Location and Characterization of Two Functions on RP1 That Inhibit the Fertility of the IncW Plasmid R388

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Two fertility-inhibition functions which reduce R388 (IncW) transfer were detected on RP1 (60 kb, IncP). The respective genes, *fiwA* and *fiwB*, were mapped by transposon insertion mutagenesis to the regions between coordinates 32.8 to 31.7 kb (*fiwA*), and 59.8 to 0.8 kb (*fiwB*). The *fiwA* function occurs in a non-essential region of RP1 whereas *fiwB* is straddled by essential plasmid-maintenance and host-range determinants and apparently coincides (or overlaps) with the gene for tellurite-resistance.

INTRODUCTION

RP1 (IncP, 60 kb) and two of its derivatives, pVS76 and pUB1601, are each capable of inhibiting the fertility of a co-resident R388 (IncW) plasmid (Yusoff & Stanisich, 1984). pVS76 consists of the 6.0 kb *Pst*I fragment of RP1 inserted in the vector pBR322. This fragment is absent in pUB1601 (49.2 kb), a ligation derivative of RP1 consisting of the two largest *Pst*I fragments (Wallace *et al.*, 1981). RP1 must, therefore, encode at least two fertility inhibition (*fiw*) functions, one of which affects only R388-transferability (the pVS76 component), while the other affects both R388-transferability and pilus production (the pUB1601 component) (Yusoff & Stanisich, 1984). In this paper the position and phenotypic characteristics of the *fiw* determinants of pVS76 and pUB1601 are further defined.

METHODS

Bacteria. The *Escherichia coli* K12 sub-lines used were UB1301 (prototroph, Rif^R) (Grinsted *et al.*, 1972), UB281 (*pro met* Nal^R) (Hedges *et al.*, 1973) and its *recA56* derivative UB5201 (Sanchez *et al.*, 1982), and various plasmid-carrying constructs of these bacteria. *Pseudomonas aeruginosa* PAO9505 (*argB18 chl-2* Rif^R) (Stanisich & Bennett, 1976) lysogenized by a Tn504⁺ derivative of phage JA1 (Elliott, 1983) was also used.

Isolation of pVS76 *fiw* insertion mutants. Tn501 (Hg^R) insertion mutants of pVS76 were constructed by selecting for the conjugal conduction of pVS76 (Tc^R) from an *E. coli* UB5201 sub-line carrying both pVS76 and a conjugally-proficient, Tn501⁺ derivative of R388. Selection was imposed for Tc^R transconjugants, and those carrying pVS76 *fiw*::Tn501 insertion mutants were identified by their ability to re-transfer the co-resident R388::Tn501 plasmid at high frequency. The putative pVS76 *fiw* plasmids were retrieved by transformation and retested to confirm their *fiw* phenotype.

Isolation of pUB1601 *fiw* insertion mutants. A *P. aeruginosa* PAO9505 sub-line carrying pUB1601 (Tc^R Km^R Tra⁺) and a chromosomally inserted Tn504 (Sm^R) was used as a conjugal donor to *E. coli* UB5201(R388). The Sm^R transconjugants obtained carried transpositional derivatives of pUB1601 and those with insertions in *fiw* were identified by their ability to transfer the co-resident R388 at high frequency. The putative pUB1601 *fiw* plasmids were retrieved by conjugational transfer and retested to confirm their *fiw* phenotype.

Isolation of pUB1601 Te^R mutants. pUB1601 derivatives which express high-level resistance to tellurite were isolated by plating overnight broth cultures of *E. coli* UB1301(pUB1601) onto nutrient agar (Oxoid Blood Agar Base 3.5%, w/v; Oxoid Yeast Extract 0.5%, w/v) supplemented with K₂TeO₃ (5 µg ml⁻¹; Sigma). The few resistant colonies that developed (about 10⁻⁶ ml⁻¹) were purified, the plasmid transferred by conjugation and the new sublines retested for expression of high-level tellurite resistance. Six mutants, each from independent cultures, were isolated.

Recombinant DNA procedures. Digestion of DNA with restriction endonucleases, ligation of DNA, small-scale and large-scale plasmid preparations, agarose gel electrophoresis and transformation were carried out by standard procedures (Maniatis *et al.*, 1982).

