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Genetic Structure of *xyl* Gene Cluster Responsible for Complete Degradation of (4-Chloro)Benzoate from *Pseudomonas* sp. S-47

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Abstract *Pseudomonas* sp. S-47 is a bacterium capable of degrading benzoate as well as 4-chlorobenzoate (4CBA). Benzoate and 4CBA are known to be degraded via a *meta*-cleavage pathway characterized by a series of enzymes encoded by *xyl* genes. The *meta*-cleavage pathway operon in *Pseudomonas* sp. S-47 encodes a set of enzymes which transform benzoate and 4CBA into TCA cycle intermediates via the *meta*-cleavage of (4-chloro)catechol to produce pyruvate and acetyl-CoA. In the current study, the *meta*-pathway gene cluster was cloned from the chromosomal DNA of S-47 strain to obtain pCS1, which included the degradation activities for 4CBA and catechol. The genetic organization of the operon was then examined by cloning the *meta*-pathway genes into a pBluescript SKII(+) vector. As such, the *meta*-pathway operon from *Pseudomonas* sp. S-47 was found to contain 13 genes in the order of *xylXYZLTEGFJQKIH*. The two regulatory genes, *xylS* and *xylR*, that control the expression of the *meta*-pathway operon, were located adjacently downstream of the *meta*-pathway operon. The *xyl* genes from strain S-47 exhibited a high nucleoside sequence homology to those from *Pseudomonas putida* mt-2, except for the *xylJQK* genes, which were more homologous to the corresponding three genes from *P. stutzeri* AN10. One open reading frame was found between the *xylH* and *xylS* genes, which may play a role of a transposase. Accordingly, the current results suggest that the *xyl* gene cluster in *Pseudomonas* sp. S-47 responsible for the complete degradation of benzoate was recombined with the corresponding genes from *P. putida* mt-2 and *P. stutzeri* AN10.

Key words: *Xyl* genes, nucleotide and amino acid sequences, (4-chloro)benzoate degradation, *Pseudomonas* sp. S-47

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Benzoate and chlorobenzoates are the major intermediate products from the aerobic catabolism of various aromatics and polychlorinated biphenyls. Therefore, the degradation of (choro)benzoates is important for the complete catabolism of such compounds. The bacterial degradation of (chloro)benzoates via (chloro)catechols is a typical degradation pathway [13, 22], through which (chloro)benzoates are converted into (chloro)catechols, then the resulting catecholic compounds are transformed via either a *meta*- or *ortho*-cleavage pathway. In addition, the subsequent reactions from the catecholic compounds lead to the production of Krebs cycle intermediates [9, 12].

The most comprehensively studied *meta*-cleavage pathway is that of the TOL plasmid pWW0, which encodes the toluene degradation pathway of *Pseudomonas putida* mt-2 [6, 14, 16]. The *meta*-cleavage pathway genes are located in the plasmid as an operon that is comprised of 13 structural genes, *xylXYZLTEGFJQKIH*, as shown in Fig. 1. The overall conserved gene order has already been reported in several bacterial strains that exhibit similar degradation activities to other (chloro)aromatic compounds, including naphthalene-degrading *Pseudomonas stutzeri* AN10 [5], phenol-degrading *P. putida* CF600 [21], phenol-degrading *Comamonas testosteroni* TA441 [1], and isopropylbenzene-degrading *P. putida* RE204 [3, 10]. However, a recent analysis of the pWW0 nucleotide sequence suggested that multiple transposition and recombination have produced a complex evolution in the *xyl* gene cluster [23].

Pseudomonas sp. S-47 was originally isolated from contaminated waste by Seo *et al.* [20] and can convert benzoate and 4-chlorobenzoate (4CBA) into the corresponding catechols, which are then utilized as the sole source of carbon and energy through a *meta*-cleavage pathway. The *xylE* and *xylJQK* genes involved in the degradation of benzoate have also been previously studied [17, 18]. Accordingly, the current study determined the complete nucleotide and

