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TOL Is a Broad-Host-Range Plasmid

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We readily isolated insertions of the carbenicillin resistance element Tn*401* into the TOL plasmid in *Pseudomonas putida*. Hybrid TOL::Tn*401* plasmids stably express the Cb^r phenotype in *Pseudomonas aeruginosa* and *Escherichia coli*. Whereas the replicative and conjugative functions are expressed in both hosts, the ability to grow on *m*-toluate is only expressed in the *Pseudomonas* species.

Degradative plasmids which encode catabolic pathways for various hydrocarbons are common in saprophytic pseudomonads. These include the CAM, OCT, NAH, SAL, and TOL plasmids (4-6, 11, 12). There is both scientific and industrial interest in these plasmids because of their role in the evolution of bacterial metabolism and their potential use in genetic engineering to produce organisms for pollution control and chemical syntheses. Hence, one important question about degradative plasmids concerns their host range and function in different bacterial species. CAM and OCT belong to the P-2 incompatibility group whose host range is limited to the genus *Pseudomonas* (9). There are no reports on transfer of the other degradative plasmids outside *Pseudomonas,* but failure to find such transfer can result from at least two problems: (i) inability to enter or replicate in other taxonomic groups or (ii) inability to express the degradative phenotype in those groups. The latter explanation is plausible because plasmid-encoded gene products must integrate into the host biochemical pathways, transcription systems, and often the cell envelope (3, 7). By integrating transposable antibiotic resistance elements with widely expressed phenotypes into degradative plasmids (1), we have been able to distinguish these possibilities for the TOL plasmid.

TOL confers the ability to utilize toluene and xylenes and their oxidation products as a sole carbon and energy source on *Pseudomonas putida* (12, 13). Aside from conjugal transfer, this approximately 75-megadalton plasmid confers no other known phenotype on its host.

To facilitate a number of genetic experiments, we isolated TOL::Tn*401* plasmids which also encode resistance to penicillins by virtue of the transposable beta-lactamase gene on the Tn*401* element (1). These plasmids were generated by placing TOL in a strain that contained the resistance plasmid R1162::Tn*401*. R1162::Tn*401* is a derivative of the Inc-P4 SmSu plasmid R1162 (9), into which the Tn*401* element has transposed from the RP1 plasmid (9) in a *P. putida* host. The Tn*401* element in R1162::Tn*401* is approximately 3.3×10^6 daltons (R. Meyer, personal communication) and therefore similar to other reported transposable beta-lactamase determinants (2, 8). Strains carrying TOL and the R1162::Tn*401* resistance plasmid were crossed to a plasmid-free recipient, and we selected for carbenicillin-resistant (Cb^r) exconjugates. Out of 420 Cb^r exconjugates tested, 45 did not have the streptomycin and sulfa resistance phenotypes determined by the R1162 plasmid. Forty-one of these clones were phenotypically Tol$^+$ and four were Tol$^-$. Apparently these clones had inherited TOL plasmids into which Tn*401* had transposed from R1162::Tn*401* (into the *tol* genes in the latter four cases). We verified this conclusion for three independent Tol$^+$ clones by showing 100% linkage of the Cb^r and Tol$^+$ determinants in conjugation and transformation experiments. Electrophoresis of plasmid DNA (Fig. 1) shows that three TOL::Tn*401* plasmids (two *tol*$^+$ and one *tol*) migrate at the same position as TOL in 0.7% agarose gels; two *tol* plasmids are clearly smaller than TOL and appear to have suffered a deletion. These smaller plasmids are incompatible with TOL in *P. putida*. This result indicates that both replicate under TOL control and shows linkage of the Cb^r and Tol$^-$ determinants.

To test the host range of one of the *tol*$^+$ TOL::Tn*401* plasmids, we crossed strain PpS1094 with *Pseudomonas aeruginosa* PAS102 (*thr* derivative of PAC1) and *Escherichia coli* QSR0 (a nonrestricting *thr*$^+$ *leu* derivative of C600). We also used plasmid DNA from PpS1093 to transform QSR0. In all cases we obtained Cb^r recombinants. With PAS102 and QSR0 recipients, we found conjugal transfer frequencies of approximately 4×10^{-7} and 4×10^{-5} Cb^r recombinants per donor, respectively. As Fig. 1 shows, Cb^r clones from both conjugation and transformation contained plasmid DNA indistinguishable from TOL::Tn*401* in this gel system. The *P. aeruginosa* strain PAS309 is

