

ISEcp1B-Mediated Transposition of *bla*_{CTX-M} in *Escherichia coli*

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Several expanded-spectrum β -lactamase *bla*_{CTX-M} genes are associated with ISEcp1-like elements in *Enterobacteriaceae*. We found that ISEcp1B was able to mobilize the adjacent *bla*_{CTX-M-19} gene by a transpositional mechanism in *Escherichia coli* by recognizing a variety of DNA sequences as right inverted repeats.

An increasing number of reports of expanded-spectrum β -lactamases of the CTX-M type and plasmid-mediated cephalosporinases of the AmpC type in *Enterobacteriaceae* raise the question of their acquisition (5, 24, 26). The *bla*_{CTX-M} genes belonging to three out of the five known clusters (the CTX-M-1, -M-2, and -M-9 clusters) are associated with ISEcp1-like insertion sequences (IS) (2, 5–7, 11, 12, 18, 21, 23). Nevertheless, the distance that separates the β -lactamase gene from ISEcp1 varies within a given cluster of CTX-M-type genes, indicating that different insertion events have occurred (13, 19). Analysis of GenBank databases reveals that some plasmid-encoded cephalosporinase genes, such as the *bla*_{CMY}-type genes, may also be associated with ISEcp1 (3, 10, 17).

ISEcp1 is an IS weakly related to other IS elements and belonging to the IS1380 family (IS Database HomePage [<http://www-is.biotoul.fr/page-is.html>]) (8). Nothing is known about the mobilization properties of ISEcp1. Recently, we characterized ISEcp1B associated with a *bla*_{CTX-M-19} gene in a *Klebsiella pneumoniae* ILT-3 clinical isolate from Vietnam (21). ISEcp1B differs from ISEcp1 (GenBank accession no. AJ242809) by three nucleotide substitutions. Their inverted repeat (IR) sequences are identical, and their transposases differ by a single amino acid change (18). Since ISEcp1-like elements are located upstream of several β -lactamase genes, analysis of the variable sequences separating these IS elements from the initiation codons of these genes allowed us to determine its boundaries.

Recently, it has been shown that ISEcp1B brought promoter sequences for high-level expression of the *bla*_{CTX-M-14/-18}, *bla*_{CTX-M-17}, and *bla*_{CTX-M-19} β -lactamase genes that belong to the same *bla*_{CTX-M-9} subgroup (5, 6, 18) and might be also responsible for their mobilization. The aim of this study was to analyze the mobilization process mediated by ISEcp1B.

ISEcp1B possesses two imperfect IRs likely made of 14 bp, previously estimated to be 18 bp (18), with 12 of these 14 bp being complementary (Table 1). A detailed analysis of a ca. 9.5-kb DNA fragment from natural plasmid pILT-3 from *K. pneumoniae* ILT-3 revealed that the gene encoding ORF1 in transposon Tn1721 was interrupted by a 4.8-kb potential trans-

poson bracketed on the left side by the left IR (IRL) of ISEcp1B and on the right side by an imperfect right IR (IRR) named IRR1 made of a 14-bp sequence belonging to a gene encoding a putative iron transport protein (Fig. 1) (18). This potential transposon comprises ISEcp1B, *bla*_{CTX-M-19}, IS903D, and part of the gene encoding an iron transport protein. We have hypothesized that ISEcp1B could mobilize adjacent sequences by transposition.

Several transposition experiments were performed to determine (i) the mobility of this potential transposon, (ii) the role of ISEcp1B in mobilizing the adjacent β -lactamase gene, and (iii) the role of IS903D. Different recombinant plasmids were constructed by inserting PCR products that encompassed either one or both ISs that bracketed the *bla*_{CTX-M-19} gene in plasmid pILT-3 into the EcoRV site of plasmid pACYC184 (Table 2) and then transformed into *Escherichia coli* reference strain DH10B. Recombinant plasmids were constructed with the pACYC184 backbone to prevent overexpression of the ISEcp1B transposase. Plasmid pC-1 contained the sequences of the potential transposon identified in plasmid pILT-3, whereas the following constructs lacked the right part of it (Fig. 1). Plasmid pC-2 possessed the ISEcp1B sequence but lacked part of IS903D, and plasmid pC-3 possessed the entire IS903D sequence and a truncated ISEcp1B transposase gene (Fig. 1; Table 2). *E. coli* RR1023 (pOX38) was transformed by electroporation with these recombinant plasmids (Table 2) (9). Plasmid pOX38 is a self-conjugative and IS-free plasmid. Transposition events were searched between inserts of several recombinant plasmids and recipient plasmid pOX38 after 24 h of growth in Trypticase soy broth. Transfer of the recombinant plasmids with the pOX38 backbone into streptomycin-resistant *E. coli* reference strain DH10B (Table 2) was then performed by conjugation as described previously (20), and transconjugants were selected on agar plates containing 100 μ g of amoxicillin per ml (plasmid resistance marker) and 20 μ g of streptomycin per ml (chromosomal marker). Several amoxicillin-resistant transconjugants were extracted and sequenced in part.