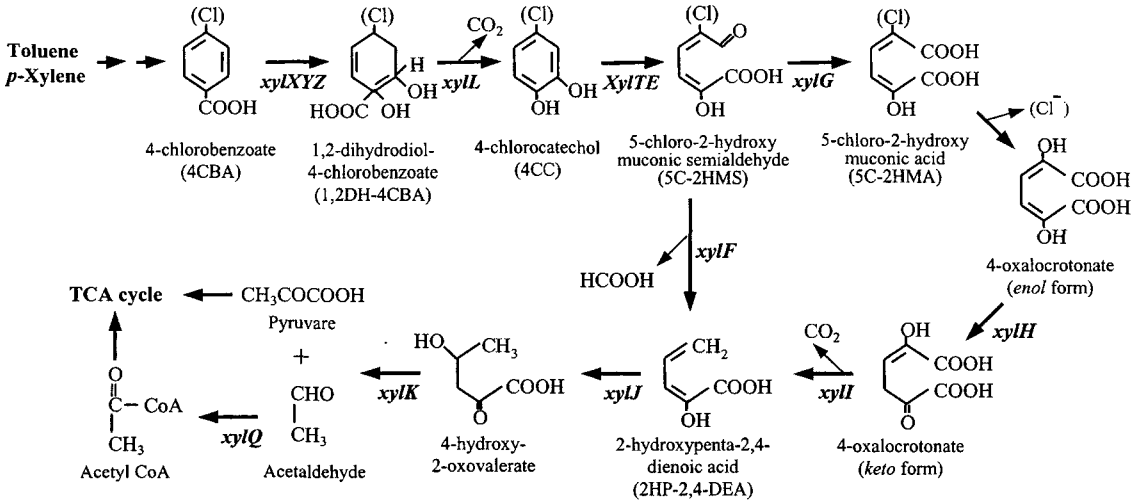

Fig. 1. Pathway for degradation of (4-chloro)benzoate by *Pseudomonas* sp. S-47.

amino acid sequences of the *xylXYZLTEGFJQKIH* gene cluster and analyzed its genetic organization based on a comparison with the corresponding genes from other strains.

MATERIALS AND METHODS

Strains and Cultivation

Pseudomonas sp. strain S-47 was grown at 30°C in an MM2 minimal medium [1 mM $FeSO_4 \cdot 7H_2O$, 100 mM $CaCl_2 \cdot 7H_2O$, 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. Meanwhile, *E. coli* XL1-Blue was used as the host strain for the recombinant plasmids and grown in a Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 1% NaCl) at 37°C. The transformation was accomplished using the calcium chloride method [19], and the transformants selected by supplementing the LB medium with ampicillin at a final concentration of 50 mg/ml. pBluescript II SK(+) was used as the cloning and sequencing vector.

Sequencing and Analysis of 16S Ribosomal DNA

The 16S rRNA gene was PCR amplified using the 27F (*E. coli* numbering 8-27) and 1492R (*E. coli* numbering 1492-4510) primers with ExTaq DNA polymerase (Takara, Japan). The PCR product was then purified using a Quantum Prep Freeze N Squeeze DNA Gel extraction spin column (Bio-Rad, U.S.A.), and a fragment of the 16S rRNA gene introduced into a pGEM-T vector (Promega Co., Madison, WI, U.S.A.). The automated DNA sequencing reactions were performed and resolved using an ABI 377 automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA, U.S.A.), utilizing 27F, 350F, 750F, and 1050F, as the forward primers and 520R, 900R, 1392R, and 1492R as the reverse primers to ensure full overlapping coverage in both directions. The resulting DNA sequences were then aligned with the DNASIS program (Hitachi version 7.0) and analyzed using the GenBank database and Ribosomal Database Project.

Cloning and Nucleotide Sequencing of *xyl* Gene Cluster

The plasmid DNA was isolated by the alkali lysis method as described by Sambrook *et al.* [19], while the DNA cleavage by restriction endonuclease and ligation of the DNA fragments by T4 DNA ligase was performed by standard procedures as recommended by the supplier (Kosco Co., Seoul, Korea). The *xyl* gene cluster from the chromosomal DNA of *Pseudomonas* sp. S-47 encodes a set of enzymes that transform 4CBA/BA into TCA cycle intermediates via the *meta*-cleavage of (4-chloro)catechol. Therefore, the *xyl* gene cluster was cloned from the chromosomal DNA of strain S-47 to obtain pCS1, which included the degradation activities for 4CBA and catechol. The genetic organization of the cluster was then characterized by cloning the *xyl* genes into a pBluescript SKII(+) vector.

