Transposon-mediated mobilization of chromosomally located catabolic operons of the CAM plasmid by TOL plasmid transposon Tn4652 and CAM plasmid transposon Tn3614

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The CAM (camphor degradation) plasmid is integrated into the chromosome of *Pseudomonas putida* PaW-line strains and is not selftransferable as a plasmid via conjugation. Our results show that the mobilization of chromosomally located CAM and the integration of *cam*-operons into the chromosome of the new Cam⁺ transconjugants is a *recA*-independent process mediated by transposons Tn4652 (17 kbp) and Tn3614 (7.2 kbp). Transposon Tn3614 is apparently identical to the left-hand and the right-hand sequences of the TOL plasmid pWW0 transposon Tn4654. The insertion of Tn401 inside the left-hand terminal IR of Tn4652 completely inhibited the mobilization of CAM. According to our data transposons Tn4652 and Tn3614 together with CAM plasmid catabolic operons are integrated into the chromosome. We propose that in pseudomonads the transposons Tn4652 and Tn3614 play a key role in the evolution and spread of new catabolic plasmids in nature.

Keywords: *Pseudomonas*, CAM plasmid, TOL plasmid, mobilization, transposon

INTRODUCTION

Catabolic plasmids in soil pseudomonads are able to mobilize chromosomal genes (Shaham *et al.*, 1973; Chakrabarty & Gunsalus, 1979) and non-conjugative plasmids (Chakrabarty, 1974; Chakrabarty *et al.*, 1978) at a low frequency (i.e. 10^{-7} to 10^{-8}). Such mobilization can be achieved by Mob proteins acting at an *oriT* site (Derbyshire *et al.*, 1987) or by the action of a transposon (Sherratt *et al.*, 1980; Crisona *et al.*, 1980; Simon, 1984; Derbyshire *et al.*, 1987). In the latter case the mobilization of non-conjugative plasmids can be either *recA*-dependent (Hooykaas *et al.*, 1982) or *recA*-independent (Broome-Smith, 1980). In either case, an initial cointegrate structure is resolved by site-specific recombination at *res* sites to yield the end products of transposition (Kitts *et al.*, 1982).

The TOL plasmid pWW0 provides good examples of recombination events between different DNA regions. This 117 kbp long catabolic plasmid encodes the ability to degrade toluene and related compounds (Williams & Murray, 1974). The catabolic genes (*xyl* genes) are located

within a 39 kbp segment. Excision of this region from pWW0 by reciprocal recombination between 1.4 kbp direct repeats leads to the formation of the cryptic plasmid pWW0-8 (Bayley *et al.*, 1977).

Plasmid pWW0 can recombine with R plasmids where the size of the inserts from the TOL plasmid varies from 56 to 104 kbp (White & Dunn, 1977; Lehrbach *et al.*, 1982). The 56 kbp region, encompassing the *xyl* genes, and the residual 17 kbp region, formed after deletion of the 39 kbp region, are transposons which have been named Tn4651 and Tn4652, respectively. The structural genes essential for transposition, *tnpA*, *tnpS* and *tnpT*, and the resolution site *res*, are located in the 17 kbp of Tn4652; there are no transposition functions in the 39 kbp catabolic region which contains the *xyl* genes (Tsuda & Iino, 1987). Another large (70 kbp) transposon, Tn4653 is also present in the TOL plasmid pWW0. This encompasses Tn4651 and shares with it *tnpT*, *tnpS* and *res*, and additionally a unique *tnpA* (the transposase) and also an adjacent *tnpR* (resolvase) (Tsuda & Iino, 1988). Both Tn4651 and Tn4653 are class-II transposons (Tn3-like) but, whereas the latter shares similarities with other class-II transposons, the former appears to be the first of a new subgroup (Tsuda *et al.*, 1989). Owing to transposition by a Tn3-type transposition mechanism, the TOL plasmid

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Table 1. Bacterial strains and plasmids

Abbreviations: Mxy⁺, Mtol⁺, Ben⁺, Cam⁺, ability to grow on *m*-xylene, *m*-toluate, benzoate and D-camphor; Trp⁻, Met⁻, Ade⁻, Leu⁻, Pro⁻, His⁻, Ile⁻, requirement for tryptophan, methionine, adenine, leucine, proline, histidine and isoleucine, respectively; Sm^r, Cb^r, Km^r, Tc^r, resistance to streptomycin, carbenicillin, kanamycin and tetracycline, respectively; RecA⁻, mutant of *recA* gene.