Susceptibility to chloramphenicol was always observed in transconjugant strains, thus ruling out full integration (chloramphenicol resistance) of the recombinant plasmids derived from pACYC184 into plasmid pOX38. Detection of ISEcp1B and IS903D was performed using two sets of primers (primer ISEcp1A [Table 2] in combination with primer ISEcp1B [5'-T TTCCGCAGCACCGTTTGC-3'] and primer IS903A [5'-CA

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TABLE 1. Sequences identified as IRR boundaries for *ISEcp1B*-mediated transposition

Description of sequence	Nucleotide sequence (5'→3') ^a	No. of base pairs identical to perfect IRR ^b	Size of transposed fragment (bp)
IRL of <i>ISEcp1B</i>	GATTCTACGTCAGT		
Deduced perfect IRR of <i>ISEcp1B</i>	<u>ACGTAGAATCTAGG</u>		
IRR of <i>ISEcp1B</i>	<u>ACGTGGAATTTAGG</u>	12	
IRR1 at the potential transposon end of pILT-3	<u>GCGTTGATTCTCGG</u>	9	4,798
IRR2 in plasmid pACYC184	<u>GCATGGATTGTAGG</u>	9	4,726
IRR3 in plasmid pBK-CMV	<u>CCACAGAATCAGGG</u>	9	4,541
IRR4 in the <i>tnpA</i> gene of IS903D	<u>GATGAACTGCGGGC</u>	3	3,273
IRR5 in the <i>iron</i> gene of pILT-3	<u>GATGTGGAACTCG</u>	4	3,817
IRR6 in the <i>iron</i> gene of pILT-3	<u>GCACCGGGATGGCG</u>	3	4,167
IRR7 in the <i>iron</i> gene of pILT-3	<u>GCGCCGCCGCGGGG</u>	6	4,273
IRR8 in the <i>iron</i> gene of pILT-3	<u>GGGTTCGAACTGGG</u>	6	4,283

^a Underlined nucleotides are nucleotides identical to those identified at the same positions in the deduced IRR of *ISEcp1B*.

^b A perfect IRR contains 14 bp.

CATGAAATCATCTGCGC-3'] in combination with primer IS903B [Table 2], respectively).

Amoxicillin-resistant transconjugants were not obtained when *E. coli* RR1023 (pC-3) lacking *ISEcp1B* (Fig. 1) was used as a donor, suggesting that the IS903D element located downstream of the β -lactamase gene did not mobilize this latter gene. On the other hand, amoxicillin-resistant transconjugants were obtained when *E. coli* RR1023 (pC-1) and *E. coli* RR1023 (pC-2) were used as donors with a transposition efficiency of ca. 10^{-7} per donor. These results indicated that *ISEcp1B* was essential for movement of the *bla*_{CTX-M-19} gene.

Using *E. coli* RR1023 (pC-1) as a donor, all amoxicillin-resistant transconjugants except one contained *ISEcp1B* and IS903D elements, with the *bla*_{CTX-M-19} gene and DNA sequences being located on the right part of IS903D in plasmid pILT-3 (Fig. 1). Surprisingly, none of the 11 transconjugants that were sequenced had the entire potential transposon identified in natural plasmid pILT-3. Nevertheless, the transposition mechanism mediated by *ISEcp1B* was evident, since 5-bp duplications were observed at the insertion sites in plasmid

pOX38 (see below). These data suggested that the IRR sequences recognized by *ISEcp1B* were located inside the potential transposon. Analysis of the boundaries of the transposed fragments identified sequences, similar to that of the IRR of *ISEcp1B* and defined as IRR-like sequences, that had been recognized as such by the transposase of *ISEcp1B* during a mobilization process.