Sequence Analysis of *xyl* Gene Cluster

The nucleotide and deduced amino acid sequences were analyzed using DNASIS and PROSIS software (Hitachi version 7.0, Japan). The amino acid sequences were compared with the GenBank database using programs based on the BLAST algorithm, and multiple alignments were generated using the Clustal X algorithm and used for a phylogenetic analysis. The data set was also subjected to a UPGMA analysis of PHYLIP (Department of Genetics, the University of Washington), and the display program TreeView used to visualize the phylogenies of the PHYLIP tree file.

Nucleotide Sequence Accession Number

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

RESULTS AND DISCUSSION

Identification and Characterization of Strain S-47

Strain S-47, originally isolated from contaminated waste by Seo *et al.* [20], is an aerobic Gram-negative rod and its optimal conditions for growth are 30°C and pH 7.0. In addition, strain S-47 degrades and grows well on toluene, p-xylene, benzoate, 4-chlorobenzoate, protocatechuate, catechol, and 4-chlorocatechol (Table 1).

Therefore, to identify strain S-47, its 16S rRNA gene was amplified and the PCR product was sequenced with internal primers. A phylogenic tree based on the neighbor-joining method is presented in Fig. 2. Based on a preliminary comparison against the GenBank and Ribosomal Database Project databases, the 16S rRNA sequence exhibited high levels of identity (above 96%) with those from several different *Pseudomonas* species. Thus, on the basis of a phylogenetic analysis of its 16S rRNA sequence, strain S-47 was positively identified as *Pseudomonas* sp. strain S-47.

Nucleotide Sequences and Genetic Organization of *xyl* Gene Cluster

pCS1 carrying the *xyl* gene cluster was previously cloned from the chromosomal DNA of *Pseudomonas* sp. S-47 [15]. However, in the current study, a DNA fragment of pCS1 digested with endonucleases was introduced into the polyclonal region of a pBluescript II SK (+) vector to create pCSP3, pCSP21, and pCS201. The nucleotide sequence (16,886 bp) of the *xyl* gene cluster for the degradation of 4CBA was then determined from the insert of pCSP3, pCSP21, and pCS201. The *xyl* gene cluster was found to contain 16 open reading frames (ORFs), as shown in Fig. 3 and Table 2, and a sequence analysis of the identified 16 ORFs revealed that the genes related to benzoate degradation had almost the same gene organization as the genes in pWW0 from *Pseudomonas putida* mt-2 [6, 13, 16], except for ORF1 adjacent to the *xylS* gene (Fig. 3). Therefore, the 16 ORFs were designated as *xylX*, *xylY*, *xylZ*, *xylL*, *xylT*, *xylE*, *xylG*, *xylF*, *xylJ*, *xylQ*, *xylK*, *xylI*, *xylH*, *orf1*, *xylS*, and *xylR*. At the junctions of *xylT*-*xylE* and *xylK*-*xylI*, the last base of the last codon of the previous gene overlapped with the first base of the ATG start codon of the next gene. The position of each gene, number of nucleotides, % G+C, terminal codon, intergenic space, and number of amino acids are all listed in Table 2. A putative promoter sequence upstream of the translational start of *xylX* exhibited a high homology to a group of promoters, including the *xyl* operon promoter of the toluene-xylene catabolic plasmid pWW0. In addition, two regulatory genes, *xylS* and *xylR*, were located adjacent to each other at the end of the *meta*-cleavage pathway gene cluster.

Table 1. Degradation of various aromatic compounds by *Pseudomonas* sp. S-47.

Aromatic compounds	Degradability
Toluene	+++
p-Xylene	++
Benzoate	+++
4-Chlorobenzoate	+++
Protocatechuate	+++
Catechol	+++
4-Chlorocatechol	+++
4-Chlorobiphenyl	-
Biphenyl	-
Phenol	-
3-Chlorobenzoate	+
4-Hydroxybenzoate	+

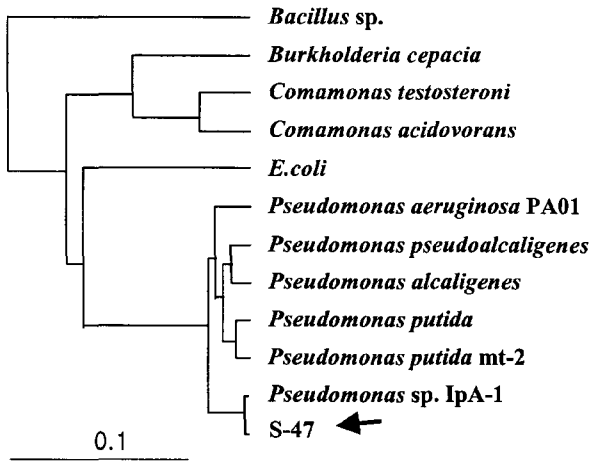

Fig. 2. Rooted neighbor-joining tree based on the nearly complete 16S rDNA sequence of *Pseudomonas* sp. S-47.

