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IS*257*-Mediated Cointegration in the Evolution of a Family of Staphylococcal Trimethoprim Resistance Plasmids

AMORNRUT LEELAPORN,† NEVILLE FIRTH, IAN T. PAULSEN,‡ AND RONALD A. SKURRAY*

School of Biological Sciences, University of Sydney, Sydney, New South Wales 2006, Australia

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Analyses of the *Staphylococcus epidermidis* multiresistance plasmids pSK697 and pSK818 have revealed them to be closely related to the trimethoprim resistance plasmid pSK639, also isolated from *S. epidermidis*. pSK697 and pSK818 were found to contain a cointegrated copy of a second plasmid related to the *S. epidermidis* multidrug antiseptic and disinfectant resistance plasmid pSK108 and the *S. aureus* tetracycline resistance plasmid pT181, respectively. In contrast to pSK639, both plasmids were found to contain a third copy of IS*257*, such that the integrated plasmids in both cases are flanked by a copy of this element. This organization and the presence of duplicated sequences at the extremities of the integrated plasmids implicate IS*257* in the formation of these cointegrate plasmids. Sequence analysis of the IS*257* elements from these plasmids has provided insights into the probable mechanism of cointegration, viz., nonresolved replicative transposition of IS*257*.

The composite transposon-like structure Tn*4003* is responsible for the high-level trimethoprim resistance mediated by the *Staphylococcus aureus* multiple antibiotic resistance plasmid pSK1 (11). This element is bounded by a single copy of IS*257* at one end and two copies of IS*257* at the other end; 8-nucleotide (nt) direct repeats flank the ends of the composite structure (11). The S1 dihydrofolate reductase responsible for trimethoprim resistance is encoded by a gene, *dfrA*, located within the central region of the element (11); a gene encoding thymidylate synthetase, *thyE*, is located upstream of *dfrA*, and an open reading frame of unknown function, *orf140*, is located downstream. The chromosomal gene encoding the Tp^s dihydrofolate reductase of a coagulase-negative organism, *S. epidermidis* ATCC 14990, shows extensive similarity to *dfrA* in both sequence and flanking genetic organization, implicating this or a closely related staphylococcal species as the origin of the Tp^r variant (4). Tp^r plasmids from coagulase-negative staphylococci may therefore be the vectors responsible for dissemination of Tp^r among staphylococci (6). We have undertaken a molecular characterization of the *S. epidermidis* Tp^r plasmids pSK639, pSK697, and pSK818 (6) to investigate relationships between them and *S. aureus* Tp^r plasmids, so as to gain insights into the evolution of Tp^r plasmids in staphylococci, particularly with respect to the role(s) played by IS*257*.

Relationships among Tp^r plasmids from coagulase-negative staphylococci. pSK639 and pSK818 restriction maps were deduced to enable comparison with each other and with the restriction map generated previously for pK697 (8). Furthermore, the complete nucleotide sequence of pSK639 has now been determined (1) (GenBank accession number U40259) and will be reported elsewhere. The resulting physical maps (Fig. 1) revealed that pSK697 and pSK818 share sequences corresponding to perhaps the entirety of pSK639. However, pSK697 and pSK818 also contain a third copy of IS*257* (hereinafter named IS*257*^{697C} and IS*257*^{818C}, respectively) (Fig. 1) and additional DNA segments of 2.5 and 4.4 kb, respectively. In the case of pSK697, the additional DNA has been shown to contain a functional *smr* (formerly known as *qacC*) gene, which confers multidrug resistance to antiseptics and disinfectants (8). Nucleotide sequencing (14, 16) of selected regions of both pSK697 and pSK818 (data not shown) reinforced the level of conservation suggested by the restriction maps of these plasmids (Fig. 1), which will henceforth be referred to as the pSK639 family of plasmids. Specifically, the ends of the central regions of the Tn*4003*-like elements from pSK639, pSK697, and pSK818 are identical to the equivalent sequences from Tn*4003* except for the previously described deletions of 32 and 286 nt adjacent to IS*257*^{639A} and IS*257*^{697A} in pSK639 and pSK697, respectively (Fig. 1) (6). Likewise, sequence identity is also evident immediately to the left of the Tn*4003*-like elements on pSK639, pSK697, and pSK818 (Fig. 1). However, the sequence identity of pSK697 and pSK818 to the sequence to the right of the Tn*4003*-like element of pSK639 extends rightward from the IS*257*C elements of pSK697 and pSK818 to at least the next *Sal*I site (Fig. 1); this segment encodes the probable replication initiation gene, *rep* (Fig. 1), of these plasmids (1). Significantly, the sequences encoding the 3′ ends and downstream flanking sequences of *rep* from these plasmids are also identical to the 404-nt DNA segment located between IS*257*R1 and IS*257*R2 of Tn*4003* from pSK1 (Fig. 1) (11).

