Two identical copies of IS1246, a 1275 base pair sequence related to other bacterial insertion sequences, enclose the *xyl* genes on TOL plasmid pWW0

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Two identical direct repeats of a 1275 bp sequence, designated IS1246, encompass the *xyl* genes, which determine the catabolism of toluene, *m*- and *p*-xylenes to central metabolites, on the TOL catabolic plasmid pWW0. IS1246 has a terminal inverted repeat of 12 bp (5'GGGCACCTCGAA3') and contains a major open reading frame of 280 codons. This ORF shows significant homology with ORFs encoded by a number of bacterial insertion sequences from *Bacteroides*, *Neisseria* and *Escherichia coli*.

Keywords: TOL plasmid pWW0, insertion sequences IS1246, toluene-xylene catabolism, *Pseudomonas putida*, catabolic plasmid

The 117 kbp TOL plasmid pWW0 confers the ability to grow on the aromatic hydrocarbon toluene and some alkyl-substituted toluenes in host *Pseudomonas putida* strains (Worsey & Williams, 1975; Kunz & Chapman, 1981). The 18 catabolic structural genes are in two adjacent operons (*xyl*/CMABN and *xyl*/XYZLTEGFJQKIH), the expression of which is regulated by the products of two genes *xyl*/R and *xyl*/S: all the *xyl* genes are clustered on a region consisting of less than one-third of the plasmid (Fig. 1; Assinder & Williams, 1990). Two types of rearrangements of the *xyl* gene cluster have been reported.

(a) Transposition. Two overlapping transposons, Tn4651 (56 kbp) and Tn4653 (70 kbp) carry the *xyl* genes as their central portion (Tsuda & Iino, 1987, 1988). Both are class II (Tn3-like) transposons which share *tnpT*, *tnpS* and *res* genes but have individual *tnpR* and *tnpA* genes and are bounded by terminal inverted repeats of 46 bp (Tn4651) and 38 bp (Tn4653) (Tsuda *et al.*, 1989). Both transposons confer the toluene⁺ growth phenotype upon *Pseudomonas* hosts.

(b) Deletion. The *xyl* genes can be lost from pWW0 by spontaneous deletion (Bayley *et al.*, 1977) forming a cryptic transmissible plasmid pWW0-8 (Downing *et al.*, 1979). The deletion is of a specific 39 kbp fragment and

occurs by recombination between two apparently homologous direct repeats originally estimated to be of about 1.4 kbp (Meulien *et al.*, 1981). None of the transposon functions are lost from the plasmid in the deletion and the residual 17 kbp of Tn4651 present (i.e. 56–39 kbp) can still act as a cryptic transposon Tn4652 (Tsuda & Iino, 1987).

Determination of the nucleotide sequences of the regions encompassing both direct repeats reveals they are identical repeats of 1275 bp (Fig. 2) with perfect inverted terminal repeats of 12 bp of 5'GGGCACCTCGAA3' which are not homologous to the terminal repeats of either of the TOL transposons. Comparison of the 1275 bp nucleotide sequence and all potential reading frames with the nucleotide and inferred protein databases was performed using the BLAST server (National Center for Biotechnology Information, Bethesda, USA) (Altschul *et al.*, 1990). The DNA shows limited homology (35% identity) over a 1200 bp region with IS1186 from *Bacteroides fragilis* (accession no. X72301) and with the very similar IS1168 from plasmid pIP417 of *Bacteroides vulgatus* (accession no. X71444).

Analysis of potential protein products shows one large open reading frame (ORF1) with an overlapping smaller open reading frame (ORF2) on the opposite strand (Fig. 2), a pattern which is found with a number of other IS sequences of similar size. The ORFs are capable of encoding proteins of 280 and 170 amino acids (32 kDa and 19 kDa), respectively. Comparison with the data-

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The GenBank accession numbers for the insertion sequences and their flanking DNA reported in this paper are L11585 and L11583, respectively.

