

Vibrio cholerae InV117, a Class 1 Integron Harboring *aac(6′)-Ib* and *bla*_{CTX-M-2}, Is Linked to Transposition Genes

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A ca. 150-kbp *Vibrio cholerae* O1 biotype El Tor plasmid includes *bla*_{CTX-M-2} and a variant of *aac(6′)-Ib* within InV117, an orf513-bearing class 1 integron. InV117 is linked to a *tnp1696* module in which IRI carries an insertion of IS4321R. The complete structure could be a potential mobile element.

A *Vibrio cholerae* O1 biotype El Tor isolate from Argentina harbors a conjugative ca. 150-kbp plasmid, named pAS1, which includes *bla*_{CTX-M-2} and genes coding for resistance to non-β-lactam antibiotics, including amikacin (12). The *bla*_{CTX-M-2} gene, commonly found in clinical isolates from South America, is most often found in orf513-bearing integrons such as InS21, In35, or In116 (1, 2, 5, 14). Our analysis of pAS1 indicated that the resistance genes are included in an orf513-bearing integron, InV117, which is linked to a transposition module.

V. cholerae O1 El Tor M1516 (Ogawa serotype), isolated in 1993 during the course of the second Argentinean cholera season, has been described previously (12). *Escherichia coli* M3099 was obtained by transfer of pAS1 from *V. cholerae* O1 El Tor M1516 to *E. coli* ER1793 (New England Biolabs, Beverly, Mass.) by conjugation (12). Transformation of *E. coli* cells was performed as described previously, and bacteria were cultured in Lennox Luria broth (15). PCR mapping was carried out using the appropriate primers as described before (7). Amplicons obtained by PCR for sequencing were either sequenced directly or sequenced after cloning into pGEM-T Easy (Promega, Madison, Wis.). DNA sequencing was performed on an ABI PRISM 3100 sequencer (Applied Biosystems, Foster City, CA) using the BigDye terminator method. Both strands of the whole DNA fragment described here were sequenced. Amino acid sequence analysis was performed using the CLUSTALW program (16). The N terminus of AAC(6′)-Ib was determined as described before (4), but the Edman degradation was carried out at the LANAIS-PRO facility (University of Buenos Aires).

Sequencing and analysis of a 15,723-bp pAS1 DNA fragment confirmed the identity of the CTX-M-type β-lactamase *bla*_{CTX-M-2} gene and a copy of *aac(6′)-Ib*. PCR-mapping experiments using DNA from *V. cholerae* M1516 confirmed that no rearrangements occurred during the transfer of pAS1 from *V. cholerae* M1516 to *E. coli* ER1793 (not shown). The *aac(6′)-Ib* gene,

present as a gene cassette with the usual *attC* locus (18), was cloned and expressed in *E. coli* from its natural promoter. The N terminus of the AAC(6′)-Ib protein was LRSSKTKLGI TKY, different from those coded for by other variants of the gene (Fig. 1). AAC(6′)-Ib variants are the most prevalent AAC(6′) type I aminoglycoside acetyltransferase among various gram-negative microorganisms (13, 18, 19). A factor contributing to this predominance may be a high flexibility in the structural requirements at the N terminus (see Fig. 1) (3).

Analysis of the nucleotide sequence of the genetic environment of *bla*_{CTX-M-2} and *aac(6′)-Ib* showed the presence of an orf513-bearing class 1 integron, named InV117, highly related to InS21, In35, and In116 (2, 5, 6, 14). Alignments carried out between InV117 and the sequenced regions of In35, InS21, and In116 (Fig. 2) showed 99.98%, 99.88%, and 99.86% identity, indicating a common origin. The differences found between the InV117 sequence and each of the other three integrons are due to single-nucleotide substitutions. A detailed diagram of the InV117 structure, a G+C content plot, and some of its characteristics are shown in Fig. 2.