pSK697 and pSK818 each contain a cointegrated plasmid. The unique DNA segment of pSK697 between IS*257*^{697B} and IS*257*^{697C} (Fig. 1) was entirely sequenced and found to represent a cointegrated plasmid nearly identical to the *smr* plasmid pSK108, previously identified in *S. epidermidis* (7). An 8-nt target duplication (TTTTTTAG), corresponding to nt 284 to 291 of the pSK108 sequence (7), is present immediately adjacent to the IS*257* elements flanking the cointegrated plasmid. Apart from three distinct nucleotide differences (corresponding to T at nt 79, A at nt 1032, and an additional G between nt 70 and 71 of the pSK108 sequence) (7), the cointegrated plasmid contains a tandem triplication of the 56-nt sequence (cor-

* Corresponding author. Mailing address: School of Biological Sciences, University of Sydney, Sydney, New South Wales 2006, Australia. Phone: 61 2 351-2376. Fax: 61 2 351-4771. Electronic mail address: skurray@extro.ucc.su.oz.au.

† Present address: Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

‡ Present address: Department of Biology, University of California at San Diego, La Jolla, California 92093-0116.

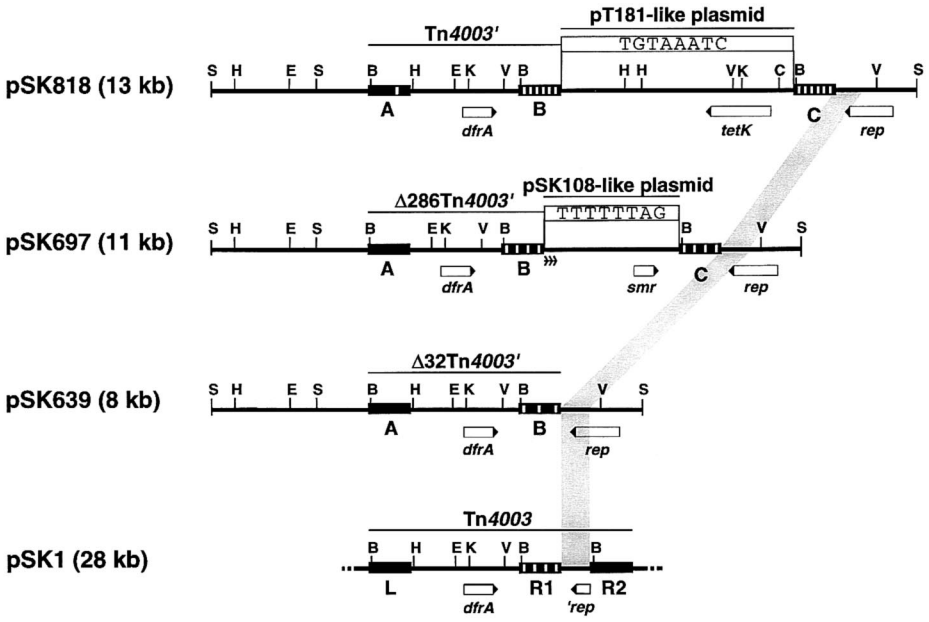

FIG. 1. Maps of pSK639 family plasmids. A map of the region containing Tn*4003* from pSK1 is shown for comparison. Plasmid names, with sizes noted in parentheses, are shown on the left. The restriction endonuclease recognition sites mapped were *Bgl*II (B), *Cla*I (C), *Eco*RI (E), *Eco*RV (V), *Hin*dIII (H), *Kpn*I (K), and *Sal*I (S). For clarity, the two *Hin*dIII sites present in each IS*257* element are not shown. The positions of the Tn*4003*-like elements (Tn*4003*, Tn*4003′*, Δ286Tn*4003′*, and Δ32Tn*4003′*, where the Δ prefix followed by a number indicates the number of nucleotides deleted from the leftmost boundary of the central region in that element) (6) and cointegrated pT181- and pSK108-like plasmids are indicated, as are the sequences of the target duplications present in these plasmids. The positions and directions of *dfrA*, *tetK*, *smr*, and the pSK639 family replication gene, *rep*, are denoted by boxes with arrowheads, whereas the position of the tandem triplication within the cointegrated pSK108-like plasmid in pSK697 is indicated by conjoined