Characterization of *Pseudomonas* Mercury-resistance Transposon Tn502, Which Has a Preferred Insertion Site in RP1

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Tn502mer differs in size and restriction map from the well-characterized Tn501mer. It also differs in its preferential and high-frequency insertion into the 6 kb *Pst*I-C region of RP1. The affinity for this region is perpetuated in pVS76, a clone of RP1 *Pst*I-C in pBR322. Restriction mapping of independent pVS76::Tn502 derivatives revealed that Tn502 inserted at the same site (or small region) in *Pst*I-C corresponding to the 35 kb coordinate in RP1. Insertion occurred in both orientations, but one was preferred. When *Pst*I-C was deleted from RP1, acquisition of Tn502 was reduced and the sites of insertion randomized.

INTRODUCTION

Tn501, originally from *Pseudomonas aeruginosa*, is the best studied of a group of similarly sized (7.2–8.4 kb) and functionally related transposons that confer resistance only to mercury(II) (Stanisich *et al.*, 1977; Foster, 1987). Tn501 is apparently identical to Tn1861 (from *P. putida*; Friello & Chakrabarty, 1980) and Tn503 (from *P. aeruginosa*; Arwas, 1982) but differs in restriction map from Tn2613 (from *Proteus mirabilis*; Tanaka *et al.*, 1983), Tn3926 (from *Yersinia enterocolitica*; Lett *et al.*, 1985) and the identical pair, Tn3402 and Tn3403 (from *Klebsiella* and *Citrobacter* species respectively; Radford *et al.*, 1981; Radford, 1984). However, one end of each of Tn2613, Tn3402, Tn3403 and Tn21 (a multi-resistant Tn501-related transposon) is similar, carrying an unusual cluster of three *Eco*RI and two *Hind*III sites, and encoding transpositional functions complementary to those of Tn501 (de la Cruz & Grinsted, 1982; Diver *et al.*, 1983; Tanaka *et al.*, 1983; Radford, 1984). Extensive similarities at the phenotypic and DNA sequence levels also exist between the *mer* operons of Tn501 and Tn21 (Misra *et al.*, 1984; Gilbert & Summers, 1988) and to a lesser extent between the *mer* and transpositional activities of Tn501, Tn21 and Tn3926 (Lett *et al.*, 1985).

In this paper a new *mer* transposon, Tn502, is described which differs from Tn501 in size and restriction map and also in its insertional preference for a site or small region in the *Pst*I-C region of RP1.

METHODS

Bacteria and plasmids. The *Escherichia coli* K12 sub-lines used were UB1301 (prototroph, Rif^R) (Grinsted *et al.*, 1972), UB1832 (*his lys trp* Str^R Rif^R) (Sanchez *et al.*, 1982), UB5201 (*pro met recA56* Nal^R) (Sanchez *et al.*, 1982) and its Rif^R derivative, LT105. The *Pseudomonas aeruginosa* PAO sub-lines used were PAO2003 (*argH32*

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Table 1. *Plasmids used*

Plasmid	Characteristics/comments	Source
<i>Conjugative plasmids</i>		
RP1	Ap ^R Tc ^R Km ^R IncP	Grinsted <i>et al.</i> (1972)
pUB307	Tc ^R Km ^R IncP; Tn801amp-deletant of RP1	Bennett <i>et al.</i> (1977)
pUB1601	Tc ^R Km ^R IncP; <i>Pst</i> I-ligation derivative; lacks <i>Pst</i> I-C, D, E and F	Wallace <i>et al.</i> (1981)
R751	Tp ^R IncP	Jobanputra & Datta (1974)
pVS991	R751::Tn502	This paper
pVS992	pUB307 <i>tet</i> ::Tn502	This paper
pVS993	pUB1601 <i>tet</i> ::Tn502	This paper
pVS6	Hg ^R ; source of Tn502 <i>mer</i>	This paper
<i>Non-conjugative plasmids</i>		
pBR322	Ap ^R Tc ^R	Bolivar <i>et al.</i> (1977)
pVS76	Tc ^R Ap ^S clone of pBR322; carries RP1 <i>Pst</i> I-C fragment	Fong & Stanisich (1989)
pVS982	pBR322::Tn501 <i>mer</i> ; Tn501 inserted at co-ordinate 2.3 kb of pBR322	This paper
pVS983	pBR322 <i>tet</i> ::Tn502; Tn502 inserted between <i>Bam</i> HI (0.4 kb) and <i>Sal</i> I (0.7 kb) sites of pBR322	This paper
pVS989	pBR322::IS21; IS21 inserted at co-ordinate 2.5 kb of pBR322	This paper
pVS994-pVS1001	pVS76::Tn502 derivatives	This paper

