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In Vitro Analysis of IS*Ecp1B*-Mediated Mobilization of Naturally Occurring β-Lactamase Gene bla_{CTX-M} of *Kluyvera ascorbata*

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IS*Ecp1B* has been reported to be associated with and to mobilize the emerging expanded-spectrum β-lactamase bla_{CTX-M} genes in *Enterobacteriaceae*. Thus, the ability of this insertion sequence to mobilize the $bla_{CTX-M-2}$ gene was tested from its progenitor, *Kluyvera ascorbata*. Insertions of IS*Ecp1B* upstream of the $bla_{CTX-M-2}$ gene in *K. ascorbata* reference strain CIP7953 were first selected with cefotaxime (0.5 and 2 μg/ml). In those cases, IS*Ecp1B* brought promoter sequences enhancing $bla_{CTX-M-2}$ expression in *K. ascorbata*. Then, IS*Ecp1B*-mediated mobilization of the $bla_{CTX-M-2}$ gene from *K. ascorbata* to *Escherichia coli* J53 was attempted. The transposition frequency of IS*Ecp1B*-$bla_{CTX-M-2}$ occurred at $(6.4 \pm 0.5) \times 10^{-7}$ in *E. coli*. Cefotaxime, ceftazidime, and piperacillin enhanced transposition, whereas amoxicillin, cefuroxime, and nalidixic acid did not. Transposition was also enhanced when studied at 40°C.

Increasing worldwide reports of expanded-spectrum β-lactamases of the CTX-M type in *Enterobacteriaceae* and mostly in *Escherichia coli* raise the question of their way of acquisition (4, 31). These enzymes are now widespread not only in nosocomial but also in community-acquired pathogens (4, 23). The 40 CTX-M-type β-lactamases may be grouped into five main subgroups according to amino acid sequence identity (CTX-M-1, -M-2, -M-8, -M-9, and -M-25) (1, 4, 13, 15, 27, 29). Most CTX-M enzymes hydrolyze cefotaxime better than ceftazidime. However, the latest reported enzymes, including CTX-M-15 (13), hydrolyze ceftazidime better than cefotaxime and are also widespread (32). It has been shown that different genetic elements are associated with bla_{CTX-M} genes. IS*Ecp1*-like insertion sequences are most frequently reported (5, 7, 11, 13, 29). This insertion sequence element has been found to be associated with four out of the five bla_{CTX-M} gene clusters (CTX-M-1, -M-2, -M-9, and -M-25 clusters) (1, 4, 13, 15, 27, 29). Nevertheless, the DNA sequence that separates the β-lactamase gene from IS*Ecp1* varies within a given cluster of CTX-M genes, indicating that different insertion events may have occurred (16). Moreover, several plasmid-encoded cephalosporinase genes, such as the bla_{CMY}- or bla_{ACC}-type genes, may be associated also with the same IS*Ecp1*-like element (2, 18).

IS*Ecp1* is weakly related to other IS elements and belongs to the IS*1380* family (IS Database home page [http://www-is.biotoul.fr/page-is.html]) (8). Since IS*Ecp1*-like elements are located upstream of several β-lactamase genes, analysis of the variable sequences separating these IS elements from initiation codons of these genes allowed us to determine its boundaries. IS*Ecp1B* possesses two imperfect inverted repeats (IR) likely made of 14 bp, with 12 of these 14 bp being complementary (Table 1), and a gene encoding a 420-amino-acid transposase. IS*Ecp1B* brings 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 (4, 6, 24). Recently, we have shown that IS*Ecp1B* is 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 (IRR) (26).

Chromosome-encoded β-lactamases of several *Kluyvera* species have been identified as progenitors of CTX-M-derived enzymes. The CTX-M-1 and CTX-M-2 subgroups are derived from *Kluyvera ascorbata* (12, 28), whereas the CTX-M-8 and CTX-M-9 subgroups are derived from *Kluyvera georgiana* (21, 25).