Five different IRRs (IRR4 to IRR8) (Table 1) were identified in five amoxicillin-resistant transconjugants that were analyzed, four of them being located in the gene encoding the iron transport protein and one of them being located in the *tnpA* gene of IS903D (Table 2; Fig. 1). The number of identical base pairs among the sequence defined as IRR varied from three to six, corresponding to less than 50%.

Analysis of *E. coli* RR1023 (pOX38) transconjugants resistant to amoxicillin, obtained with plasmid pC-2 as a donor, and thus lacking the sequence used as IRR in pILT-3, also showed that transposition had occurred using nearby sequences of pACYC184. The IRR-like sequences bracketing the transposed fragment in these transconjugants remained unchanged.

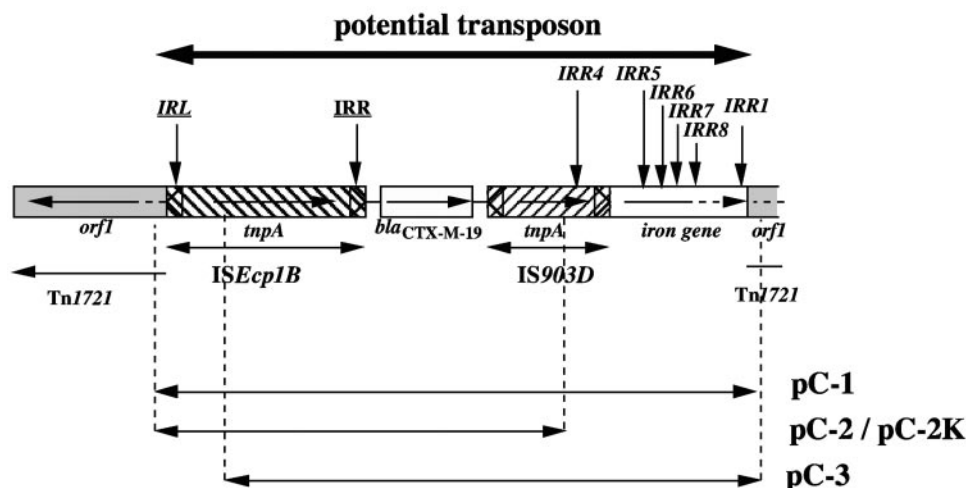


FIG. 1. Schematic map of the potential transposon structure in natural plasmid pILT-3 of *K. pneumoniae* ILT-3 (13). Open reading frames and genes are shown as boxes with an arrow indicating the orientation of each coding sequence and the gene name shown under the corresponding box. IRL and IRR motifs are indicated by vertical arrows. The IRL and IRR extremities of the *ISEcp1B*-mobilized fragments are in italics, whereas those of the *ISEcp1B* element are underlined. The sequences cloned in recombinant plasmids are indicated by double-headed arrows with the corresponding recombinant plasmid names.

TABLE 2. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Source or reference
Strains		
<i>E. coli</i> RR1023	pOX38 <i>recA56</i> ; susceptible strain	9
<i>E. coli</i> DH10B	<i>araD139</i> Δ (<i>ara leu</i>)7697 <i>deoR endA1 galK1 galU nupG recA1 rpsL F' mrcA</i> Δ (<i>mrr-hsdRMS-mrcBC</i>) ϕ 80 <i>dlacZ</i> Δ M15 Δ <i>lacX74</i> ; streptomycin resistant	Stratagene
<i>K. pneumoniae</i> ILT-3	Extended-spectrum cephalosporin-resistant isolate	18
Plasmids		
pOX38	55 kb, F derivative, conjugative	9
pACYC184	Chloramphenicol resistant	New England Biolabs
pILT-3	Natural ca. 50-kb plasmid of <i>K. pneumoniae</i> ILT-3 producing β -lactamase CTX-M-19	18
pC-1	Recombinant plasmid pACYC184 with a 4,877-bp PCR fragment (primers PreA [5'-GCAGGTCTTTTCTGCTCC-3'] and PreB [5'-TTTCCGAGCACCAGTTTGC-3']) encompassing the potential transposon identified in pILT-3	This study
pC-2	Recombinant plasmid pACYC184 with a 3,635-bp PCR fragment (primers PreA and IS903B [5'-CCGTAGCGGGTTGTGTTTC-3']) containing <i>ISEcp1B</i> , <i>bla</i> _{CTX-M-19} gene, and truncated <i>IS903D</i>	This study
pC-2K	Recombinant plasmid pBK-CMV with the same 3,635-bp PCR fragment inserted into pC-2	This study
pC-3	Recombinant plasmid pACYC184 with a 4,606-bp PCR fragment (primers ISEcp1A [5'-GCAGGTCTTTTCTGCTCC-3'] and PreB) containing <i>bla</i> _{CTX-M-19} and <i>IS903D</i> but lacking part of <i>ISEcp1B</i>	This study