rec-2 Chl^R Str^R) (Chandler & Krishnapillai, 1974), PAO9503 (*met-28 ilv-202* Str^R) (Stanisich & Bennett, 1976) and PAO9501 (*pur-136 leu-8* Chl^R Rif^R) (Stanisich & Ortiz, 1976) together with derivatives of the latter carrying Tn502 inserted between the *met-28* and *trp-6* chromosomal loci (i.e. PAO9529) or the B3 prophage. The plasmids used are listed in Table 1. pVS6 (Hg^R, Tra⁺) is a new plasmid which confers resistance to mercury(II) but not to organomercurials. It was derived from a Gm^R Km^R Tc^R Sm^R Su^R Hg^R clinical strain of *P. aeruginosa* (provided by R. D. Meyer, Wadsworth Hospital Centre, Los Angeles, California, USA) which transferred only the Hg^R determinant to PAO9501 (frequency 5×10^{-8} per donor in a 12 h broth mating). The pBR322::IS21 derivative was obtained from a rare Ap^R[Str^R] transconjugant from the cross UB5201(pUB307, pBR322) $\times$ UB1832. Acquisition of IS21 was confirmed by the plasmid's size (6.5 kb) and the presence of the IS21-derived *Hpa*I, *Kpn*I, *Sma*I and *Pst*I sites.

Conjugational procedures. (a) *Broth matings.* These were carried out according to Stanisich & Bennett (1976). (b) *Filter matings.* Volumes (0.5 ml) of growing donor and recipient cultures (about 10^8 cells ml⁻¹) were filtered through a 25 mm nitrocellulose membrane (0.45 μ m pore size) and the latter placed onto nutrient agar, bacteria uppermost. After incubation (37 °C, 1–2 h) the bacteria were resuspended, diluted and plated onto selective medium. (c) *Determination of transposition frequency.* IncP plasmids were introduced into PAO9529 by conjugation. The transconjugants were isolated and purified once on selective medium, then donor cultures immediately prepared for mating with PAO9503. Selection in the latter mating was for the transposon marker. (d) *Determination of conduction frequency.* IncP plasmids, with or without a *mer* transposon, were introduced by conjugation into UB5201 carrying pBR322 or a derivative. Transconjugants and donor cultures derived from them were handled as in (c) except that in matings with UB1832 selection was imposed for a pBR322 marker.

Recombinant DNA procedures. Small-scale and large-scale plasmid preparations, transformation, restriction endonuclease digestion and ligation of DNA, and electrophoresis using agarose (0.8% or 1.2%) or polyacrylamide gels (6%) were carried out by the standard procedures described in Maniatis *et al.* (1982).

Abbreviations. Resistance phenotypes are symbolized as follows: Ap, ampicillin; Gm, gentamicin; Hg, mercury(II); Km, kanamycin; Sm, streptomycin; Su, sulphathiazole; Tc, tetracycline; Tp, trimethoprim. The genotype, *ter*, represents resistance to tellurite.