Strain	Plasmid	Notes	Reference/source
<i>P. putida</i>			
PaW1	pWW0	Mxy ⁺ Mtol ⁺ Ben ⁺ Cam ⁻	Williams & Murray (1974)
PpG1	CAM	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁺	Rheinwald <i>et al.</i> (1973)
PaW8	pWW0-8	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁻ produced by growth on benzoate	Williams & Murray (1974)
	pEST3030	Mxy ⁻ Mtol ⁻ Ben ⁺ Cb ⁺ Tn401 in <i>HindIII</i> G/ <i>EcoRI</i> -C fragment of pWW0-8	This study
	pEST3036	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁻ Cb ^r Tn401 in <i>HindIII</i> -E/ <i>EcoRI</i> -I fragment of pWW0-8	This study
	pEST3051	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁻ Tn401 in <i>HindIII</i> -f/ <i>EcoRI</i> -I fragment of pWW0-8	This study
	pEST3065	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁻ Cb ^r Tn401 in <i>HindIII</i> -f/ <i>EcoRI</i> -b fragment of pWW0-8	This study
	pEST3070	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁻ Cb ^r Tn401 in <i>HindIII</i> -B/ <i>EcoR</i> -K fragment of pWW0-8	This study
KT2440	pKG206	2.3 kbp <i>PstI</i> fragment (<i>camC</i>) of CAM in pKT240	Koga <i>et al.</i> (1986)
PaW85	–	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁻	Bayley <i>et al.</i> (1977)
PaW340	–	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁻ Trp ⁻ Sm ^r	Franklin & Williams (1980)
PpG1400	–	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁻ Trp ⁻ RecA ⁻	Bagdasarian & Timmis (1982)
PpG1400	CAM	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁺ Trp ⁻ RecA ⁻ transconjugant from the mating AC793CAM × PpG1400	This study
AC793	CAM	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁺ Met ⁻	Gift of P. M. A. Broda (UMIST, Manchester, UK)
AC793	CAM, pEST3617	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁺ Met ⁻ Cb ^r Km ^r Tc ^r transconjugant from the mating AC793 × PAO 1822, insertion of Tn3614 into RP1	This study
EST3000	CAM	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁺ Trp ⁻ Sm ^r transconjugant from the mating AC793 CAM × PaW340	This study
EST3001	CAM, pWW0-8	Mxy ⁺ Mtol ⁺ Ben ⁺ Cam ⁺ Trp ⁻ Sm ^r transconjugant from the mating PaW1 × EST3000	This study
EST3002	CAM, pEST3012	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁺ deleted derivative of EST3002 isolated after growth on benzoate	This study
EST3081	CAM, pEST3614	Mxy ⁻ Mtol ⁻ Ben ⁺ Cam ⁺ Trp ⁻ Sm ^r Cb ^r Km ^r Tc ^r transconjugant from the mating EST3001RP1 × PaW85, insertion of Tn3614 into the RP1 <i>tra2</i> gene	This study
<i>P. aeruginosa</i>			
PAO1822	RP1	Ade ⁻ Leu ⁻ Cb ^r Km ^r Tc ^r	Gift of B. Holloway (Monash Univ., Victoria, Australia)
<i>E. coli</i>			
GCB	pOX38::Tn5	Pro ⁻ His ⁻ Km ^r Sm ^r	Gift of L. I. Glatman (Gamaleya Institute, Moscow, Russia)

pWW0 transposons can be used for the mobilization of non-conjugative plasmids.

We discovered that the CAM (which encodes *cam* genes necessary for degradation of camphor) plasmid locates in the chromosomes of PaW-line strain of *P. putida* and that Cam⁺ character is easily transferable to isogenic strains with the aid of conjugative plasmids like pWW0-8 (Mäe *et al.*, 1991). However, because the mobilization efficiency of the TOL plasmid pWW0 is at least a hundred times lower

than that for pWW0-8, in the present paper we studied the role of TOL plasmid transposons in mobilization of the CAM plasmid.

METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used or constructed in this study are described in Table 1.