InV117 is associated with a potentially mobile element. Upstream of the integron, there is a module that includes transposition-related genes and shares homology with the *tnp1696* module, a genetic structure found adjacent to In34 in pRMH760 (9) (Fig. 2). The *tnp1696* module includes a copy of the Tn1696 *tnpA* and *tnpR* genes with the insertion of an IS4321-like element within IRI (9). IS4321_{pAS1} and the *tnp1696* module showed different percents GC, suggesting different origins (Fig. 2). While the *tnpA*_{pAS1} and *tnpR*_{pAS1} genes were identical to *tnpA*_{pRMH760} and *tnpR*_{pRMH760}, the sequence of IS4321_{pAS1} shares higher homology with another IS4321 variant, IS4321_{pHCM1}, as compared to the homology shared with IS4321_{pRMH760} (100% and 97% identity, respectively) (Fig. 2) (8, 9, 11). The finding of an IS4321 inserted within a Tn1696 inverted repeat is in keeping with the previous observation that IS4321 and IS5075, both members of the IS1111 family, target terminal inverted repeats (TIRs) of transposons belonging to the Tn501/Tn21 family (11). Two nonidentical copies of IS4321 (IS4321L and IS4321R) are located at the ends of the composite transposon Tn4321, found in the R751 plasmid (17). IS4321_{pAS1}

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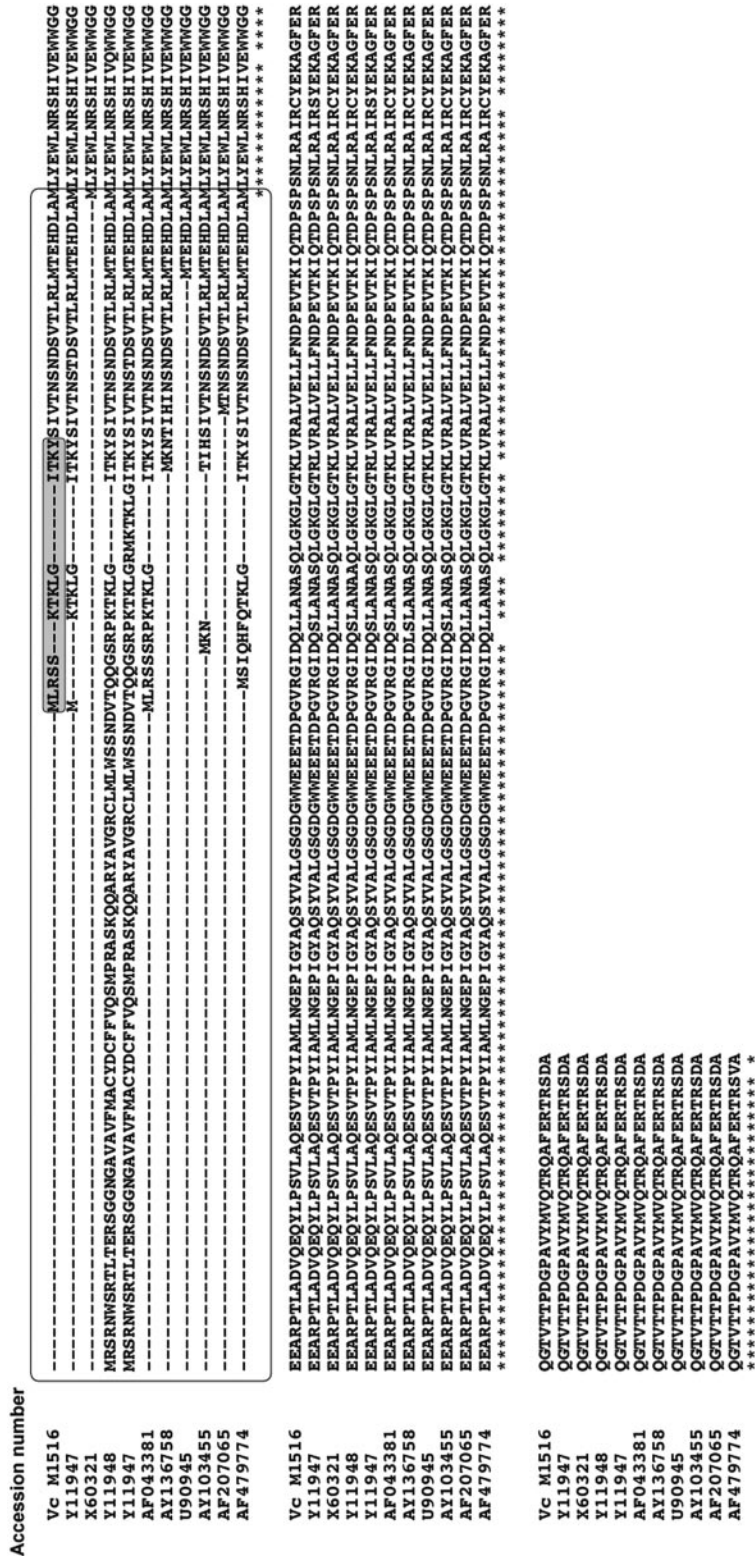