RESULTS

Characterization of Tn502

pVS6 (Hg^R, Tra⁺) does not transfer from *P. aeruginosa* PAO to *E. coli* K12 ($< 10^{-8}$ per donor). Its carriage of the mercury-resistance transposon, Tn502, was recognized by the recovery of Hg^R[Rif^R] progeny in intergeneric matings between PAO2003(RP1, pVS6) and LT105. These progeny carried RP1::Tn502 derivatives as shown by conjugational linkage analysis and plasmid size. Differences in the insertional specificity of Tn502 and Tn501 were suggested by the following observations. (i) Both elements transposed efficiently to RP1 (see next section) but

Fig. 1. (a) The map of RP1 (60 kb) (outer circle) is based on that of Thomas & Smith (1987). The positions of the transfer (Tra) regions, resistance functions and transposable elements (Tn801amp, Tn522ter and IS21) are indicated by the dark and hatched regions. The RP1 derivatives, pUB1601 (49.2 kb) and pUB307 (54.8 kb), are represented by the inner circles. pUB1601 is a ligation derivative consisting of the two largest *Pst*I fragments (Wallace *et al.*, 1981); pUB307 is a Tn801 deletant (Bennett *et al.*, 1977). (b) Plasmid pVS76 (10.4 kb) is a *Pst*I ligation derivative of pBR322 (dark region) and the 6 kb *Pst*I-C fragment of RP1 which includes part of IS21 (hatched region). The preferred site of insertion of Tn502 in *Pst*I-C and the preferred orientation of Tn502 are indicated by the triangle. (c) The map of Tn502mer (9.6 kb). (d) The map of Tn501mer (8.3 kb) is based on that of Bennett *et al.* (1978) and Lett *et al.* (1985) and shows the regions responsible for mercury-resistance (Hg) and transpositional activity (Tnp). The location of *Bgl*I sites is shown below the map by the open-circle symbols. These occur at the following kb map locations: 0.42, 0.51, 1.49, 1.65, 2.05, 3.20, 5.78, 6.17, 6.41, 6.57, 6.95, 7.47, 7.88 and are taken from the Tn501 nucleotide sequence of Brown *et al.* (1983, 1985, 1986) and Lund *et al.* (1986). In all figures cleavage sites for various restriction enzymes are as follows: *Eco*RI (E), *Hinc*II (Hc), *Hind*III (Hd), *Hpa*I (Hp), *Kpn*I (K), *Pst*I (P), *Sal*I (Sa), *Sma*I (Sm), *Sst*II (S). The location of only one of the four *Kpn*I sites of RP1 is shown.

Tn502 failed to inactivate any of the RP1 resistance functions (Ap^R Km^R Tc^R) (<0.05%) under conditions that yielded insertional mutants with Tn501 (0.2–2%; Stanisich *et al.*, 1977). (ii) Previous studies showed that in matings between RP1⁺ donors and B3-lysogenic recipients the transconjugants inherited plasmids with deletions of the Km^R or (some) *tra* loci (Stanisich & Bennett, 1976). When six independent RP1::Tn502 plasmids were subjected to deletion formation, *mer* was lost together with the Tra and/or Km^R functions in 10–42% of the transconjugants. This suggested that Tn502 occurred in the Tra 1–Km^R–Tra 2 region (Fig. 1a) of all six plasmids but at a site(s) that did not affect either Tra or Km^R activities. A corresponding loss of *mer* was not found when three RP1::Tn501 plasmids were similarly tested. (iii) Conduction of pBR322 (a non-conjugative mobilization-deficient plasmid; Finnegan & Sherratt, 1982) was mediated efficiently by Tn501 but not by Tn502 (i.e. frequencies of 3×10^{-5} and 2×10^{-8} from the respective pUB307::Tn⁺ pBR322⁺ donors).