The genetic organization of the *xyl* genes from *Pseudomonas* sp. S-47 was compared with those of analogous genes reported from other strains as shown in Fig. 4. There are several different nomenclatures (cf. *nah*, *dmp*, *aph*, *ipb*, *ben*, *bph*, etc.) for the isofunctional *xyl* genes specifying the catabolic pathway of benzoate and related aromatic compounds as shown in Table 3. The *nah* and *bph* genes have been reported to degrade two-ring aromatic compounds, while other genes degrade one-ring aromatic compounds, such as benzoate (*Pseudomonas putida* F1), xylene (*Pseudomonas putida* mt-2), and phenol (*Pseudomonas testosteroni* TA441), which are oxidized to produce catechol, followed by the *meta*-cleavage pathway

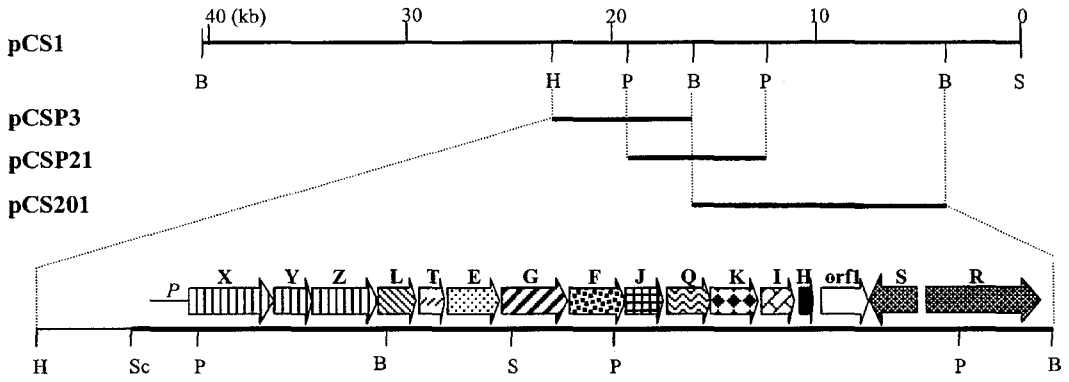

Fig. 3. Genetic maps of pCS1 and genetic organization of *xyl* genes from *Pseudomonas* sp. S-47.
Abbreviations: B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; S, *Sac*II; Sc, *Sac*I.; *p*, promoter site.

of catechol [1, 9, 14]. The *xylXYZL* genes from S-47 strain degrading benzoate exhibited a homology to the *xyXYZL* genes from *P. putida* mt-2, *ipbAbAcB*, and *benABCD*, yet not to the *dmp* and *aph* genes. However, the *xylTEGFJQKIH* genes from S-47 strain encoding the *meta*-cleavage pathway of catechol showed a relatively high homology to the corresponding genes from most previously reported strains [1, 5, 10, 21]. Furthermore, the products of *xylXYZLTEGF* and *xylIHSR* from strain S-47 revealed very high identities (93% to 100%) with the corresponding enzymes from strain mt-2 as regards the amino acid sequence. Meanwhile, the XylXYZ from strain S-47 was found to include an aromatic ring dioxygenase, belonging to class IB of Batie's classification reported for *Pseudomonas* spp. [2]. In addition, the XylF product, hydroxymuconic semialdehyde hydrolase, from *Pseudomonas* sp. S-47 was a serine hydrolase that contained a Ser-107, Asp-228, and His-256 catalytic triad, belonging to a group of enzymes known as the α/β hydrolase-fold family [8].