The aim of this study was to experimentally evaluate the ability of IS*Ecp1B* to mobilize a chromosome-encoded β-lactamase gene from its reservoir, *K. ascorbata*, to a plasmid location in *Escherichia coli*. The effects of addition of different antibiotics (mostly β-lactams) and of growth at various temperatures were also tested.

MATERIALS AND METHODS

Bacterial strains. Clinical strain *Klebsiella pneumoniae* ILT-3 (expressing the $bla_{CTX-M-19}$ gene associated with IS*Ecp1B*) has been described previously (24). *Kluyvera ascorbata* CIP7953 reference strain, the recombination-deficient strain *E. coli* DH5α (harboring pOX38-Gen, a self-conjugative, IS-free, and gentamicin-resistant plasmid), and the azide-resistant *E. coli* J53 were used for transposition and conjugation experiments (10, 17). The low-copy-number cloning vector pBBR1MCS.3 was used for cloning experiments (14). Bacterial cells were grown in Trypticase soy (TS) broth or onto TS agar plates (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) with antibiotics when required.

Antimicrobial agents and susceptibility testing. Routine antibiograms were determined by the disk diffusion method on Mueller-Hinton agar (Sanofi Diagnostics Pasteur). The antimicrobial agents and their sources have been referenced elsewhere (22). The antibiotic concentrations used for selection were as follows: cefotaxime (CTX; 0.5 and 2 μg/ml), amoxicillin (AMX; 100 μg/ml), tetracycline (TET; 15 μg/ml), kanamycin (KAN; 30 μg/ml), and gentamicin (GEN; 7 μg/ml).

Nucleic acid extraction. Recombinant plasmids and pOX38-Gen derivative plasmids were extracted using QIAGEN Plasmid Midi kits and the very-low-copy plasmid purification protocol, respectively (QIAGEN, Courtaboeuf, France). Extraction of whole-cell DNA was done as described elsewhere (22).

PCR experiments. PCR experiments were performed as previously described (30). The entire IS*Ecp1B* gene was amplified using the primers preTnCTXM-1 (5′-CTAACAGAGCTTAAGCTTCC-3′) and preISEcp1-2 (5′-CTCCCAATAC GGTCAATCCG-3′) and subsequently cloned into the SmaI site of plasmid pBBR1MCS.3.

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TABLE 1. Sequences identified as IRR boundaries after ISEcp1B transposition

Description of sequence	Nucleotide sequence (5′→3′)[a]	No. of base pairs identical to perfect IRR	Size of transposed fragment (bp)
IRL of ISEcp1B	GATTCTACGTCAGT		
Deduced perfect IRR of ISEcp1B	ACGTAGAATCTAGG		
IRR of ISEcp1B	ACGTGGAATTTAGG	12	
IRR-1	CGTCATATAGCTGG	4	5,464
IRR-2	ATATGGATAAGGAG	5	3,450
IRR-3	CTTTGTAAAGAACG	5	3,951
IRR-4	GAGAAGAAAATGGG	8	3,582
IRR-5	GCTCTTTTTTCTGG	4	2,667

[a] Underlined nucleotides correspond to those identified at the same positions in the deduced IRR of ISEcp1B.

Cloning experiments and sequencing. T4 DNA ligase and restriction endonucleases were used according to the recommendations of the manufacturer (Amersham Biosciences, Orsay, France). The recombinant plasmid pISE was constructed by inserting the PCR product of the ISEcp1B gene into the SmaI site of plasmid pBBR1MCS.3, which was then electroporated into electrocompetent *Kluyvera ascorbata* CIP7953 cells, as previously described (22), and selection was performed on TET (15 μg/ml)-containing plates (Fig. 1). In order to study the transposition of ISEcp1B, an omega fragment (ΩKm) from plasmid pHP45Ω-Km, made of a kanamycin resistance gene [*aph*(3′)-*IIa*] flanked by transcriptional and translational termination sequences, was introduced into ISEcp1B. The recombinant plasmid pISE was digested by NsiI enzyme (into ISEcp1B between the stop codon of the transposase gene and the IRR). The digested plasmid was mixed with an EcoRI-restricted ΩKm fragment (2.2 kb) in order to create the tagged insertion sequence ISEcp1B.Kan, yielding the plasmid pISEcp1B.Kan.