A pBR322-Tn plasmid from each conductional experiment from (iii) above was used to compare the molecular features of the two transposons. Tn502 was larger than Tn501 (9.6 kb vs

8.3 kb) and differed in the number and distribution of *Eco*RI, *Hinc*II and *Sal*I sites (Fig. 1c, d) although both carried a *Hind*III site at one end. Further disparity in the molecular structure of these transposons was evident from *Bgl*I digests subjected to polyacrylamide gel electrophoresis (data not shown). pBR322::Tn501 produced 11 bands, of which five were doublets as deduced from the Tn501 *Bgl*I map (Fig. 1d); pBR322::Tn502 produced 17 bands including one possible doublet. Whilst the positions of the Tn502 *Bgl*I sites were not determined, the 14 internal transposon fragments could be identified and compared with the 12 from Tn501. Only two of these, of 90 bp and 160 bp, were of similar mobility. Thus at the gross level reflected by these various genetic and molecular tests, Tn501 and Tn502 are clearly distinguishable. Tn502 also bears no obvious similarity to other *mer* transposons for which sizes and molecular maps have been reported.

Transposition of Tn502 to RP1 and derivative plasmids

Information regarding the insertional specificity of Tn502 in RP1 was obtained by comparing its transposition frequency to RP1 and the derivative plasmids pUB307 and pUB1601. The latter two have sustained deletions involving the four small *Pst*I fragments (Fig. 1a). When each plasmid was passaged through PAO9529, which carries a chromosomal Tn502, transposition to RP1 and pUB307 occurred at frequencies of about 3×10^{-3} per donor, compared to a significantly reduced frequency to pUB1601 of 10^{-6} per donor. This suggests that loss of the 6.0 kb *Pst*I-C and/or the adjacent 0.8 kb *Pst*I-E fragment of RP1, which occur in the Km^R-Tra 2 region, leads to reduced acquisition of Tn502.

The location of Tn502 in eight RP1::Tn502 and six pUB307::Tn502 derivatives was determined by comparing their *Pst*I profiles with those of the parent plasmids. In all cases, Tn502 (which has no *Pst*I sites) occurred in the *Pst*I-C fragment. Since the Tn502⁺ plasmids had originated from three transpositional donors (i.e. pVS6, pVS983 and PAO9529) including Rec⁺ and Rec⁻ lines, it appears that Tn502 has a strong insertional preference for the *Pst*I-C region. In pUB1601, where the *Pst*I-C region is absent, acquisition of Tn502 was reduced and the sites of insertion random. This was suggested by the isolation of Tc^S mutants (6/150 tested) (but no Km^S or Tra⁻ mutants), and the location of Tn502 in three pUB1601::Tn502 plasmids tested. In *Pst*I/*Sst*II digestions of these, Tn502 (which lacks *Pst*I or *Sst*II sites), occurred in the 1.8 kb *Sst*II fragment of the *tet* gene in a mutant with a Tc^S phenotype, and in the 3.4 kb or 16.3 kb *Pst*I/*Sst*II fragments adjacent to the *tet* gene in two plasmids with unaltered RP1-phenotypes (Fig. 1a) (data not shown).

Tn502-mediated conduction of pBR322 and its derivatives

Tn502-mediated conduction of pBR322 occurs only rarely (data presented above). This feature provided a system for the further analysis of Tn502 behaviour in terms of its affinity for *Pst*I-C and the possible role of IS21 in this interaction given that part of it occurs at one end of *Pst*I-C (Fig. 1a). The ability of pBR322 and two derivatives, pBR322-*Pst*I-C (i.e. pVS76; Fong & Stanisich, 1989) and pBR322::IS21 (see Methods), to be transferred via cointegrate formation with various resident Tn502⁺ conjugative plasmids was then assessed in a Rec⁻ host. Efficient conduction occurred only with the pBR322-*Pst*I-C derivative (Table 2). Thus relocation to pBR322 of *Pst*I-C DNA, but not of IS21, markedly enhances the interaction of this plasmid with Tn502.