arrows. IS*257* elements are shown as solid boxes and are labelled (A, B, C, L, R1, and R2) to facilitate differentiation; white strokes indicate the number of nucleotide differences with respect to IS*257*L. Shading indicates the extent of identity between the segment between IS*257*R1 and IS*257*R2 of Tn*4003* on pSK1 and the replication regions of the pSK639 family plasmids.

responding to nt 292 to 347 of the pSK108 sequence) (7) immediately adjacent to the 8-nt target duplication abutting IS*257*^{697B} (Fig. 1). The intimate proximity of the triplicate sequence and IS*257*^{697B} suggests that the latter may have been involved in the processes resulting in this arrangement; an analogous situation, consisting of 10 copies of a 40-nt sequence, has been reported to occur adjacent to IS*257* (IS*431*) in the *mec*-associated hypervariable region of *S. aureus* BB270 (13).

Nucleotide sequencing at both ends of the unique DNA segment in pSK818 adjacent to the flanking IS*257* elements revealed identity with a contiguous portion of the replication gene (*repC*) from the 4.4-kb *S. aureus* tetracycline resistance plasmid pT181 (5, 10). Approximately 160 nt of the pT181-like plasmid adjacent to IS*257*^{818B} and a further 1 kb adjacent to IS*257*^{818C}, including the 5′ end of the tetracycline resistance gene, *tetK*, were sequenced, corresponding to nt 3890 to 4060 and 2900 to 3898 of the pT181 sequence, respectively (5, 10). An 8-nt target duplication (TGTAAATC) was found adjacent to the flanking IS*257* elements (Fig. 1) and corresponds to nt 3891 to 3898 of the pT181 sequence (5, 10). As expected, a derivative of *S. aureus* SK982 harboring only pSK818 was found to exhibit resistance to both trimethoprim and tetracycline. These data and the similarities between the sizes (4.4 kb) and restriction site patterns of the unique segment and pT181 are consistent with the notion that pSK818 contains a complete copy of a plasmid identical or closely related to pT181. The presence of an additional copy of IS*257* (compared with pSK639) in pSK697 and pSK818 so that the IS*257* elements flank integrated plasmids bearing 8-nt target duplications at their extremities implicates replicative transposition of IS*257* as a step in the formation of these cointegrate plasmids.