Sequencing of the insert was performed using laboratory-designed primers on an ABI PRISM 3100 automated sequencer (Applied Biosystems, Les Ulis, France).

Transposition experiments. Several transposition experiments were performed to determine (i) the mobility of ISEcp1B alone (i.e., without the β-lac-

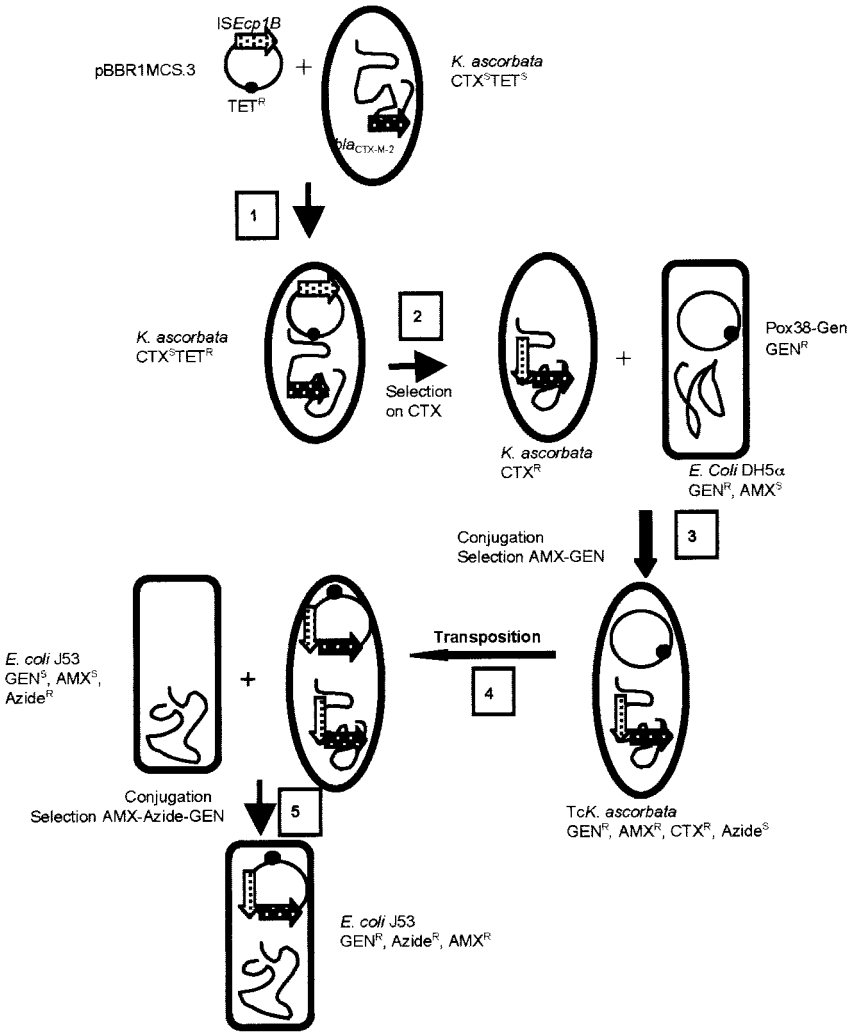

FIG. 1. Schematic representation of ISEcp1B-mediated mobilization of the naturally occurring CTX-M-2 β-lactamase gene of *Kluyvera ascorbata* CIP7953. 1. Introduction of ISEcp1B in *K. ascorbata* by electroporation. 2. Selection of *K. ascorbata* strains with ISEcp1B inserted upstream of the $bla_{CTX-M-2}$ gene. ISEcp1B enhances the gene expression by bringing promoter sequences. 3. Transfer of recipient plasmid in cefotaxime-resistant *K. ascorbata* strains. 4. ISEcp1B mobilizes the $bla_{CTX-M-2}$ gene on the plasmid under various conditions: with or without amoxicillin, piperacillin, cefuroxime, cefotaxime, ceftazidime, or nalidixic acid at 22, 30, 37, or 40°C. 5. Plasmid-located events of transposition are isolated. White arrow with black dots, ISEcp1B; black arrow with white dots, bla_{CTX-M}.