Tn502 site-specificity in PstI-C DNA

In order to determine the specificity of insertion of Tn502 in the *Pst*I-C region, eight pVS76::Tn502 derivatives, obtained by conduction from independently constructed pUB1601*tet*::Tn502⁺ pVS76⁺ donors and recovered by transformation, were analysed. All eight were larger than pVS76 by about 10 kb (the approximate size of Tn502) and had insertions in the *Pst*I-C DNA. These insertions were all in the smaller 2.6 kb *Sal*I-*Pst*I segment and, in seven cases, the orientation of insertion was the same (i.e. with the *Hind*III end of Tn502 nearest the *Sal*I site in *Pst*I-C). The site of Tn502 insertion was also apparently identical and was

Table 2. *Tn502*-mediated conduction of *pBR322* and its derivatives

Mobilizing plasmid	Transfer frequency* of:		
	pBR322	pBR322::IS21	pBR322- <i>Pst</i> I-C
pUB307 <i>tet</i> :: <i>Tn502</i>	$<2.0 \times 10^{-8}$	$<2.0 \times 10^{-8}$	1.6×10^{-2}
pUB1601 <i>tet</i> :: <i>Tn502</i>	2.2×10^{-7}	8.6×10^{-7}	2.5×10^{-3}
R751:: <i>Tn502</i>	$<2.0 \times 10^{-8}$	4.0×10^{-7}	8.0×10^{-3}

* Donors were UB5201 sub-lines carrying an IncP plasmid and either pBR322 or a derivative of it. The recipient was UB1301. Filter-matings were carried out and $\text{Tp}^{\text{R}}[\text{Rif}^{\text{R}}]$ or $\text{Km}^{\text{R}}[\text{Rif}^{\text{R}}]$ transconjugants selected to determine the transfer frequency of the R751 derivative and the RP1-deletant plasmids respectively. In all cases the transfer frequency was 0.4–0.8. Conductional transfer of pBR322 or its derivatives was determined from the number of $\text{Tc}^{\text{R}}[\text{Rif}^{\text{R}}]$ transconjugants. Control experiments in which pUB307, pUB1601 and R751 were used yielded low or no ($<5 \times 10^{-7}$) conductional transfer of pBR322 or its derivatives as determined from the recovery of $\text{Ap}^{\text{R}}[\text{Rif}^{\text{R}}]$ transconjugants.

determined precisely from *Sal*I/*Hind*III digests. In all cases these generated a 0.7 kb *Pst*I-C-*Tn502* junction fragment. Since *Hind*III occurs 0.2 kb from the *Tn502* terminus, the transposon insertion site must be 0.5 kb from the *Sal*I site (Fig. 1*b*). *Tn502* was also inserted in the same site in the remaining plasmid, although in the opposite orientation. In this case junction fragments of 2.2 kb and 1.8 kb were found in *Hind*III/*Pst*I and *Hind*III/*Hpa*I digestions respectively. Thus, *Tn502* insertions into the 2.6 kb *Pst*I-*Sal*I segment of the *Pst*I-C fragment are not randomly distributed but occur in a preferred orientation and at a site or small region 0.5–0.6 kb from the *Sal*I end. This site corresponds to co-ordinate 35 kb in RP1.

DISCUSSION

In this study, genetic and molecular differences between the *P. aeruginosa mer* transposons, *Tn501* and *Tn502*, have been identified. Both elements transpose efficiently to RP1 but differ in their target specificity. Grinsted *et al.* (1978) have shown that *Tn501* insertions occur at many sites flanking the *Tn801amp* element but rarely (about 5% of events) in the Km^{R} -Tra region. The latter observation is consistent with our finding that deletions in RP1::*Tn501* plasmids that involved the Km^{R} region did not affect *mer*. In contrast, the *Pst*I-C fragment of this region or, more precisely, a site within it, is strongly preferred by *Tn502*. This was shown by: (i) the co-deletion of *mer* with Tra and/or Km^{R} functions in RP1::*Tn502* plasmids; (ii) the low affinity of *Tn502* for pUB1601 compared to RP1 and pUB307; (iii) the ability of *Tn502*⁺ conjugative plasmids to mobilize a pBR322-*Pst*I-C construct but not pBR322 or an IS21⁺ derivative; and (iv) the mapping of a target site at the 35 kb co-ordinate of RP1.