The deduced amino acid sequences of the XylJ, XylQ, and XylK genes from *Pseudomonas* sp. S-47 exhibited 93%, 99%, and 99% identities to the corresponding gene products from *Pseudomonas stutzeri* An10, respectively [5]. A recent study on the crystal structure of enoyl-CoA hydratase (XylJ) suggested that a glutamate serves as the catalytic acid for providing the alpha-protein, while another glutamate serves as the catalytic base for the activation of water molecules in the hydratase reaction [11]. As such, the XylJ from strain S-47 revealed two glutamates (E106 and E108) and four aspartates (D78, D154, D158, and D178), representing potential active site residues. In addition, the XylQ exhibited a short region that coincided with a fold fingerprint identified as an ADP binding site [21]. Consequently, the degree of agreement

Table 2. Summary of the *xyl* gene cluster in *Pseudomonas* sp. S-47.

Gene	No. of nucleotide	% G+C	Terminal codon	Intergenic spacing (bp)	No. of amino acids	Characteristics
xylX	1,368	59	TAA	-	455	Rieske-type [2Fe-2S] motif
xylY	489	57	TGA	0	162	
xylZ	1,011	61	TAG	11	336	Chloroplast-type [2Fe-2S] motif, FAD binding domain, NAD binding domain
xylL	777	61	TGA	116	257	Short-chain alcohol dehydrogenase pattern
xylT	339	61.9	TGA	146	112	Chloroplast-type [2Fe-2S] motif
xylE	927	57.5	TGA	-1	307	Fe^{2+} binding domain
xylG	1,461	66.8	TGA	34	486	NAD binding domain
xylF	846	67	TGA	12	281	Nucleophilic elbow motif
xylJ	786	65	TGA	13	261	Glu-106, Glu-108, Asp-78, Asp-158, Asp-178
xylQ	924	65	TAA	16	307	βαβ fold fingerprint region
xylK	1,041	65	TGA	14	346	
xylI	795	64.6	TGA	-1	264	
xylH	192	60.4	TGA	50	63	Pro-2, Arg-12, Arg-40
Orf1	1,023	60.9	TGA	206	340	Transposase
xylS	929	51.6	TGA	89	321	
xylR	1,701	57.7	TGA	400	566	

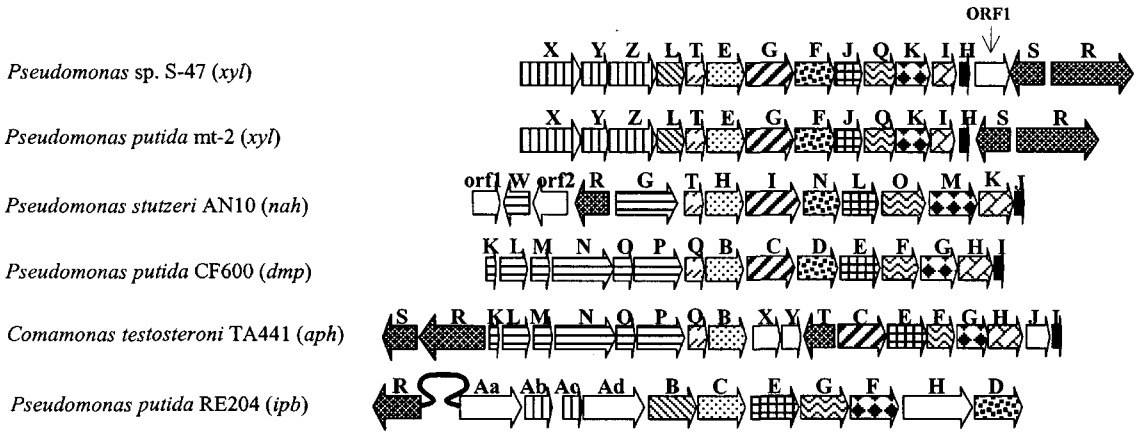

Fig. 4. Genetic organization of *meta*-pathway genes from *Pseudomonas* sp. S-47 and other strains.

Table 3. Products of *xyl* genes and identity with other corresponding gene products.