They shared 9 of 14 bp of the perfect IRR sequence (Table 1) and corresponded to a segment belonging to the cloning vector. In order to rule out that the specific sequence of pACYC184 may play a role in this transposition process, the same inserted PCR product of pC-2 was cloned into the *Sma*I site of pBK-CMV, giving rise to pC-2K (Table 2; Fig. 1). Analysis of several amoxicillin-resistant transconjugants obtained with plasmid pC-2K as a donor also showed that transposition had occurred by involving sequences of pBK-CMV. The IRR-like sequences bracketing the transposed fragment in these transconjugants were always the same and shared 9 of the 14 bp of a perfect IRR (Table 1). These results indicated that sequences of pACYC184 (IRR2) or of pBK-CMV (IRR3) might be used as IRRs by *ISEcp1B*, but further repeated experiments are needed to assess this hypothesis. The reason why the IRR1 of the potential transposon of pILT-3 was not the boundary of the transposed fragments in these in vitro experiments remains intriguing. One explanation could be that IRR2 and IRR3 share greater nucleotide identity in their 3' extremities (7 and 8, respectively, of last 10 bp) to a perfect IRR than IRR1 (only 6 of last 10 bp).

No consensus sequence could be determined by comparing these 14-bp-long IRR sequences of recombinant plasmids and the IRR sequences of *ISEcp1B* in pILT-3 (Table 1). Nevertheless, a guanosine residue located at the 3' end of these IRRs was always found, likely indicating that this nucleotide was necessary in the transposition process. In addition, the last two nucleotides of these IRR sequences were either GG or CG. These data indicated that *ISEcp1B* was involved in a transposition process with weakly related IRRs.

Analysis of the insertion sites of *ISEcp1B*-mediated transposition revealed variable sequences in recombinant plasmids (AAGAA, TATAA, TATTA, GCTGA, ATATC, TAATA, TTTAC, AATGA, TTATA, ATAAG, and TGATT). No consensus sequence was identified among the 5-bp duplicated sites, whereas an A+T-rich content that may target *ISEcp1*-mediated transposition as observed for *IS1* was noticed (16,

27). In silico analysis of six *ISEcp1*-like sequences associated with *bla*_{CTX-M}-like or *bla*_{CMY}-like β -lactamase genes available in the GenBank databases did not identify target site duplications just bracketing the insertion sites of *ISEcp1*-like sequences. This result likely indicates that after insertion, *ISEcp1*-like elements may be responsible for mobilization of the adjacent sequences. In addition, this in silico analysis confirmed that the 5-bp sequences adjacent to the IRRs and IRRs of *ISEcp1* elements were always A+T rich.

ISEcp1B may use a wide range of DNA sequences as IRRs during a mobilization process that involves its adjacent sequences, therefore explaining its ability to mobilize structurally unrelated β -lactamase genes. Similar observations have been made for *IS1247* of *Xanthobacter autotrophicus*, which also belongs to the *IS1380* family (25). A single copy of *IS1247* may mobilize sequences located at its right-end extremity in what is considered a one-ended transposition process since only a single IS is involved.

These results may also be compared to those obtained with other IS elements, such as *IS91* and *IS911*, that are responsible for mobilization of adjacent sequences by a one-ended transposition mechanism (4, 22). In addition, transposition of other elements belonging to the Tn3 family, such as Tn21, generates variable endpoints as a consequence of one-ended transposition (1, 15). This peculiar process is the consequence of recognition of several different IR sequences by the Tn21 transposase, generating novel joint molecules (14). However, in our case, some homology between the different IRRs used by *ISEcp1B* is noticed, whereas one-ended transposition (for example, with Tn21) requires one fixed end and another random one.

In the present study, we have demonstrated the ability for *ISEcp1B* to mobilize the *bla*_{CTX-M} β -lactamase gene. In addition, the *IS903D* element present in the potential transposon in pILT-3 had no role in the mobilization process.

In conclusion, it remains to be determined why the emerging expanded-spectrum β -lactamases of the CTX-M type that are

structurally related to several chromosomally located β -lactamase genes of enterobacterial species are associated specifically with *ISEcp1*-like sequences.

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