tamase gene), (ii) the ability of IS*Ecp1B* to mobilize a chromosome-encoded β-lactamase gene from *K. ascorbata* to a plasmid location in *E. coli* by transposition, and (iii) the effects of antibiotics and of temperature on the transposition events. The transposition of IS*Ecp1B*.Kan onto the conjugative plasmid pOX38-Gen was investigated with a mating-out technique in liquid medium (22). The recombinant plasmid pIS*Ecp1B*.Kan was electroporated into *E. coli* DH5α(pOX38-Gen) for transposition experiments. Transfer of the recombinant plasmids with the pOX38 backbone into the *E. coli* J53 azide-resistant (AZ^r) strain was then performed by conjugation. One colony obtained after 24 h of growth was cultured under weak agitation in 1-ml of TS broth at 37°C for 3 h and was used as a donor for the mating assay with *E. coli* J53 as recipient. Conjugation was done by incubating 800 μl of recipient and 200 μl of donor under low agitation at 37°C for an additional 3-h step. Mating was stopped by vigorous vortexing and cooling on ice. The transconjugants were selected on agar plates containing 7 μg per ml of GEN (plasmid marker), 30 μg per ml of KAN (transposon marker), and 100 μg per ml of azide (chromosomal marker). The transposition frequency was calculated by dividing the number of transconjugants by the number of donors.

The MIC of cefotaxime for the wild-type *K. ascorbata* strain is 0.06 μg/ml. To select for IS*Ecp1B* upstream of bla_{CTX-M}, *K. ascorbata* harboring the recombinant plasmid pISE was screened on agar plates containing 0.5 or 2 μg of cefotaxime per ml after 24 h of growth in TS broth (Fig. 1). This strategy was based on the previous observations demonstrating IS*Ecp1B* mediated high-level expression of bla_{CTX-M} β-lactamases genes, with the IS element providing promoter sequences. The transposition frequency was calculated by dividing the number of CTX^r transformants by the total number of bacteria. The second step consisted of mobilization by IS*Ecp1B* of the $bla_{CTX-M-2}$ gene to the *E. coli* J53 recipient strain. Plasmid pOX38-GEN was used as a target for transposition events. Transfer of pOX38-GEN into the *K. ascorbata* transformants was performed, and transconjugants were selected on agar plates containing 7 μg per ml of GEN (plasmid marker) and 100 μg per ml of AMX (chromosomal marker) (Fig. 1). Transposition events were searched for between the chromosomal $bla_{CTX-M-2}$ gene and the recipient plasmid pOX38-GEN after overnight growth in TS broth with and without antibiotics at different subinhibitory concentrations and after 3 h of growth at various temperatures (22°C, 30°C, 37°C, and 40°C). Several structurally unrelated β-lactams were studied, since it is unknown whether any β-lactam might enhance mobilization of the $bla_{CTX-M-2}$ gene. Nalidixic acid was also studied, since quinolone resistance is frequently associated with extended-spectrum β-lactamase (ESBL)-mediated resistance in *Enterobacteriaceae* (23) and since quinolones are known to induce antibiotic resistance through the SOS response (3). Transfer of the recombinant plasmids with the pOX38-GEN backbone into the *E. coli* J53AZ^r strain was then performed by conjugation, and transconjugants were selected on agar plates containing 7 μg per ml of GEN (plasmid marker), 100 μg per ml of AMX (transposon marker), and 100 μg per ml of azide (chromosomal marker) (Fig. 1). The transposition frequency was calculated by dividing the number of transconjugants by the number of donor bacteria. All the GEN^r AMX^r AZ^r colonies were screened for tetracycline susceptibility to exclude those that may have resulted from nontransposition events.

Insertion site determination. The regions where for insertions of IS*Ecp1B* upstream of the $bla_{CTX-M-2}$ gene were amplified by PCR and sequenced. Plasmid pOX38-GEN carrying various transposed structures was extracted and sequenced in part.