Gene	Gene product	Identity with other gene products (%)[a]							
		Xyl	Nah	Dmp	Aph	Ipb	Ben	Bph	Others
xylX	B1,2O large (α) subunit	**XylX (97)**	-[b]	-	-	IpbAb (31)	BenA (65)	-	AntA (49), CbdA (55), CmtAb (27)
xylY	B1,2O small (β) subunit	**XylY (96)**	-	-	-	IpbAc (17)	BenB (59)	-	AntB (36), CbdB (53), CmtAc (17)
xylZ	B1,2O reductase component	**XylZ (91)**	-	-	-	-	BenC (51)	-	AntC (43), CbdC (45)
xylL	Benzoate *cis*-dihydrodiol dehydrogenase	**XylL (97)**	-	-	-	IpbB (26)	BenD (59)	-	CmtB (31)
xylT	Chloroplast-type ferredoxin	**XylT (98)**	NahT (55)	DmpQ (50)	AphQ (19)	-		-	PhhQ (43)
xylE	Catechol 2,3-dioxygenase	**XylE (100)**	NahH (94)	DmpB (83)	AphB (43)	-		-	AtdB (72), PhnE (48)
xylG	Hydroxymuconic semialdehyde dehydrogenase	**XylG (90)**	NahI (83)	DmpC (77)	AphC (61)			-	AtdC (70)
xylF	Hydroxymuconic semialdehyde hydrolase	**XylF (93)**	NahN (90)	DmpD (73)		IpbD (57)		BphD (27)	
xylJ	2-Oxopent-4-enoate hydrotase	XylJ (79)	**NahL (93)**	DmpE (85)	AphE (64)	IpbE (64)		-	AtdE (63)
xylQ	Acetaldehyde dehydrogenase	XylQ (57)	**NahO (99)**	DmpF (57)	AphF (79)	IpbG (76)		BphG (56)	
xylK	2-Oxo-4-hydroxy pentanoate aldolase	XylK (55)	**NahM (99)**	DmpG (53)	AphG (76)	IpbF (75)		BphI (75)	CdoJ (56)
xylI	4-Oxalocrotonate decarboxylase	**XylI (97)**	NahK (950	DmpH (87)	AphH (59)	-		BphH (40)	CdoJ (57)
xylH	4-Oxalocrotonate tautomerase	**XylH (98)**	NahJ (90)	DmpI (79)	AphI (46)	-		-	PhnL (43)
xylS	Regulatory protein	**XylS (98)**	-	-	-	IpbR (56)	BenR (58)	-	CbdS (42)
xylR	Regulatory protein	**XylR (95)**	-	DmpR (66)	AphR (46)	-	-	BphR (40)	TmbR (95)

[a]Percentage identity is given at the amino acid level.
[b]The homology with *xyl* (S-47 strain) was not detected.
Xyl is from TOL plasmid pWW0 of *Pseudomonas putida* mt-2; Nah, *Pseudomonas stutzeri* AN10; Dmp, *Pseudomonas putida* CF600; Aph, *Comamona testosteroni* TA441; Ipb, *Pseudomonas putida* RES204; Ben, *Pseudomonas putida* F1; Bph, *Pseudomonas* sp. LB400; Ant, *Pseudomonas* sp. CA10; Cbd, *Burkholderia cepacia* 2CBS; Cmt, *Pseudomonas putida* F1; Phh, *Pseudomonas putida* P35X; Atd, *Acinetobacter* sp. YAA; Phn, *Sphingomonas chungbukensis* DJ77; Cdo, *Comamonas* sp. JS765; Tmb, *Pseudomonas putida*.

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1     CTTGGAGAGTGGGCGGCGGCAAAAACTGAGTGCAACACCT
                  IR
                      ***
41    GACCTTTCCGGTAAGGTGCTGCCCCATGTCCTATTCCGAA--

          ***
1085  --AATGACCGTGTTGCACTCAGCTCCTGCAACCGCCGTGTCT
                 IR
1125  AAGAAAGCCGGGGCGATTCAACCCATCCCTTCTTCGGCTA
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Fig. 5. Nucleotide sequence of ORF1 in inverted repeats (IR) indicated by arrows.
Putative initiation (ATG) and termination (TGA) codons of ORF1 are indicated by asterisks.

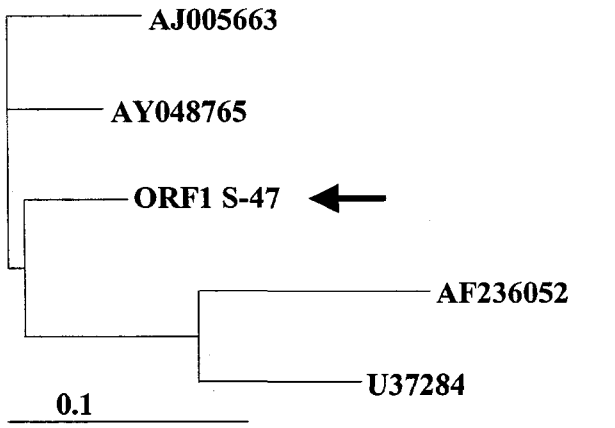

Fig. 6. Phylogenic relationship between ORF1 from *Pseudomonas* sp. S-47 and 4 transposase homologues.
Abbreviations: AF236052, putative transposase [*Pseudomonas aeruginosa* PAO17]; AJ005663, putative transposase [*Pseudomonas stutzeri* OX1]; AY048765, transposase-like protein [*Pseudomonas fluorescens*]; U37284, putative transposase [*Pseudomonas alcaligenes*].