RESULTS AND DISCUSSION

*Localization and characterization of *fiwA**

The 6.0 kb *Pst*I region of RP1, which carries a *fiw* determinant, has previously been cloned into the plasmid vector pBR322 to construct pVS76 (Fig. 1*b*; Yusoff & Stanisich, 1984). Ten independently derived *fiw* mutants of pVS76 were obtained by Tn501 transposon mutagenesis (see Methods). Comparison of the restriction profiles of pVS76 and its derivatives revealed that all the latter carried Tn501 inserted in the 1.7 kb *Sst*II/*Sma*I fragment of RP1-derived DNA (Fig. 1*b*). The orientation and precise location of Tn501 in each derivative was determined relative to the asymmetrically placed *Sal*I site within Tn501 (Fig. 1*d*) and the *Sst*II and *Sal*I sites within pVS76. Clustering of the Tn501 insertions in the region 0.2–1.3 kb proximal to the *Sst*II site suggests a single *fiw* determinant, here designated *fiwA*, with the corresponding location between coordinates 32.8 kb to 31.7 kb on RP1. *fiwA* does not include the *Sst*II site at coordinate 33 kb since pVS517, a mutant of pVS76 deleted for the 1.5 kb *Sal*I–*Sst*II fragment of RP1-derived DNA, retains *fiwA* activity. Whether or not *fiwA* includes the *Sma*I site at coordinate 31.3 kb has not been tested.

*Localization and characterization of *fiwB**

Plasmid pUB1601, which consists of the two largest *Pst*I fragments of RP1, carries a *fiw* determinant (Fig. 1*a*; Yusoff & Stanisich, 1984). Seven independently derived *fiw* mutants of pUB1601 were obtained by Tn504 transposon mutagenesis (see Methods). Comparison of the restriction profiles of pUB1601 and its derivatives showed that the latter consisted of two types. (i) pVS474, pVS475 and pVS480 were additive insertion mutants. The position and orientation of Tn504 in each mutant was determined from *Eco*RI/*Apa*I and *Pst*I restriction profiles for pVS474, and *Eco*RI/*Sma*I and *Kpn*I/*Sma*I restriction profiles for pVS475 and pVS480. Insertion occurred 0.2 kb left of the *Eco*RI site (pVS474) or 0.5 kb right of this site (pVS475 and pVS480) (Fig. 1*c*). (ii) pVS476 to pVS479 inclusive had sustained deletions associated with acquisition of Tn504. This was deduced from *Eco*RI/*Kpn*I restriction profiles in which the 6.3 kb restriction fragment had increased in size, but not to the 11.5 kb expected from the addition of Tn504. The position of each deletion was determined (Fig. 1*c*) and the smallest, of 0.6 kb in pVS478 and pVS479, extended from coordinate 0.8 kb across the *Sma*I site at 0.9 kb. These observations, together with those from the class (i) mutants suggests that a single *fiw* determinant, here

Table 1. *Tellurite-resistance and fertility-inhibition phenotypes of plasmid-carrying derivatives of E. coli K12*

IncP plasmid carried	MIC of tellurite ($\mu\text{g ml}^{-1}$)*	Transfer frequency of R388†
None	0.03	1.2
RP1	0.3	2.5×10^{-5}
pUB1601	0.3	9.0×10^{-3}
pUB1601 <i>fiwB</i> ::Tn504	0.03	9.0×10^{-1}
pUB1601Te ^R	> 5	7.5×10^{-2}

* Nutrient agar was supplemented with K_2TeO_3 using 0.01 $\mu\text{g ml}^{-1}$ increments over the range 0.01–0.1 $\mu\text{g ml}^{-1}$ and 0.1 $\mu\text{g ml}^{-1}$ increments over the range 0.1–1 $\mu\text{g ml}^{-1}$. Samples (20 μl) of serially diluted overnight broth cultures of *E. coli* UB1301 and its IncP⁺ derivatives were spotted onto the supplemented plates. The MIC of tellurite was taken as the minimum concentration required to inhibit the growth of single colonies after 24–48 h incubation.

† Spot plate matings were carried out in which 20 μl of serial dilutions of the donor cultures (UB1301 derivatives carrying R388 alone or together with an IncP plasmid) were inoculated onto the surface of selective medium previously seeded with UB281. R388⁺ transconjugants were isolated on Oxoid DST Agar supplemented with nalidixic acid (10 $\mu\text{g ml}^{-1}$) and trimethoprim (1 $\mu\text{g ml}^{-1}$). The data presented for the pUB1601 mutants are representative of those obtained for the seven *fiwB*::Tn504 mutants (range = 6.5×10^{-1} –1.2) and the six Te^R mutants (range = 6.0×10^{-2} – 1.0×10^{-1}).