IS*257* elements of pSK639 family plasmids. Previous studies have revealed considerable variation among the sequences of both chromosome- and plasmid-located IS*257* elements (2, 9, 12). In contrast, the IS*257* elements of Tn*4003* on pSK1 were found to be very similar to each other (11). The relationships among IS*257* elements of the pSK639 family of plasmids (Fig. 1) are illustrated in the multiple-sequence alignment shown in Fig. 2. Of all the nucleotide differences, only the change at nt 484 (Fig. 2) would result in an amino acid change in the putative IS*257* transposase, viz., alanine to valine. It should be noted that the elements flanking the cointegrated plasmids in pSK697 and pSK818 are more similar to IS*257*^{639B} than they are to IS*257*^{639A} (Fig. 2).

The sequence identity between the IS*257* copies on pSK697 and those from Tn*4003* on pSK1 (IS*257*^{697A} and IS*257*^{697B}-IS*257*^{697C} are identical to IS*257*L-IS*257*R2 and IS*257*R1, respectively [Fig. 2]) (11) and the presence of a remnant of the putative pSK639 plasmid family *rep* gene between IS*257*R1 and IS*257*R2 of Tn*4003* (1, 11) implicate a pSK639-like plasmid (possibly analogous to the precursor of pSK697 prior to cointegration with a pSK108-like plasmid) in the evolution of this transposon-like element. A structure equivalent to Tn*4003*, including the presence of flanking duplications evident in pSK1, could have resulted from IS*257*^{697A}-mediated cointegration of a pSK697 progenitor into a pSK1 progenitor, followed by an IS*257*^{697A} (corresponding to IS*257*L of Tn*4003*) (Fig. 2)-mediated flanking deletion(s) that removed much of the pSK639-like sequence except for the IS*257*-flanked *dfrA*-encoding region and a truncated segment of the *rep* gene (Fig. 1).

Features of the plasmid cointegrates described here and elsewhere, in particular the generation of replication-inactivating insertions (e.g., pSK818) (3) and identical flanking IS*257* elements (e.g., in pSK697 and pSK818), are more satisfactorily explained by a single cointegrative event resulting from nonresolved replicative transposition than by a scenario involving