with the fingerprint (10 to 11 amino acid residues) and requirement of the co-factor NAD^+ for enzymatic activity strongly suggested that this region participates in NAD^+ binding, thereby indicating that the *xylJQK* genes from strain S-47 were transposed from other strains through a transposase including ORF1.

Analysis of ORF1

The position of ORF1 in strain S-47 was unique between the *xylH* and *xylS* genes (Fig. 4) and showed the highest amino acid sequence identity (96%) to that of the transposase-like protein from *Pseudomonas fluorescens* (AY048765). Including two imperfect 25 bp inverted repeats (IR), as shown in Fig. 5, the ORF1 in strain S-47 was composed of 1,023 bp and encoded a polypeptide chain with a molecular mass of 38 kDa consisting of 340 amino acid residues. Yet, no such ORF has been observed in *Pseudomonas putida* mt-2, where the *xyl* gene organization is almost identical to that in S-47 strain. However, the pWW0 from *Pseudomonas* sp. mt-2 has been reported to carry insertions including an IS*Ppu12* transposable element in various locations, usually within the catabolic genes [23]. In addition, analysis of pWW0 would seem to suggest that the plasmid has had a complex evolution and that multiple transposition and recombination events by horizontal transfer of the genes have made a major contribution to its present structure.

The ORF1 from strain S-47 demonstrated a high homology to the transposase-like protein (94%) from *Pseudomonas stutzeri* OX1 [4], putative transposase (86%) from *Pseudomonas alcaligenes* [24], and transposase-like protein (85%) from *Pseudomonas aeruginosa* PAO17 [7] as shown in Fig. 6. Bosch *et al.* [5] reported that the ORF1 and ORF2 encoding TnpA3 and TnpA2, respectively, in *Pseudomonas stutzeri* AN10 functioned as transposases. The *xyl* genes responsible for the complete degradation of (4-chloro)benzoate in *Pseudomonas* sp. S-47 exhibited higher than 90% homology with the corresponding *xyl* genes from *Pseudomonas putida* mt-2, except for *xylJQK*, which showed 93% to 99% homology with *Pseudomonas stutzeri* AN10. However, the *xylJQK* genes from *Pseudomonas* sp. S-47 showed only 55% to 79% homology with the corresponding genes from *P. putida* mt-2, even though the remainder of the *xyl* gene cluster organization was the same. Therefore, the current results would seem to suggest that the product of ORF1 is a transposase enzyme that facilitates the transposition of genetic elements, like *xylJQK* genes, into *Pseudomonas* sp. S-47 from *P. stutzeri* AN10.

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REFERENCES

1. Arai, H., T. Ohishi, M. Y. Chang, and T. Kudo. 2000. Arrangement and regulation of the genes for *meta*-pathway enzymes required for degradation of phenol in *Comamonas testosteroni* TA441. *Microbiology* **146:** 1707-1715.
2. Batie, C. J., E. LaHaie, and D. P. Ballou. 1987. Purification and chracterization of phthalate oxygenase and phthalate oxygenase reductase from *Pseudomonas cepacia*. *J. Biol. Chem.* **262:** 1510-1518.
3. Berendes, F., N. Sabarth, B. Averhoff, and G. Gottschalk. 1998. Construction and use of an *ipb* DNA module to generate *Pseudomonas* strains with constitutive trichloroethene and isopropylbenzene oxidation activity. *Appl. Environ. Microbiol.* **64:** 2454-2462.