FIG. 1. Amino acid sequence alignment of AAC(6)-Ib variants. Alignments were generated using the CLUSTALW program (16). The accession numbers for each sequence are shown to the left. The amino acids identified by Edman degradation in the pAS1 variant are boxed. Vc, *V. cholerae*.

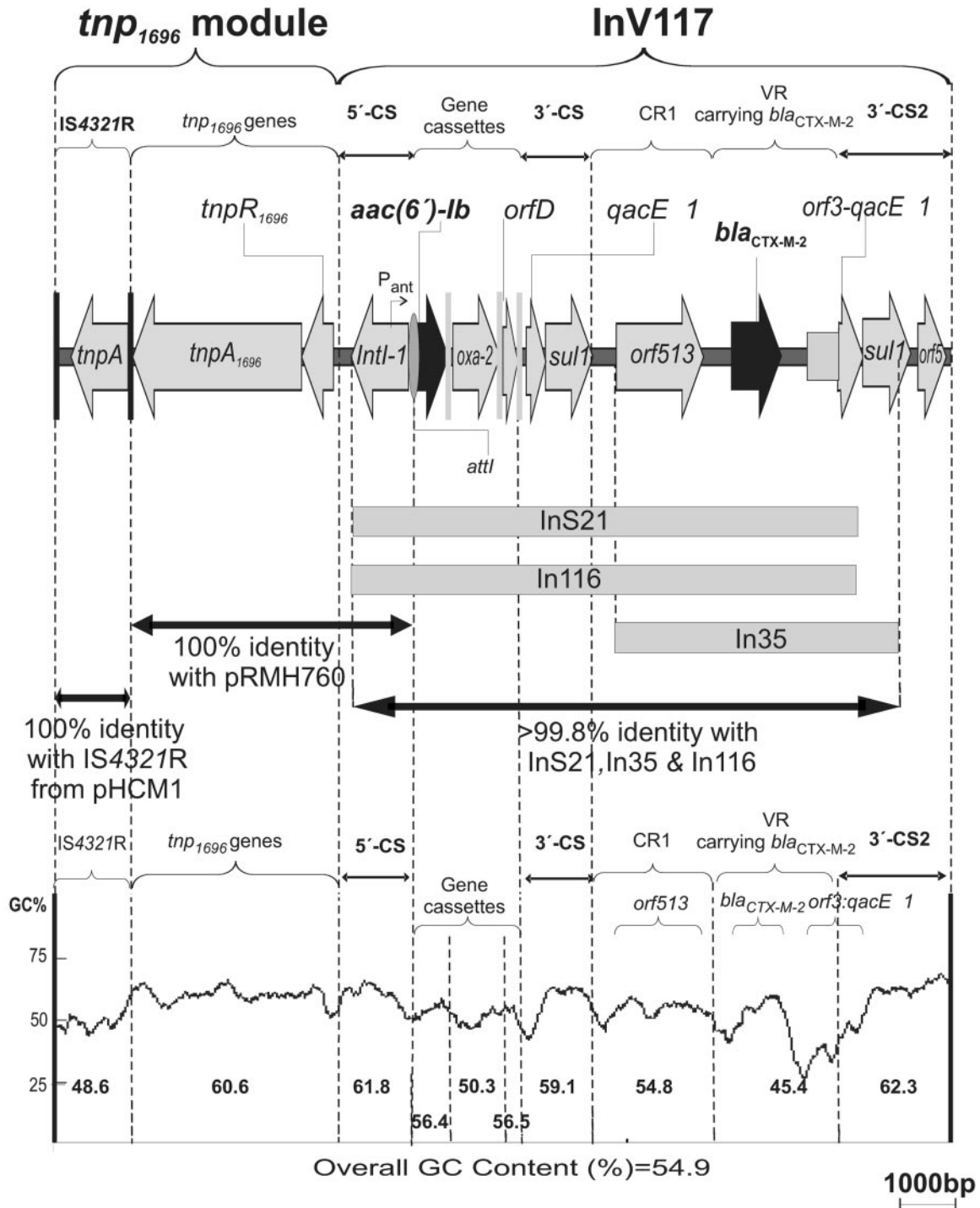


FIG. 2. Genetic structure of InV117 and the adjacent region. The diagram shows the genes and other elements present in the 15,723-bp pAS1 DNA fragment sequenced. The arrows indicate genes and their direction of transcription. Black and gray vertical bars represent inverted repeats and *attC* loci. The gray oval represents *attI*. Relevant regions are grouped and named: CR1, common region 1 (10); VR, variable region. Thick arrows with double arrowheads indicate the degree of homology with the specified element. Gray bars show the sequenced portions of integrons InS21, In116, and In35; >99.8% identity refers to identity between the sequenced portion of each integron and the cognate region in InV117. The GC content plot was generated using a window size of 300 bp, except for gene cassettes where the window size used was 50. Numbers between dotted lines indicate the GC content for each region.

and IS4321_{pHCM1} share higher identity (99%) with IS4321R than with IS4321L (96%). On the other hand, IS4321_{pRMH760} is more closely related to IS4321L than to IS4321R. Therefore, it was of interest that in the pAS1 structure, the sequence of the TIR of

tnp1696 is interrupted by an IS4321R copy, while so far all cases described showed an insertion of an IS4321L or an incomplete IS4321R within the TIR (11). Although the insertion of IS4321 within the TIR would most probably inactivate the