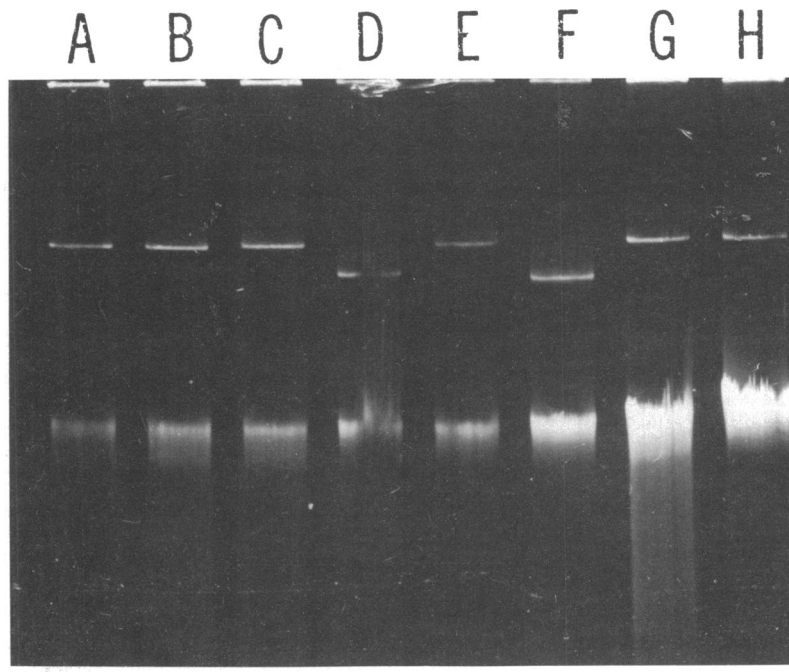

FIG. 1. *Agarose gel analysis of plasmid DNA in strains carrying TOL and TOL::Tn401 plasmids. We extracted plasmid DNA by a cleared lysate procedure (1). After deproteinization and ethanol precipitation, 15-μl samples were subjected to electrophoresis for 2 h at 150 V through a 0.7% agarose gel, visualized by staining with ethidium bromide, and photographed under long-wavelength UV light (10). The various slots contained DNA of the following strains: A, PpS1066 (TOL); B, PpS1093 Tol$^+$ (TOL::Tn401); C, PpS1094 Tol$^+$ (TOL::Tn401); D, PpS1092 Tol$^-$ (TOL::Tn401); E, PpS1115Tol$^-$ (TOL::Tn401); F, PpS1116 Tol$^-$ (TOL::Tn401); G, PAS309 (TOL::Tn401); H, QSR164 (TOL::Tn401). PpS1093 and 1094 are separate P. putida Tol$^+$ Cb^r clones from one experiment. Tol$^+$ Cb^r clones from three independent experiments all have plasmids with the same electrophoretic mobility. PpS1092, -1115, and -1116 are independent Tol$^-$ Cb^r P. putida clones. PAS309 is a Tol$^+$ P. aeruginosa strain that received TOL::Tn401 from PpS1094 by conjugation. QSR164 is an E. coli Cb^r clone that received TOL::Tn401 from PpS1093 by transformation. Its Tol phenotype is discussed in the text. Crosses and transformations were done as previously described (1). The diffuse band of chromosomal DNA fragments closer to the bottom of the gel covers a size range of about 10 to 20 megadaltons (10). The Tol$^+$ phenotype was determined by growth on 0.2% m-toluate plates (13).*

Tol$^+$ at both 32 and 42°C (but weak at the higher temperature). Two of the Cb^r *E. coli* transformants were Tol$^-$ and unable to grow on *m*-toluate, but they readily transferred the Tol$^+$ phenotype back to *P. putida* in conjugal crosses at a frequency of approximately 10^{-5}. Hence, these clones contained TOL::Tn*401* DNA. These plasmids are compatible with R1162 in *P. putida* and show unique incompatibility properties in *E. coli*. TOL::Tn*401* is lost after introduction of IncP, IncQ, or IncF_1 plasmids (all of which are mutually compatible). Together with the fact that TOL::Tn*401* plasmids lack the SmSu determinants of R1162::Tn*401*, these results indicate that TOL::Tn*401* does not use R1162 functions to replicate in *E. coli*. Growth overnight in carbenicillin-free nutrient medium leads to loss of TOL::Tn*401* in less than 2% of the *E. coli* QSR164 (TOL::Tn*401*) cells. We know that QSR0 will grow on pyruvate, one of the end products of the plasmid-determined *m*-toluate oxidation pathway (13), and QSR164 will grow on 0.2% *m*-toluate if glucose is provided as an alternative carbon source. So the Tol$^-$ phenotype of QSR164 is not due to plasmid loss, a metabolic defect in QSR0, or toxicity of the *m*-toluate. The inability to grow on *m*-toluate must be due to a lack of functional expression of the *tol* genes in *E. coli* genetic background.

Although the exact blocks in *tol*$^+$ expression in *E. coli* remain to be elucidated, our results show that there can be significant differences between functional and replication host ranges for metabolic plasmids. It will be necessary to consider this phenomenon in future studies of plasmid host range and in devising schemes for the construction of bacteria to carry out specific applications.

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ADDENDUM IN PROOF

A recent report has shown by enzyme assays that the *tol*$^+$ genes of an RP4-TOL hybrid plasmid are expressed at very low levels in an *E. coli* K-12 host (T. Nakazawa, E. Hayashi, T. Yokota, Y. Ebina, and A. Nakazawa, J. Bacteriol. **134**:270–277, 1978). These results will account for the inability of QSR164 (TOL::Tn*401*) to grow on *m*-toluate.

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