Media and culture conditions. Luria broth (LB) was used as

the complete medium and basal salt medium based on M9 salt solution (Maniatis *et al.*, 1982) supplemented with trace elements (Bauchop & Elsdén, 1960) was used as the minimal medium (MS). The final concentrations of antibiotics added to the media were: ampicillin (Ap), 30 µg ml⁻¹; carbenicillin (Cb), 1000 µg ml⁻¹; kanamycin (Km), 25 µg ml⁻¹; streptomycin (Sm), 1000 µg ml⁻¹; and tetracycline (Tc), 12.5 µg ml⁻¹. *m*-Toluolate was added to MS at a final concentration of 5 mM and glucose was added to a final concentration of 10 mM. D-Camphor was added as a solid in the lid of the Petri dish. *Pseudomonas* strains were incubated at 30 °C and *Escherichia coli* strains at 37 °C.

DNA transformation. The transformation of *P. putida* cells with plasmid DNA was as described by Bagdasarian & Timmis (1982) and the transformation of *E. coli* cells was as described by Hanahan (1983).

Mating experiments. Conjugation experiments were carried out in LB as described by Ougham *et al.* (1983). Transconjugants were detected by growth on *m*-toluolate and D-camphor plates. Transfer frequencies were calculated as the ratio of transconjugants to donor cells.

Transposon insertions into plasmids. The source of transposon Tn401 (Cb^r) was plasmid RP1 (IncP1) in strain *P. aeruginosa* PAO1822(RP1). RP1 was transferred via conjugation into pWW0-8 or pEST3012 containing strains. The resulting transconjugants were selected on plates containing carbenicillin and used as donor strains for subsequent matings with isogenic plasmid free PaW strains. The source of transposon Tn5 (Km^r) was *E. coli* mini-F plasmid pOX38::Tn5 (Km^r), which does not replicate in *P. putida*. pOX38::Tn5 DNA was transformed to EST3002 (pEST3012) Cam⁺ and Cam⁺ Km^r transformants were used as donor strains for the conjugal transfer of Cam⁺ and Km^r to other host strains.

DNA isolation, analysis and manipulations. Plasmid DNA was isolated by the procedure of Hansen & Olsen (1978) and Connors & Barnsley (1982). DNA cleavage by restriction fragments from low melting point agarose gels and agarose gel electrophoresis were performed as described by Maniatis *et al.* (1982). The sizes of DNA fragments in agarose gels were calculated by comparing their mobilities with restriction fragments (*Hind*III, *Eco*RI) of pWW0 (Downing & Broda, 1979).

Southern blotting and hybridization. DNA fragments were separated in 1.0% agarose gels and transferred to nitrocellulose filters according to Southern (1975). DNA probes were labelled to a specific activity of 1–3 × 10⁷ c.p.m. (µg DNA)⁻¹ by nick-translation (Maniatis *et al.*, 1982).

RESULTS

Mobilization of CAM with plasmid pWW0-8 and its Tn401 derivatives

Plasmid DNA was not detected in EST3000 CAM. Thus the hybridization between *Hind*III digested chromosomal DNA of EST3000 CAM and pKG206 containing the *camC* gene was investigated. pKG206 hybridized with 4.7 kbp and 2.6 kbp fragments of *Hind*III digested DNA (Fig. 1) which are indicative of the *cam* operon (Koga

et al., 1986). The ability to utilize camphor (Cam⁺) was transferable via conjugation from EST3000 CAM to other recipient strains at a frequency of less than 10⁻⁸ (Table 2). After transformation of the TOL plasmid pWW0 into EST3000 CAM, the resulting Cam⁺ Tol⁺ transformants transferred the Cam⁺ character to isogenic PaW strains at a frequency of 10⁻⁷ to 10⁻⁸ (Table 2). However, Cam⁺ Tol⁺ transconjugants containing plasmid pWW0-8 (for example EST3001) transferred the Cam⁺ character via conjugation at a higher frequency (10⁻³) (Table 2).

Hybridization data (not shown) revealed the presence of the *camC* gene in the chromosomal DNA of the Cam⁺ transconjugants.

To verify the role of the TOL plasmid transposon Tn4652 in the mobilization of CAM, we isolated 42 independent pWW0-8::Tn401 (Cb^r) harbouring strains in which the insertion of Tn401 was outside the Tra- and Rep-regions of this plasmid. The insertion sites were mapped by restriction analysis and six Tn401 insertions in pWW0-8 (including pEST3030) were located outside the TOL plasmid transposons Tn4652 and Tn4654 in restriction fragments *Hind*III-G/*Eco*RI-C (Fig. 2a). These Tn401 inserts did not lower the mobilization frequency of the Cam⁺ character from EST3000 CAM (Table 2).