Fig. 1. Restriction map of TOL plasmid pWW0 showing relative location of the two directly repeated copies of IS1246 and the *xyI* genes encoding the catabolism of toluene and the xylenes; the arrows represent the length and direction of the four known transcripts. The IS sequences run from left to right in the 5'–3' direction of Fig. 2. This map has been constructed from an assemblage of published restriction maps and nucleotide sequences.

<u>EcoRI</u>	
GGGCACCTCG <u>AA</u> TAAACGGA GGTITTCAGT <u>GAATCCGACC</u> GCCAATGAT GGCCATGATC 60	
GAATTCGGTT CGATTCTCGG GCGCAATCAC CGCTTCTGCG GCGGTGTCGC CCGTTTTTCT 120	
<u>PstI</u>	
TCCGAATCGC <u>CCTCTGCAGC</u> GCGGATTCGG CCCACTCCGG GCGTCAGAAG GCCTTGATTC 180	
CCGCCCTCTG GAAGTACACC AGCCGCTTCA AGTTGTAGCA GCGCGCCATC ATCGTCATCG 240	
CAAACTTCGG GCGGCCCTGT CCGATGGTAC GGATCAACTT GCGGCCCATC TGCTCGATGC 300	
TCCGGAACAC GTGCTCCACT GCGGACGCGC TCTTGGCGAT GCGGTGGTTG CGCCGTTGCT 360	
<u>KpnI</u>	
GACACTCGGA AAGGGGCTTG TTGCGGTACC CTTTCTCTG GATCTGATTA CGATAGCCGT 420	
GATCCTTGAG CCAGCCCTCC CGGTCTGCGC TGGTGTGCCC TCCGTCCGCA TAGACATCGC 480	
ORF2-	
GGCTCGTGT GCTCCGGTCG ATGAGCGCAT CGAAGTGCTT GCTGTCA ^{atg} ACGCTGGCCG 540	
TATCAGTCTC GAATTTCGGG ATGATCTTGT ACITCTTGTG CACGTTGACC GAGAGCTTGT 600	
AGCCGAAGTG GTTCTTGGCG TGCTTCTGCG TCCAGGTGGC ATCCGTATCC TTCTCGGCCG 660	
GTTTGGCTGG CTTCCAGTTA GCGGCGATCG CGCCTTCCCT GACCAGCTTG TTCTCTCTAC 720	
GGCTGTTATG TTGCTTGGGA GCAGGCACCA GCGTGGCATC GACGATTGG CCGCGCGGGG 780	
CGATGAATCC GTGCGGAGAG AGTTGGGCGG TGACACCGTC GAACAGGACC TTCGCGCCGG 840	
CGTCGCCGAT GCGGTTCTCG AACGTCCACA CGGTGCTTGG ATCCGGAAAT TTGACCGCGC 900	
TGGCCAAACC GCAGAATCGC TTGTAGTCA TCCGGTCAAG CAATGGTAC TCCATCTGTT 960	
-ORF1	
CATCCGACAG GTTGACAGC CGCTTGACCA CCAGAATCCG CACCATCGTC TCCGTGGAT 1020	
ACGGCGGGCG ACCGCTT ^{gag} GGCTGACAGG GCGCGGCGCT ACCCGATCGA CTTCCGCGCG 1080	
AAGTGCGCGG AAATCGATGT ACGACTCGAT CTGGGCCAGC GGATCTCCAA GCGTGTGAT 1140	
CTTCTTCGGA TGATGTTCTG CAGCGAACAG GTCCGTCTTG ATGGCAGTGC GCTTCTTCAT 1200	
GGCTGTGTGC AGAGAACGAA ATCAGGACAA CGCAATTTTA CCAATCGCAG GGGCGCGAGG 1260	
TTTTTCGAGG TGCCC 1275	