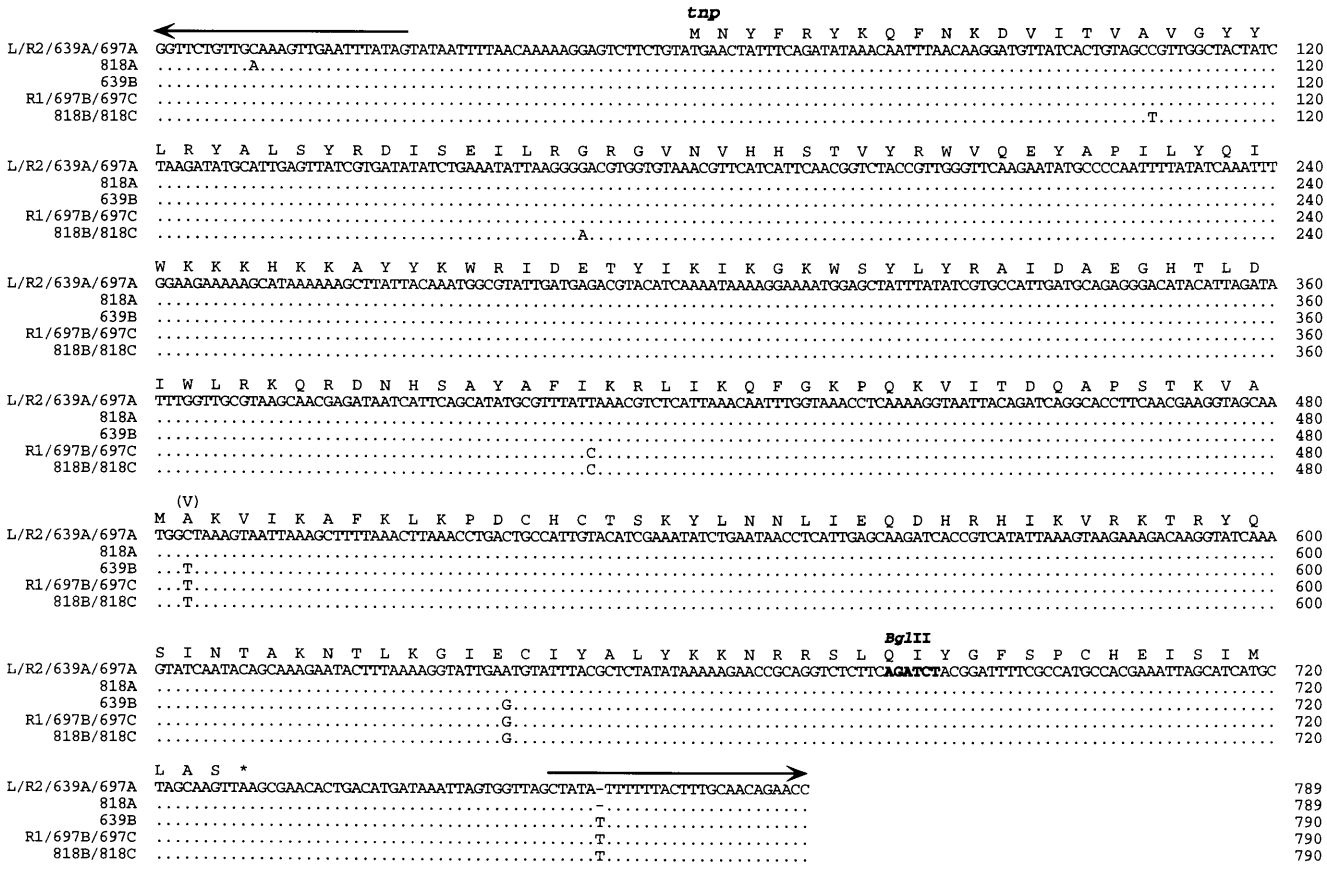

FIG. 2. Comparison of IS*257* elements associated with staphylococcal Tp^r determinants. The IS*257* elements shown are IS*257*L (L), IS*257*R1 (R1), and IS*257*R2 (R2) from Tn*4003* on pSK1 (11); IS257^{639A} (639A) and IS257^{639B} (639B) from pSK639; IS257^{697A} (697A), IS257^{697B} (697B), and IS257^{697C} (697C) from pSK697; and IS257^{818A} (818A), IS257^{818B} (818B), and IS257^{818C} (818C) from pSK818. The pSK639 sequences are taken from GenBank entry U40259, whereas those of IS257^{697A}, IS257^{697B}, IS257^{697C}, IS257^{818A}, IS257^{818B}, and IS257^{818C} are taken from the entries U40381, U40382, U40383, U40384, U40385, and U40386, respectively. Dots indicate identity to IS*257*L, whereas dashes indicate the positions of insertions or deletions. The deduced amino acid sequence of the putative IS*257* transposase (*tnp*) is shown above the alignment; the stop codon is denoted by an asterisk, and the amino acid difference encoded by IS257^{639B}, IS*257*R1, IS257^{697B}, IS257^{639C}, IS257^{818B}, and IS257^{818C} is shown in parentheses. The terminal inverted repeats of IS*257* are indicated by arrows, and the *Bgl*II site is shown in boldface type.

homologous recombination between preexisting IS*257* elements. Indeed, the proficiency of IS*257* to mediate replicon fusions has recently been demonstrated experimentally, although the data obtained could not discriminate between the possible mechanisms discussed above. However, the apparent scarcity of individual elements flanked by potential target duplications (15) suggests that cointegrate formation may represent a common outcome of IS*257* transposition. Of course, the probability that IS*257* utilizes nonresolved replicative transposition in no way diminishes the likelihood of rearrangements resulting from homologous recombination between preexisting IS*257* elements.

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