Fourteen Tn401 inserts in pWW0-8 (including pEST3036) did not mobilize the Cam⁺ character from EST3000 CAM (Table 2). In these cases the insertion was located inside the left-hand terminal IR of Tn4652 in restriction fragments *Hind*III-E/*Eco*RI-I (Fig. 1a).

Nineteen of the Tn401 inserts in pWW0-8 mobilized Cam⁺ character from EST3000 CAM at the lower frequency of 10⁻⁵ (Table 2). These Tn401 inserts were either in the restriction fragments *Hind*III-f/*Eco*RI-I (as in pEST3051) or in the restriction fragments *Hind*III-f/*Eco*RI-b (as in pEST3065) (Fig. 2a).

The remaining three Tn401 inserts (including pEST3070) were in the *Eco*RI-K fragment (Fig. 2a) leading to a lower Cam⁺ mobilization frequency from EST3000 CAM (Table 2).

Hybridization of chromosomal DNA to pKG206 showed that all of the Cam⁺ transconjugants arising from the mobilization of Cam⁺ by pWW0-8 or pWW0-8::Tn401 derivatives had the *camC* gene integrated into the chromosome (results not shown).

Mobilization of CAM with the plasmid pEST3012

The plasmid pEST3012 is a conjugative helper plasmid which is derived from the CAM plasmid. It does not contain the catabolic operons of CAM but does contain the 17 kbp transposon Tn4652. The plasmid pEST3012 has a molecular size of 86 kbp and its restriction pattern differs from that of pWW0-8. As for pWW0-8, pEST3012 mobilizes Cam⁺ in PaW strains at the frequency of 10⁻³. Ten Tn401 (Cb^r) insertion mutations of pEST3012 and

Fig. 1. Agarose gel electrophoresis of genomic DNA restriction fragments (left panel) and the corresponding Southern blot hybridization (right panel). Hybridization analysis of various genomic digests using pK206 (*camC*) as the labelled probe. Lane 1, *P. putida* PpG1 (*Hind*III); lane 2, *P. putida* PaW340 (*Hind*III); lane 3, *P. putida* AC793 (*Hind*III); lane 4, *P. putida* PpG1400 CAM (*Hind*III); lane 5, *P. putida* EST3001 (*Hind*III); lane 6, *P. putida* EST3000 (*Hind*III).

Table 2. Conjugal mobilization of *Cam*⁺ character from EST3000 CAM with plasmids pWW0-8::Tn401 (*Cb*^r) to *P. putida* PaW85

Non-conjugative catabolic plasmid	Helper plasmid	Transfer frequencies of <i>Cam</i> ⁺ character on the selection of transconjugants on D-camphor
CAM	—	$\leq 10^{-8}$
CAM	pWW0	$10^{-7} - 10^{-8}$
CAM	pWW0-8	10^{-3}
CAM	RP1	$< 10^{-8}$
CAM	pEST3030	1.0×10^{-3}
CAM	pEST3036	$< 10^{-8}$
CAM	pEST3051	1.3×10^{-5}
CAM	pEST3065	1.0×10^{-5}
CAM	pEST3070	0.6×10^{-8}

five Tn5 (*Km*^r) insertion mutations of pEST3012 were selected. All of the Tn401 and Tn5 insertions were located in a 7.0 kbp region in pEST3012 at the left-hand side of

Tn4652 (Fig. 2a). Transformation of ten pEST3012::Tn401 and five pEST3012::Tn5 derivatives into EST3000 CAM resulted in mobilization of *Cam*⁺ at a frequency of between 10^{-3} to 10^{-4} ; this is comparable to the frequencies mediated by the helper plasmids pWW0-8 and pWW0-8::Tn401.

Mobilization of CAM with RP1::Tn3614

Four *Eco*RI fragments of AC793 CAM chromosomal DNA gave strong hybridization with pWW0-8 DNA (data not shown). Three fragments were the same size as *Eco*RI fragments W, S, Z and the fourth (*n*) was 2.65 kbp of N and G. Because these fragments cover the right-hand and left-hand sequences of Tn4654 in pWW0-8 (Fig. 2a), it was considered to be a putative transposon, Tn3614. To study the properties of this putative transposon in more detail it was introduced into plasmid RP1 as follows.