4. Bertoni, G., M. Martino, E. Galli, and P. Barbieri. 1998. Analysis of the gene cluster encoding toluene/*o*-xylene monooxygenase from *Pseudomonas stutzeri* OX1. *Appl. Environ. Microbiol.* **64:** 3626-3632.
5. Bosch, R., E. Garcia-Valdes, and E. R. B. Moore. 2000. Complete nucleotide sequence and evolutionary significance of a chromosomally encoded naphthalene-degradation low pathway from *Pseudomonas stutzeri* AN10. *Gene* **245:** 65-74.
6. Burlage, R. S., S. W. Hooper, and G. S. Sayler. 1989. The TOL (pWW0) catabolic plasmid. *Appl. Environ. Microbiol.* **55:** 1323-1328.
7. Dean, C. R. and J. B. Goldberg. 2000. The *wbpM* gene in *Pseudomonas aeruginosa* serogroup O17 resides on a cryptic copy of the serogroup O11 O antigen gene locus. *FEMS Microbiol. Lett.* **187:** 59-63.
8. Diaz, E. and K. N. Timmis. 1995. Identification of functional residues in a 2-hydroxymuconic semialdehyde hydrolase. *J. Biol. Chem.* **270:** 6403-6411.
9. Eaton, R. W. 1996. p-Cumate catabolic pathway in *Pseudomonas putida* F1: Cloning and characterization of DNA carrying the *cmt* operon. *J. Bacteriol.* **178:** 1351-1362.
10. Eaton, R. W., O. V. Selifonova, and R. M. Gedney. 1998. Isopropylbenzene catabolic pathway in *Pseudomonas putida* RE204: Nucleotide sequence analysis of the *ipb* operon and neighboring DNA from pRE4. *Biodegradation* **9:** 119-132.
11. Engel, C. K., M. Mathieu, J. P. Zeelen, J. K. Hiltunen, and R. K. Wierenga. 1996. Crystal structure of enoyl-coenzyme A (CoA) hydratase at 2.5 angstroms resolution: A spiral fold defines the CoA-binding pocket. *EMBO J.* **15:** 5135-5145.
12. Geshcher, J., A. Zaar, M. Mohamed, H. Schägger, and G. Fuchs. 2002. Genes coding for a new pathway of aerobic benzoate metabolism in *Azoarcus evansii*. *J. Bacteriol.* **184:** 6301-6315.
13. Harayama, S. and M. Rekik. 1990. The *meta* cleavage operon of TOL degradative plasmid pWW0 comprises 13 genes. *Mol. Gen. Genet.* **221:** 113-120.
14. 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.
15. Kim, K. P., D. I. Seo, L. 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. strain S-47. *J. Microbiol.* **36:** 99-105.
16. Marques, S. and J. L. Ramos. 1993. Transcriptional control of the *Pseudomonas putida* TOL plasmid catabolic pathways. *Mol. Microbiol.* **9:** 923-929.
17. 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.
18. Park, D.-W., J. H. Lee, D. H. Lee, K. Lee, and C.-K. Kim. 2003. Sequence characteristics of *xylJQK* genes responsible for catechol degradation in benzoate-catalyzing *Pseudomonas* sp. S-47. *J. Microbiol. Biotechnol.* **13:** 700-705.
19. Sambrook, J., E. F. Fritisch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual.* 2nd. Ed., Cold Spring Harbor, New York, U.S.A.
20. Seo, D. I., J. C. Chae, K. P. Kim, Y. S. Kim, K. S. Lee, and C. K. Kim. 1998. A pathway for 4-chlorobenzoate degradation by *Pseudomonas* sp. S-47. *J. Microbiol. Biotechnol.* **8:** 96-100.
21. Shingler, V., J. Powlowski, and U. Marklund. 1992. Nucleotide sequence and functional analysis of the complete phenol/3,4-dimethylphenol catabolic pathway of *Pseudomonas* sp. strain CF600. *J. Bacteriol.* **174:** 711-724.
22. Song, B., J. K. Lee, and M. M. Häggblom. 2002. Characterization of bacterial consortia capable of degrading 4-chlorobenzoate and 4-bromobenzoate under denitrifying condition. *FEMS Microbiol. Lett.* **213:** 183-188.
23. Williams, P. A., R. M. Jones, and L. E. Shaw. 2002. A third transposable element, IS*Ppu12*, from the toluene-xylene catabolic plasmid pWW0 of *Pseudomonas putida* mt-2. *J. Bacteriol.* **184:** 6572-6580.
24. Yeo, C. C. and C. L. Poh. 1996. IS1394 from *Pseudomonas alcaligenes* N.C.I.B. 9867: Identification and characterization of a member of the IS30 family of insertion elements. *Gene* **175:** 109-113.