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 (Tn801Ap^R and IS21) are indicated. Plasmid pUB1601 (49.2 kb) (inner circle) is a ligation derivative of RP1 consisting of the two largest *Pst*I fragments (Wallace *et al.*, 1981). (b) Plasmid pVS76 (10.4 kb) is a *Pst*I ligation derivative of pBR322 (light region) and the 6 kb *Pst*I fragment of RP1 (dark region). The locations of Tn501 in the various pVS76::Tn501 derivatives are shown by arrowheads in the linearized extension. (c) A linearized expansion of the 55 kb to 5 kb region of RP1, with the positions of plasmid-maintenance and host-range determinants (*kil*, *kor* and *trf* loci) shown. Arrowheads mark the position of Tn504, and the bars above the map indicate the extent of deletions associated with the inheritance of this transposon by various pUB1601::Tn504 derivatives. (d) The simplified map of Tn501 (Hg^R) (8.2 kb) is based on that of Bennett *et al.* (1978). (e) The map of Tn504 (Sm^R) (5.2 kb) is shown. This transposon was detected on plasmid pVS5 (IncP2) carried by a clinical *Pseudomonas aeruginosa* strain from Germany (Arwas, 1982) and resembles Tn904 (McCombie *et al.* 1983). In (b) and (c) open arrowheads represent transposon insertions in the orientation shown on the maps in (d) and (e); filled arrowheads represent the opposite orientation. In all figures cleavage sites for various restriction enzymes are as follows: *Pst*I (P), *Sma*I (S), *Apa*I (A), *Kpn*I (K), *Eco*RI (E), *Sst*II (Ss), *Sal*I (Sa). In the case of RP1, only the *Sst*II and *Sal*I sites that occur in the 6.0 kb *Pst*I fragment are shown.

designated *fiwB*, occurs in pUB1601 in the region 0.2 kb left of the *Eco*RI site to at least 0.8 kb right of this site. This determinant may, in addition, include the *Sma*I site at 0.9 kb.

The location of *fiwB* in the *Eco*RI (59.8–0.8 kb) region of RP1 is unexpected since it coincides (or overlaps) with that of the tellurite-resistance (Te^R) determinant. The latter has been mapped in the apparently identical plasmid RP4 (Thomas & Smith, 1987) and just spans the *Eco*RI (0 kb) and *Sma*I (0.9 kb) sites (Taylor & Bradley, 1987). Te^R function is normally 'silent', unlike

that of *fiwB*, and is confined to IncP α plasmids (Bradley, 1985; Taylor & Bradley, 1987). Members of the IncP β group carry neither Te^R (i.e. R751 and R772; Bradley, 1985) nor *fiwB* (i.e. R751 and R906; Yusoff & Stanisich, 1984). We examined the possible relationship between the Te^R and *fiwB* functions of RP1 by testing plasmids which exhibited one of these functions for the alternative property (Table 1). Contrary to previous descriptions of RP4 (Summers & Jacoby, 1977; Bradley, 1985; Taylor & Bradley, 1987), both RP1 and pUB1601 conferred constitutive resistance to tellurite, albeit at a low level (MIC 0.3 $\mu\text{g ml}^{-1}$; Table 1). This resistance was reduced to the host level (0.03 $\mu\text{g ml}^{-1}$) in all sub-lines carrying pUB1601 *fiwB*::Tn504 mutants. Thus in pUB1601 (*fiwB*⁺), and presumably also in RP1 (*fiwA*⁺*fiwB*⁺), the expression of *fiwB* correlates with that of low-level tellurite-resistance. In the converse situation, spontaneous mutants of pUB1601 which exhibited high-level resistance to tellurite were found to be less effective than the parent *fiwB*⁺ plasmid in inhibiting the fertility of R388. These various observations raise the possibility that tellurite-resistance and *fiwB*-mediated fertility inhibition are effected by the same RP1 determinant or by two closely linked determinants with interacting effects. However, the mechanism(s) underlying these phenotypes remains to be determined.

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