Firstly, plasmid RP1 was transferred via conjugation at a frequency of 10^{-4} into AC793 CAM. Then 20 independent AC793 CAM RP1 clones were mated individually with the recipient strain PaW340. One out of a hundred clones tested contained the plasmid RP1 with an insertion of 7.2 kbp of DNA. DNA/DNA hybridization and restriction mapping revealed that this 7.2 kbp CAM plasmid transposon is apparently identical to *Eco*RI fragments

Fig. 2. (a) *EcoRI* and *HindIII* cleavage map of pWW0-8 showing the insertion sites for Tn401 (□). The region of Tn401 insertion into pWW0-8 and the number of different clones studied are shown in open boxes. The filled boxes indicate the Tn4652 and Tn4654 genes: *tnpA* (transposase), *tnpR* and *tnpT*, *tnpS* (resolvase), *res* (resolution site) (Tsuda & Iino, 1987, 1988). The hatched boxes indicate DNA present in transposons Tn4654, Tn4652 (Tsuda & Iino, 1987, 1988) and Tn3614 (this paper). (b) *EcoRI/SacII* cleavage map of RP1 (Nakazawa et al., 1980) showing the insertion sites of Tn3614 in EST3080 (pEST3617) and EST3081 (pEST3614) (■). The filled boxes indicate the transfer genes (*tra1*, *tra2*, *tra3*) and the genes for carbenicillin (Cb), tetracycline (Tc) and kanamycin (Km) resistance.

Table 3. Conjugal mobilization of Cam⁺ character with plasmids RP1::Tn3614

Mating		Transfer frequencies of Cam ⁺ character on the selection of transconjugants on D-camphor
EST300 CAM RP1	× PaW85	< 10 ⁻⁸
AC793 CAM RP1	× PaW340	2.0 × 10 ⁻⁸
AC793 CAM pEST3617	× PaW340	1.7 × 10 ⁻³
EST3000 CAM pEST3617	× PaW85	3.0 × 10 ⁻³
AC793 CAM pEST3614	× PaW340	3.9 × 10 ⁻⁶
EST3081 CAM pEST3614	× PaW340	2.0 × 10 ⁻⁶

W, S, Z, n and locates in RP1 close to the *tet* genes not interrupting the *tra* functions of RP1 (Fig. 2b). This derivative of RP1 (pEST3617) mobilized the Cam⁺ character to a frequency of 10⁻³. Plasmid RP1 does not mobilize Cam⁺ character at a detectable level (Table 3).

In the second series of experiments EST3001(RP1) was used as a donor in matings with PaW85 and trans-

conjugants were selected on D-camphor and kanamycin containing MS media. One out of 257 transconjugants contained a single plasmid, pEST3614, which was larger than RP1, and an insertion of DNA in the *tra2* gene of RP1 consistent with transposon Tn3614 was detected (Fig. 2b). The Cam⁺ character was transferred from this strain into the recipient strain PaW85 at a frequency of 10⁻⁶ (Table 3). These new Cam⁺ transconjugants did not contain pEST3614 but the plasmid RP1 and are not able to mobilize the Cam⁺ character. The difference of 10³ in Cam⁺ mobilization efficiencies between pEST3617 and pEST3614 is probably due to the frequency of excision of Tn3614 from RP1.

RecA-independent mobilization of CAM with transposons Tn4652 and Tn3614

The relative efficiencies of transpositional mobilization of CAM by transposons Tn4652 and Tn3614 either into *recA*⁻ strain PpG1400 or from PpG1400 into PaW strains were determined. The three plasmid variants pEST3617, pEST3012::Tn401 and pWW0-8::Tn401 (pEST3030) produced similar results both in the *recA*⁻ strain PpG1400 CAM and in the *recA*⁺ strain AC793 (Table 4). These three plasmids mediated the mobilization of the Cam⁺ character at a frequency of between 10⁻³ and 10⁻⁴ into the new recipients.