Fig. 2. Nucleotide sequence of IS1246. The two main open reading frames are denoted. The complement of ORF1 is shown in the 3'–5' direction from nucleotide 1006 to 164 and its start and stop codons are in underlined italics. The minor reading frame ORF2 runs in the 5'–3' direction from nucleotide 527 to 1039 and its start and stop codons are in underlined lower case. The 12 bp inverted repeats at the termini of the sequence are in bold and underlined. The characteristic *EcoRI*, *PstI* and *KpnI*

ORF encoded by:	
IS1246	MVRILVLKRL YNLSDEQMEY QLDRMSYKR FCGLASAVNI PDRTTVWTFE 50
IS1186	MFKILFLQRY YGLSDHQIEY QIVDRSFRA FLGIENVDVY PDEKTIWKYR
IS1106	MRA EAPSESVPPY SYCLR..LRR LVLI.....F VNPVLFMYR
IS5	MLRIHCMQHW YNLSDGAMED ALYEIASMRL FARLSLDSAL PDRTTIMNFR
Consensus	m-ril-lqr- ynlSd-qmey ql-dr-s-rr f-g----- pdrtti----
51	
IS1246	NRIGDAGA.K VLFDGVTALQ LRHGFAR.. GGQIVDATLV ..PAPKQRNS 100
IS1186	EALTSVGAYD KLFEDFRFTM EAKGLQFN.. EGRIIDASFV ..VAPRQRNT
IS1106	NWLAQDDTLS ELLELINRQL TEKGLKVEKA SAAVVDATII QTAGSKQRQA
IS5	HLLEQHQLAR QLFKTINRWL AEAGVMM..T QGTLVDATII EAPSSTK...
Consensus	n-l-q-ga-- -Lfe-inr-l -ekGI----- g-ivDat-- --pa-qqrn-
101	
IS1246	RE..ENKLVR EGAMPANW.. KPAKRRQKDT DATWTQKHGK NHFGYKLSVN 150
IS1186	RE..ENAKIK EGKGDELWND NPHKKFHKDV DARWTEKRD TFYGYKHVFG
IS1106	IEVDEEGQIS GQTTPS.... ..KDK DARWIKKNGL YKLGYKQHTR
IS5	NKEQQRDP EMHQTCKGNQ WHFGMKAHIG
Consensus	re--en-i- eg--p--w-- -p-k--qkD- darwtK-g- -hfGyKqhv-
151	
IS1246	VDDKYKIIRK FETDTASVHD SKHFDALIDR SNTSRDVIAD RGHTSADREG 200
IS1186	VDKGNKVILS YATTPANVHD SKGFQELLDE SDKOKDLYLD AGY..AGQES
IS1106	TDAE.GYTEK LHITPANAHE CKHLPLLEG LPKGTTVYAD KGYDSAENRQ
IS5	VDKASGLTHS LVTTAANEHD LNQLGNLLHG ..EEQFVSAD AGYQGAQPRE
Consensus	vD-k----- l-ttpAnvHd skh---Lldg s-k--dvyaD aGy-sA-q--
201	
IS1246	WLKDHGYRNQ IQRKGYRNKP LSECQRRNHRIAK TRARVEHVFA 250
IS1186	TVKEHGMNPI ICEKGRRNHP LTEKQKAENRRKSK TRCLVEHVFG
IS1106	HLKEHQLQDG IMRKACRNRP LTETQTKRNRYLSK TRYVVEQSFG
IS5	ELAEVDVDWL IAERPGKVRT LKQHPKRNKT AINIEYMKAS IRARVEHPFR
Consensus	-lkehg--- I--kg-rnrp lte-q-krnr -----rk-k tR-rVEhvFg
251	
IS1246	SIEQ.MGGKL IRTIGQGRAN FAMTMAACY NLKRLVVF... ..QKAGIKAF 300
IS1186	FEEQSMHGLI VRTIGIVRAK ANVAMTNLT NIFRYQIVC NKRELAIAQ.
IS1106	TLHRKFYAR AAYFGLIKVS AQSHLKAMCL NLLKAANRLS APASA.....
IS5	IIKRQFGFVK ARYKGLKND NQLAMLFELA NLFRAQDMIR QWERSH....
Consensus	-ie---g--- -r-iGL--a- aq-am-a-cy Nlfra-q--- ----a-i-