The observation that IS21 did not enhance the affinity of *Tn502* for pBR322 was significant since the right end of IS21 is part of the *Pst*I-C DNA. It was possible, therefore, that this end of IS21 'directed' transposition of *Tn502* into the *Pst*I-C DNA. Such a situation occurs with *Tn7*, where nucleotides some 12 bp from the target site, but not within it, influence transposition to the *E. coli* K12 chromosome (Gringauz *et al.*, 1988). 'Directed' transposition by IS21 acting alone has been excluded as a possibility and was probably an unlikely one given the 1 kb distance between the IS21 terminus and the *Tn502* target site. The extreme site-specificity of *Tn7* for the *E. coli* K12 chromosome or of IS2 for λ is accompanied by total orientational specificity (Saint-Girons *et al.*, 1981; Gringauz *et al.*, 1988). This does not occur with *Tn502* although one orientation is preferred (1:7 in pVS76) compared to that found with *Tn501* (about 1:1 for RP1 and 1:2 for pUB307; Grinsted *et al.*, 1978).

Only two functions (*mrs*, Thomas & Smith, 1987; and *fiwA*, Fong & Stanisich, 1989) have been ascribed to the *Pst*I-C region of RP1 and none is known involving the *Tn502* target site. This site may also be favoured by the *P. aeruginosa* transposon *Tn2521* ($\text{Cb}^{\text{R}}\text{Sm}^{\text{R}}/\text{Sp}^{\text{R}}\text{Su}^{\text{R}}$; 6.8 kb), which inserts 0.6 kb distal to the *Sal*I site in *Pst*I-C (Sinclair & Holloway, 1982). Insertions of *Tn2521* occurred in one orientation, but as only four events were studied this difference to *Tn502* may not be significant. However, these elements differ in three further respects: (i) *Tn2521* does not transpose in a Rec⁻ host; (ii) the affinity of *Tn2521* for R18

(a plasmid identical to RP1; Thomas & Smith, 1987) is low (10^{-6}) compared to that of Tn502 (10^{-3}); and (iii) the chromosomal location of Tn2521 in *P. aeruginosa* clinical isolates is confined to a site or region co-transducible with the *P. aeruginosa* PAO *pur-136* locus (map position 25 min) (Sinclair & Holloway, 1982). Of two Tn502 chromosomal insertions studied in strain PAO, one occurred between the *met-28* (31 min) and *trp-6* (28 min) loci, as deduced from chromosome mobilization data, and the other between *argB* and *pyrE* (both about 19 min) and co-transducible with these loci (Arwas, 1982). The single feature of similarity between Tn502 and Tn2521 may thus be their preference for the same site or small region in RP1.

Our molecular comparisons of Tn501 and Tn502 were limited to an examination of their restriction maps and *Bgl*I profiles. The latter, in particular, did not identify strong similarities with Tn501 either in the *tnpA* region, which generates six *Bgl*I fragments, or the *mer* region, which generates five. Differences in the target specificities of Tn501 and Tn502 for RP1 and pBR322 could, therefore, be due to different *tnpA* activities. The mercuric reductases specified by Tn501 and Tn502 are also known to be different. Such reductases from different Gram-negative bacteria are immunologically related but comprise two sub-classes – a type I enzyme is specified by Tn501 and a type II enzyme by Tn502 (Silver, 1983; Foster, 1987). Thus, at the gross level, Tn502 appears to differ in both genetic and molecular features from the small group of transposons that encode single resistance to mercury(II) and are all related to Tn501.

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