Table 4. Conjugal mobilization of Cam⁺ character in *recA*⁺ and *recA*⁻ strains

Mating		Transfer frequencies of Cam ⁺ and Cb ^r characters depending on the selection conditions	
		Glucose + carbenicillin	D-Camphor
AC793 CAM	× PpG1400	0	< 10 ⁻⁸
AC793 CAM pEST3617	× PpG1400	0.5 × 10 ⁻⁴	2.0 × 10 ⁻⁴
AC793 CAM pEST3012::Tn401	× PpG1400	4.4 × 10 ⁻²	1.8 × 10 ⁻²
AC793 CAM pEST3030	× PpG1400	2.7 × 10 ⁻⁴	4.8 × 10 ⁻³
PpG1400 CAM	× PaW85	0	2.1 × 10 ⁻⁷
PpG1400 CAM pEST3617	× PaW85	0.8 × 10 ⁻³	1.4 × 10 ⁻²
PpG1400 CAM pEST3012::Tn401	× PaW85	5.7 × 10 ⁻³	1.5 × 10 ⁻³
PpG1400 pEST3030	× PaW85	1.0 × 10 ⁻⁴	2.6 × 10 ⁻⁴

DISCUSSION

The results presented here show that mobilization of chromosomally located CAM could be achieved by conjugative helper plasmids via the TOL plasmid transposon Tn4652 and the CAM plasmid transposon Tn3614. Tn3614 is not a spontaneous derivative of Tn4654 (Tsuda & Iino, 1988) from which Tn4652 (Tsuda & Iino, 1987) has been deleted. The fragments of Tn4654 *Eco*RI-G, the part of *Eco*RI-N and the part of *Rco*RI-X (i.e. DNA from both ends of Tn4652) are not in Tn3614 (Kivisaar *et al.*, 1988). Tn3614 is an independent transposon that is present in the derivatives of RP1 or in the chromosome of AC793.

The TOL plasmid transposons Tn4651 and Tn4653 are both Tn3-like (class-II) transposons but the former does not share similarities with other class-II transposons and appears to be the first of a new subgroup (Tsuda *et al.*, 1989).

The transposons Tn4651 and Tn4653 contain the iso-functional transposase gene *tnpA* determining the formation of cointegrates as intermediates of transposition (Tsuda & Iino, 1988). The dissociation of cointegrates needs three components determined by Tn4652: the *cis* active *res* site and the *trans* active products of the *tnpS* and *tnpR* genes.

Comparison of the maps of Tn4651 and Tn4653 (Tsuda & Iino, 1988) with the inserts of pWW0 in the eight R::pWW0 cointegrates studied by Lehrbach *et al.* (1982) revealed that only two correspond to the 56 kbp Tn4651, but four to the 69 kbp transposon Tn4653. One of the remaining two cointegrates shares one terminus common to Tn4651 and the other with Tn4653. Although Tn4651 and Tn4653 share the same *tnp* genes, the majority of recombinational events already described did not explain the transpositions from pWW0 (Assinder & Williams, 1990) completely.

Transposon mediated mobilization of a chromosomally located CAM plasmid is *recA* independent. Several experimental results lead to this conclusion. First, hybridization of chromosomal and plasmid DNA of Cam⁺ transconjugants with ³²P-labelled cloned fragments of Tn4652 and *camC* containing plasmid pKG206 showed that after the mobilization of CAM to the new recipient strain only the conjugative helper plasmid pWW0-8, pEST3012 or RP1::Tn3614 are detectable. Also, the catabolic operons of the CAM plasmid are present in the chromosome (Fig. 1). Secondly, the insertion of transposon Tn401 (Cb^r) from plasmid RP1 inside the left-hand terminal IR of Tn4652 inactivates the mobilization of Cam⁺ character (Fig. 2a).

Thirdly, independently isolated pWW0-8::Tn401 clones in which Tn401 is located between the *tnpA* and *tnpT/tnpS* genes of Tn4652 mobilized chromosomal CAM at a frequency of 10⁻⁵ (Table 2, Fig. 2a). This is comparable with the mobilization frequency (10⁻⁶ to 10⁻⁷) of CAM by TOL plasmid pWW0 in which catabolic genes of TOL are located in the same region as Tn4652. However, this is more than a hundred times lower than the frequency achieved with pWW0-8 or pWW0-8::Tn401 plasmids in which Tn401 insertion is outside the TOL plasmid transposons (Fig. 2a). A suitable explanation is that this area in Tn4652 is somehow important for the transpositional mobilization of CAM.

Our results explain the mechanism of the mobilization of non-conjugative plasmids by helper plasmids with the formation of unstable cointegrates before conjugal transfer. Cointegrate molecules could not be detected in the samples of plasmid DNA because the linked molecules were up to 300 kbp in size and could not be detected intact. Also, cointegrates probably have a very short life span in the cell.

The fact that Tn4652 and Tn3614 are present in different conjugative helper plasmids should facilitate their key

role in the development and spread of catabolic plasmids in natural bacterial populations.

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