Fig. 3. Multiple alignment of similar ORFs translated from several insertion sequences using PILEUP. The consensus sequence derived by analysis of the alignment by PRETTY is shown below, with identical residues in upper case and similar residues in lower case. The ORFs derived from each IS element were: IS1246 (ORF1 translated from the complementary strand to base pairs 1006–164 of Fig. 2), IS1186-hypothetical protein (S34201), IS1106-hypothetical protein (S22628), IS5-hypothetical protein (P03837).

banks reveals that ORF1 has significant homology to other ORFs encoded by IS1186 and IS1168 from *Bacteroides* and to a lesser extent with IS1106 from *Neisseria meningitidis* (Knight *et al.*, 1992; accession no. Z11857) and IS5 from *Escherichia coli* (Kröger & Hobom, 1982; accession no. J01735). The amino acid sequences of all the

sites are overlined and underlined. The DNA carrying the two IS elements was obtained by cloning fragments *SacI*-G and *XhoI*-F (Fig. 1) in both orientations into pUC18 in its *SacI* and *SaI* sites respectively. The approximate locations of the direct repeats were inferred from the identical configuration of *EcoRI*-*PstI*-*KpnI* sites on the two fragments (Meulien *et al.*, 1981). A series of appropriate nested deletions were generated from all four recombinant plasmids using exonuclease III and the sequences determined by dideoxy sequencing using the two pUC universal primers. The complete sequence of each IS, with its flanking DNA, was obtained from overlapping sequences on both strands.

ORFs were analysed using the GCG suite of programs running at the Daresbury Laboratory, UK (Devereux *et al.*, 1984). A pairwise alignment using the GAP program showed that IS1246 ORF1 and a hypothetical protein coded by IS1186 and IS1168 showed 42% amino acid identity and an additional 17% similarity. A multiple alignment for all four sequences was produced using the program PILEUP, from which a consensus protein sequence was obtained using the default parameters of PRETTY. This shows 24 identical amino acid residues as well as many conservative replacements (Fig. 3) and the great similarity between IS1246 and IS1186 can clearly be seen as they share 112 identical amino acids in this alignment. The function of none of these putative gene products is known. A computer search using the PROSITE database for protein motifs (Bairoch, 1993; Devereux *et al.*, 1984) failed to identify recognizable features.

As a result of the above similarities we propose that the directly repeated TOL plasmid sequence be designated IS1246, although we have no direct evidence that it is active as an insertion sequence.

The evidence suggests that IS1246 plays no role in the function of the two TOL transposons: there is no homology between its terminal repeats and those of the two TOL transposons, and the reciprocal recombination between its two directly repeated copies which leads to the loss of the 39 kbp *xyI* coding region, and therefore to loss of one copy of IS1246, has no effect upon the transposability of either transposon. Presumably their presence is the fortuitous result of some other event in the past history of plasmid pWW0.

We have also sequenced 497 bp on the 5' side and 285 bp on the 3' end of the left-hand copy of IS1246 (Fig. 1) and 264 bp on the 5' side and 221 bp on the 3' side of the right-hand copy and within these flanking regions have located one end of the *xyI* genes. At bases 259–264 on the 3' side of the right-hand IS1246 is the *HpaII* site at the beginning of the sequence identified as the upstream activator region for the *xyI*/CMABN operon (Inouye *et al.*, 1984): this site is 208 bp upstream of the start of transcription of the operon. At the other end of the region between the repeats (i.e. 5' of the right-hand IS1246) we were unable to find any homology with published sequences around the *xyI*/R gene, so this direct repeat lies more than 264 bases from the *xyI* coding region. Restriction maps indicate that this distance is considerably larger, about 3 kbp (Fig. 1).

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