amino acid sequences of the XylT, XylE, and XylG showed 55%, 94%, and 83% identity, respectively, compared with those of the corresponding enzymes from *Pseudomonas stutzeri* An10 (Table 2). However, the XylJ, XylQ, and XylK of the strain S-47 showed 93%, 99%, and 99% identity to those from *P. stutzeri* An10, respectively. The *xylJQK* genes of S-47 strain showed relatively lower identity, as compared with the *xyl, dmp*, and *aph* genes.

Thus, the *xylJ*, *xylQ*, and *xylK* genes of *Pseudomonas* sp. S-47 are more closely related to the *nahT*, *nahH*, and *nahI* of *Pseudomonas stutzeri* An10, even though the genes (*xylLTEGF*) located upstream of *xylJQK* show high homology with those of *Pseudomonas putida* mt-2. These findings suggest that the *xylLTEGI* genes might originate from the TOL plasmid of *P. putida* mt-2 and the *xylJQK* genes might come from *P. stutzeri* An10, resulting in the recombination of the *xylLTEGFIJQK* gene cluster in order for the strain S-47 to catabolize benzoate and catechol completely as the carbon and energy sources.

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