RESULTS AND DISCUSSION

Transposition of IS*Ecp1B* upstream of the chromosome-located $bla_{CTX-M-2}$ gene. Selection of the *K. ascorbata* CIP7953 reference strain, in which IS*Ecp1B* was inserted upstream of the $bla_{CTX-M-2}$ gene, was obtained at a frequency of $(1 \pm 0.5) \times 10^{-7}$ per donor, whereas the overall transposition frequency of IS*Ecp1B*.Kan was 10^{-6} (data not shown). Thus, transposition of IS*Ecp1B* upstream of the chromosomal β-lactamase gene occurred at a high frequency (10% of overall transposition events of IS*Ecp1B*.Kan). Among the *K. ascorbata* strains overexpressing their $bla_{CTX-M-2}$ gene, the IS*Ecp1B* element was inserted in such a manner that its transposase gene was transcribed in the same orientation as the β-lactamase gene. As observed in clinical isolates, IS*Ecp1B* brought promoter sequences enhancing bla_{CTX-M} expression. The transposition of IS*Ecp1B* generated a 5-bp duplication that was located at various insertion sites in the chromosome of *K. ascorbata* CIP7953 (TACTA, TAATA, and AATAC). Twenty transformants were analyzed, including 11 obtained on agar plates containing 2 μg/ml of cefotaxime and 9 on plates with 0.5 μg/ml of cefotaxime. On one hand, detailed analysis of the target sites of transformants selected on CTX (2 μg/ml) revealed a preferential location 22 bp upstream of $bla_{CTX-M-2}$ (64%), but other insertions were observed located 19 bp and 43 bp upstream of the $bla_{CTX-M-2}$ gene. The insertion sites of most of the transformants (five of nine) selected on CTX at 0.5 μg/ml were located 19 bp upstream of the $bla_{CTX-M-2}$ gene as the insertion site of IS*Ecp1B* upstream of $bla_{CTX-M-5}$, described on a natural plasmid (12). Moreover, IS*Ecp1* insertions located 43 bp upstream of bla_{CTX-M} have been identified upstream of $bla_{CTX-M-9}$, $bla_{CTX-M-14}$, $bla_{CTX-M-17}$, and $bla_{CTX-M-19}$ (6, 7, 24, 29). Nevertheless, no IS*Ecp1* insertion has been identified 22 bp upstream of the bla_{CTX-M} gene in clinical isolates.

IS*Ecp1B*-mediated transposition of bla_{CTX-M}. A conjugation was realized with the gentamicin-resistant plasmid pOX38-GEN (GEN^r) (a transfer-proficient F plasmid derivative) as donor and the *K. ascorbata* strain harboring IS*Ecp1B*-bla_{KLUA} as recipient (Fig. 1). Susceptibility to tetracycline was systematically observed in transconjugant strains, ruling out full integration or cointegration (TET resistance) of the recombinant plasmids derived from pBBR1MCS.3 into plasmid pOX38. The transposition ability of IS*Ecp1B*-bla_{KLUA} was investigated by conjugation with the *K. ascorbata* strain, harboring IS*Ecp1B*-bla_{KLUA} and the plasmid pOX38-GEN as donor, and *E. coli* J53 AZ^r as recipient (Fig. 1). Transposition of the IS*Ecp1B*-$bla_{CTX-M-2}$ fragment occurred at a frequency of $(6.4 \pm 0.5) \times 10^{-7}$ in *E. coli*. Analysis of the genetic environment of several transposition events leading to insertions of IS*Ecp1B*-$bla_{CTX-M-2}$ revealed in all cases a 5-bp duplication that very likely confirmed the acquisition of that structure through a transposition mechanism. These observations also indicated that IS*Ecp1B* was able to mobilize by itself the $bla_{CTX-M-2}$ gene; this was consistent with previous results (26).

Target site preference of the IS*Ecp1B*-$bla_{CTX-M-2}$ transposon. Several GEN^r AMX^r AZ^r *E. coli* isolates from independent experiments were analyzed. The insertion sites of IS*Ecp1B*-bla_{KLUA} were determined by sequencing the neighboring regions of the inverted repeats. A 5-bp target site duplication, consistent with a transposition event, was observed in all the studied insertion events.

