

Identical resolvases are encoded by Pseudomonas TOL plasmids pWW53 and pDK1

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The independently isolated plasmids pWW53 (1) and pDK1 (2) both confer the ability to grow on toluene and some alkyl-substituted toluenes on the host bacterium *Pseudomonas putida*. Both plasmids carry apparently identical regions of about 26 kbp, based on our analysis of 73 recognition sites for eight restriction endonucleases. As a result we have suggested that the plasmids share a common evolutionary origin (3). We have determined the DNA sequence of a small part of this common region on the two plasmids by the Sanger dideoxy approach (4). An identical open reading frame was present on these plasmids which appears to encode a previously unidentified protein of 181 amino acids in length with molecular weight of 20 123. A search using the programme PROSRCH (Biocomputing Research Unit in Molecular Biology, Edinburgh University, Scotland) was performed which revealed that the open reading frame was very similar to the *E.coli* R46 recombinase (Res4) (5). The comparison found 134 identical residues, 20 conservative replacements and 27 dissimilar amino acids. Further analysis revealed homologies with several known resolvases as shown in Figure 1.

The presence of the same ORF on the two catabolic plasmids further supports our suggestion that the two TOL plasmids carry a large homologous region. On each plasmid the resolvase gene is positioned upstream of a region (designated *xylS2*) which is homologous to part of the gene *xylS* whose product positively regulates transcription (3, 6) of the toluene catabolic pathway on the archetypal TOL plasmid PWW0 (7). The *xylS2* gene is truncated and lacks approximately one third of the 5′ coding sequence found on the PWW0 homologue (3).

These results indicate that this common region on the two plasmids could have suffered an insertion of an unknown transposon into a full-length *xylS* gene prior to the separate existence of the two plasmids.

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PWW53 RES       MRLFGYARVSTSQQSLDVQIKALKAENVRATRIFTDKVSGSHVNREGLRMLRLKVEEGDV
RES4 RECOM      MRLFGYARVSTSQQSLDIQIKGLKEAGVKASRIFTDKASGSSTDRKGLDLLRMKVEEGDV
TNP1 ECOLI      MRLFGYARVSTSQQSLDIQVRALKDAGVKANRIFTDKASGSSSDRKGLDLLRMKVEEGDV
TNP3 ECOLI      MRIFGYARVSTSQQSLDIQIRALKDAGVKANRIFTDKASGSSTDREGLDLLRMKVEEGDV
TNP3 KLEPN      MRIFGYARVSTSQQSLDIQIRAVKDAGVKANRIFTDKASGSSTDREGLDLLRMKVEEGDV
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PWW53 RES       VLVKKLDRLGRDTADMIQLIKEFDDMGVAIRFLDDGISTEGTMGKMVVTILSAVAQAERL
RES4 RECOM      TLVKKLDRLGRDTADMIQLTKEFDAQGVAVRFIDDGISTDGEMGKMVVTILSAVAQAERR
TNP1 ECOLI      ILVKKLDRLGRDTADMIQLIKEFDAQGVSIRFIDDGISTDGEMGKMVVTILSAVAQAERQ
TNP3 ECOLI      ILVKKLDRLGRDTADMIQLIKEFDAQGVAVRFIDDGISTDGDMGQMVVTILSAVAQAERR
TNP3 KLEPN      ILVKKLDRLGRDTADMIQLIKEFDAQGVAVRFIDDGISTDGDMGQMVVTILSAVAQAERR
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PWW53 RES       RILERTNEGRLEAKAKGVKFGRKPTVDKAEVFTLHGQGISAMEIAKRLKIGRSTVYKVL-
RES4 RECOM      RILERTNEGRQEAKLKGIRFGRKRIIDRNSVLALHQQGTGATDIARRLSIARSTVYKILE
TNP1 ECOLI      RILERTNEGRQEAMAKGVVFGRKRKIDRDAVLNMWQQGLGASHISKTMNIARSTVYKVIN
TNP3 ECOLI      RILERTNEGRQEAKLKGIKFGRRRTVDRNVVLTLHQKGTGATEIAHQLSIARSTVYKILE
TNP3 KLEPN      RILERTNEGRQEAKLKGIKFGRRRTVDRNVVLTLHQKGTGATEIAHQLSIARSTVYKILE
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Figure 1. Alignment of the pWW53/pDK1 deduced protein sequence (PWW53 Res) with *E.coli* recombinase, Res4 Recom (5) and *E.coli* resolvases TnP1 (8), TnP3 (9) and *Klebsiella pneumoniae* TnP3 resolvase (10). The symbol * denotes identical residues and . denotes a conservative substitution. The program CLUSTAL was used to align the sequences (accessed via SEQNET at the SERC Daresbury Laboratory, UK).

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