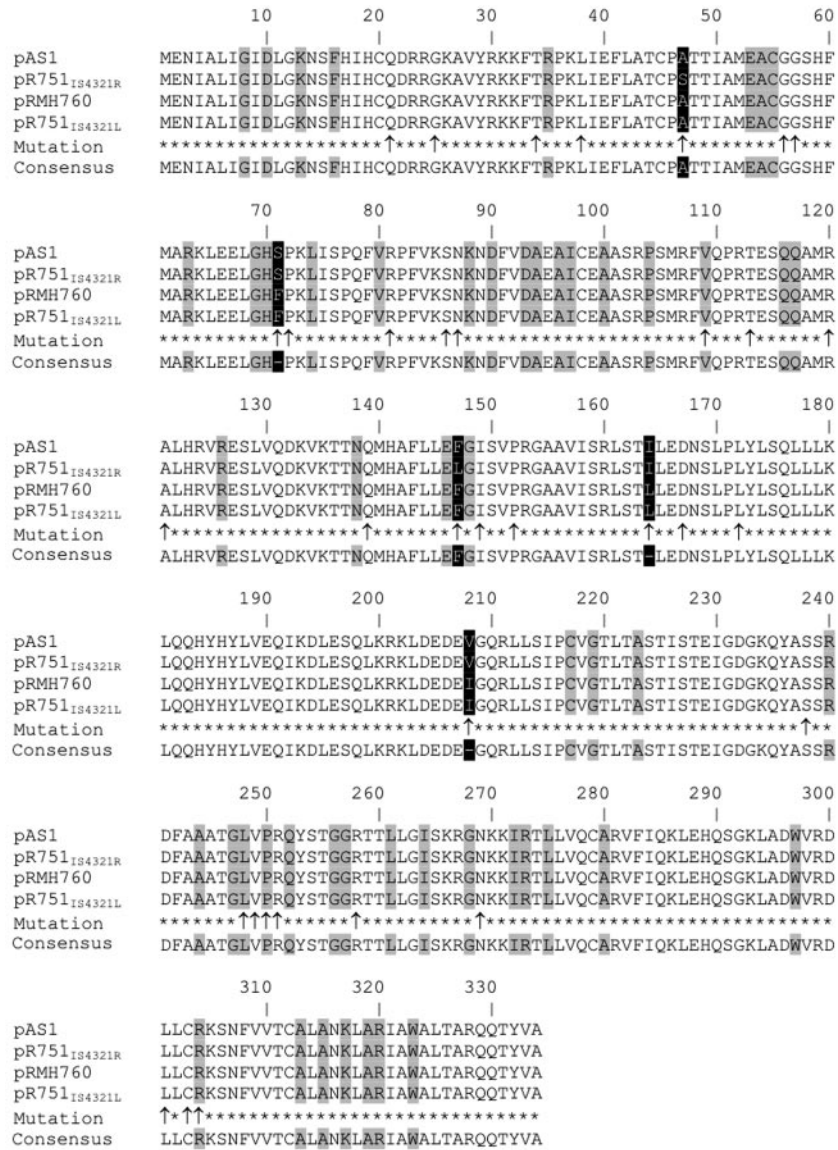


FIG. 3. Amino acid sequence alignment of TnpA variants. The amino acid sequences encoded by the *IS4321R*_{pAS1}, *IS4321R*_{pR751}, *IS4321L*_{pRMH760}, and *IS4321L*_{pR751} *tnpA* genes were aligned using the CLUSTALW program (16). Differences with respect to pAS1 are marked in white letters on a black background. Conserved residues among *IS1111* family transposases are highlighted in gray (11). Arrows indicate positions with different codons.

transposition capacity of *Tn1696*, the ability of *IS4321* to reconstitute the target TIR by precise excision (11) should reverse this inactivation. A comparison of the nucleotide sequences of the transposase genes encoded by *IS4321*_{pAS1}, *IS4321*_{pRMH760}, and *IS4321*_{pR751} showed several differences. *IS4321*_{pAS1} and *IS4321*_{pRMH760} showed 97.1% identity at the nucleotide level. Although 33 out of 38 different nucleotides found between both insertion sequences were located within the *tnpA* gene, most of them did not result in an amino acid change (Fig. 3). High degrees of conservation were also observed when comparing the nucleotide sequences of *IS4321*_{pAS1} and *IS4321R*_{pR751} (99.69% identity) or *IS4321*_{pAS1} and *IS4321L*_{pR751} (97.01% identity). Furthermore, the few amino acid substitutions found among the different trans-

posase protein versions did not occur at positions highly conserved among this family of transposases (Fig. 3). Our observations suggest that the *IS4321*_{pAS1} transposase is well conserved, and it is most probably functional. Therefore, the TIR could be regenerated by precise excision of *IS4321*_{pAS1}. An attractive theory has recently been proposed: *Tn21* family transposases could recognize the *IS4321* ends, and as a result, when *IS4321* inserts into one end of a *Tn21* family transposon, it may promote the transposition of this element along with the *IS4321* (17). If this is the case, the whole structure, including the *tnp1696* module and *InV117*, could potentially be able to transpose.

Nucleotide sequence accession number. The nucleotide sequence data for pAS1 reported in this work have been submitted to GenBank under accession no. DQ310703.

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