To determine whether the IS*Ecp1B*-bla_{KLUA} transposon had a target site preference, the locations of five insertion events were mapped onto recombinant plasmid pOX38-GEN. Insertions of IS*Ecp1B*-bla_{KLUA} sequences had occurred into five different sites which were distantly located on the plasmid. Alignment of the insertion site sequences revealed variable sequences in recombinant plasmids (TATGA, TATCA, TACAT, TATAC, and TTCAT). No consensus sequence was identified among the 5-bp duplicated sites, whereas an AT-rich content that may target IS*Ecp1B*-mediated transposition was identified again (26).

Characterization of IS*Ecp1B*-$bla_{CTX-M-2}$ transposons. Five different transposons were analyzed (Table 1). Their sizes varied from 2,667 to 5,464 bp. IS*Ecp1B* possesses two imperfect

TABLE 2. IS transposition frequency with and without antibiotics[a]

Antibiotic (μg/ml)	Transposition frequency (mean ± SD)
None	$(4.3 \pm 2.3) \times 10^{-7}$
Amoxicillin (256)	$(3.4 \pm 2.3) \times 10^{-7}$
Amoxicillin (128)	$(4.2 \pm 3.8) \times 10^{-7}$
Amoxicillin (52)	$(5.2 \pm 2.5) \times 10^{-7}$
Cefuroxime (128)	$(6.5 \pm 8.2) \times 10^{-7}$
Cefuroxime (64)	$(4.6 \pm 3.9) \times 10^{-7}$
Cefuroxime (26)	$(2.0 \pm 0.4) \times 10^{-7}$
Cefotaxime (8)	$(6.6 \pm 3.8) \times 10^{-6}$
Cefotaxime (4)	$(1.9 \pm 1.4) \times 10^{-6}$
Cefotaxime (1.5)	$(5.4 \pm 1.4) \times 10^{-7}$
Ceftazidime (0.5)	$(5.2 \pm 2.7) \times 10^{-5}$
Ceftazidime (0.25)	$(5.6 \pm 3.2) \times 10^{-6}$
Ceftazidime (0.1)	$(6.2 \pm 2.7) \times 10^{-7}$
Piperacillin (64)	$(2.9 \pm 2.8) \times 10^{-6}$
Piperacillin (32)	$(6.5 \pm 7.4) \times 10^{-7}$
Piperacillin (16)	$(2.8 \pm 2.8) \times 10^{-7}$
Nalidixic acid (2)	$(7.4 \pm 1.3) \times 10^{-7}$
Nalidixic acid (1)	$(2.9 \pm 1.4) \times 10^{-7}$
Nalidixic acid (0.4)	$(2.9 \pm 1.4) \times 10^{-7}$

[a] In each case, three independent experiments were performed and the mean and standard deviation were calculated.

TABLE 3. IS transposition frequency at different temperatures[a]

Temp (°C)	Transposition frequency (mean ± SD)
22	$(4.9 \pm 2.8) \times 10^{-7}$
30	$(6.8 \pm 5.8) \times 10^{-7}$
37	$(6.4 \pm 0.5) \times 10^{-7}$
40	$(2.6 \pm 2.2) \times 10^{-6}$

[a] In each case, three independent experiments were performed and the average and standard deviation were calculated.

IRs likely made of 14 bp, with 12 of these 14 bp being complementary (Table 1) (26). A detailed analysis of the boundaries of the transposed fragments identified five different IRR-like sequences downstream of the β-lactamase gene (Table 1). These IRR-like sequences had been recognized by the transposase of IS*Ecp1B* during the mobilization process. The number of identical base pairs among the sequences defined as IRR boundaries varied from 4 to 8 bp, corresponding to less than 50% identity with the IRR of IS*Ecp1B*. These results indicated that IS*Ecp1B* might use different sequences as IRRs downstream of the β-lactamase gene of the *K. ascorbata* chromosome. No consensus sequence could be determined by comparing the 14-bp-long IRR sequences identified in recombinant plasmids (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, as already reported (26).

Transposition frequencies under different growth conditions. Since changes of growth conditions may affect transposition efficiency of several mobile elements (19), the transposition of IS*Ecp1B*-bla_{KLUA} was examined in the presence of several concentrations of different antibiotics at different subinhibitory concentrations and under different temperatures.

Studied antibiotic concentrations were 1/2, 1/4, and 1/10 of the MICs. For *K. ascorbata* strain CIP7953, harboring IS*Ecp1B*-bla_{KLUA}, the MICs were as follows: amoxicillin, 512 μg/ml; piperacillin, 128 μg/ml; cefuroxime, 512 μg/ml; cefotaxime, 16 μg/ml; ceftazidime, 1 μg/ml; nalidixic acid, 4 μg/ml. No significant difference of transposition frequency was found with or without amoxicillin, cefuroxime, and nalidixic acid at the studied concentrations (Table 2). In contrast, the transposition frequency of the IS*Ecp1B*-bla_{KLUA} element was 100-fold higher when ceftazidime was added at 0.5 μg/ml (half of the MIC), 10-fold higher with ceftazidime at 0.25 μg/ml (one-quarter of the MIC) or cefotaxime at 8 μg/ml (half of the MIC), 7-fold higher with piperacillin at 64 μg/ml (half of the MIC), and 4-fold higher with cefotaxime at 4 μg/ml (one-quarter of the MIC) (Table 2). Under these experimented conditions, ceftazidime seemed to enhance the transposition of IS*Ecp1B* at a higher level than cefotaxime. No difference of transposition frequency was found with ceftazidime (0.1 μg/ml), cefotaxime (1.5 μg/ml), or piperacillin (32 and 13 μg/ml). Although amoxicillin and cefuroxime are widely prescribed for community-acquired infections and cefuroxime has been described as a risk factor in selection of ESBLs in the community (9), our results indicated that, under the experimental conditions described, those β-lactams might not select for those transposition events. By contrast, cefotaxime and ceftazidime may induce those transposition events, but they are not given frequently for treating community-acquired gram-negative infections.

Nalidixic acid, which may induce the SOS repair system and recombination events (3), did not play a role in IS*Ecp1B*-related transposition events. Thus, although there is somewhat of a strong association between quinolone resistance and ESBL phenotype in *Enterobacteriaceae* (23), this result would indicate that quinolone might not enhance the transposition of the bla_{CTX-M} gene. The association between quinolone resistance and ESBLs may rather result from clonal selection of specific strains by quinolone or expanded-spectrum cephalosporins.

The transposition frequency was increased at 40°C compared to that determined at 37°C, whereas no difference was observed at 22°C and 30°C (Table 3). These results may emphasize the induction of IS*Ecp1B* transposition under high-temperature conditions, as recently observed for four IS elements, IS*401*, IS*402*, IS*406*, and IS*Bmu3* in *Burkholderia multivorans* ATCC 17616 (20). This is the first report demonstrating increased transposition frequencies at a high temperature for an IS belonging to the IS*1380* family. This result contrasts with the decreased transposition activities of several mobile elements (e.g., Tn*3*, IS*1*, IS*30*, and IS*911*) in *E. coli* at this temperature (8, 19). The temperature sensitivity of transposition in *E. coli* has been considered to be a feature of each transposase (8, 19). It is tempting to speculate that global warming may increase transposition of IS*Ecp1B*. In addition, although growth curves did not differ significantly for *E. coli* and *K. ascorbata*, a slight increase of growth of both donor (*Kluyvera* sp.) and recipient (*E. coli*) at temperatures ranging from 22°C to 37°C may account also for dissemination of bla_{CTX-M} genes.

The reservoir of most bla_{CTX-M} genes has been identified in *Kluyvera* spp. (12, 21, 25, 28). Their translocation process has been studied here, which represents the first evidence of in vivo mobilization of a clinically important antibiotic resistance gene from its natural reservoir. Thus, there is now an urgent need for identification of the environmental location (if any) of *Kluyvera* spp.

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