Antimicrobial Agents and Chemotherapy, Sept. 2010, p. 4026–4028 Vol. 54, No. 9
0066-4804/10/$12.00 doi:10.1128/AAC.00075-10
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Association of the Extended-Spectrum β-Lactamase Gene $bla_{\text{TLA-1}}$ with a Novel IS*CR* Element, IS*CR20*▽

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Received 19 January 2010/Returned for modification 12 May 2010/Accepted 20 June 2010

The $bla_{\text{TLA-1}}$ gene encoding an extended-spectrum β-lactamase was identified in 11 enterobacterial isolates from Mexico City, Mexico. This gene was located on different plasmids and plasmid types with different sizes and incompatibility groups. It was associated with a novel insertion sequence, IS*CR20*, encoding a putative transposase that shared only 20% amino acid identity with the most closely related transposase of IS*CR1*. The IS*CR20* element provided specific promoter sequences for expression of the $bla_{\text{TLA-1}}$ gene.

Although the majority of Ambler class A extended-spectrum β-lactamases (ESBLs) identified in the family *Enterobacteriaceae* worldwide are mostly of the TEM, SHV, and CTX-M types, some "minor" ESBLs have been reported, such as VEB-like, GES-like, BES-1, SFO-1, and TLA-1 enzymes (5). The $bla_{\text{TLA-1}}$ gene was first reported in 2000 from an *Escherichia coli* clinical isolate from Mexico (8). From April 2000 until February 2002, the $bla_{\text{TLA-1}}$ gene was detected from an epidemic *Klebsiella pneumoniae* clone in Mexico, and both the $bla_{\text{TLA-1}}$ and $bla_{\text{SHV-5}}$ ESBL genes were located on the same plasmid (1). The $bla_{\text{TLA-1}}$ gene was identified on a 150-kb conjugative plasmid named pRZA92, but its genetic context had not been further characterized (8). Considering that many ESBL genes may be transposon or integron associated, the aim of this study was to characterize the genetic elements at the possible origin of acquisition of the $bla_{\text{TLA-1}}$ gene from isolates of members of the family *Enterobacteriaceae* recovered from several hospitals in Mexico City, Mexico.

The 150-kb plasmid pRZA92 of *E. coli* R170 (8) was extracted by using the Qiafilter plasmid purification maxikit (Qiagen, Courtaboeuf, France) and partially sequenced using the primers indicated in Table 1. A sequence of 5,923 bp was identified through a primer-walking approach and contained several open reading frames (ORFs) (Fig. 1). Upstream of the $bla_{\text{TLA-1}}$ gene, another gene (named *orf432*) was identified; this gene encoded a 432-amino-acid protein sharing 86% sequence identity with an uncharacterized *orf430* gene (83% amino acid identity) located in a class 1 integron from a *Proteus mirabilis* isolate recovered from Mediterranean herring gulls in Italy (GenBank accession number DQ520941) (3). The *orf432* gene encoded a putative transposase belonging to the IS*91* family of transposases that includes the newly described IS*CR* elements. The Orf432 transposase shared 20%, 21%, and 18% amino acid identity with the transposases encoded by the IS*CR1*, IS*CR2*, and IS*CR3* elements, respectively. Detailed analysis allowed us to identify the *orf432* gene as part of a novel IS*CR* element, termed IS*CR20* according to the official nomenclature (http://medicine.cf.ac.uk/en/research/research-groups/i3/research/antibacterial-agents/iscr-elements/). The IS*CR20* element is 1,705 bp long and possesses a G+C content of 47.1% (Fig. 1). A putative ori*IS* sequence was identified 255 bp downstream of the stop codon of the transposase gene and shared 10 identical bp (5′ACTG**ATAGGAA**C**T**GTC**A**TTT**C**3′ [the identical bases are shown in boldface type and underlined]) with the 19-bp consensus sequence reported for the other IS*CR*s (5′XXGTATAGGAAGTTCAAACGC3′) (10) (Fig. 1). A putative ter*IS* sequence forming a hairpin structure (5′GG**ACCC**GCACGCA**GGGT**GTT3′ [the boldfaced and underlined nucleotides indicate the complementary nucleotides forming the hairpin structure]) was also evidenced 136 bp upstream of the transposase start codon. The transposase of IS*CR20* shared an overall significant degree of identity with members of the IS*91* family transposases especially in the five functional domains previously described (Fig. 2). A tyrosine residue at position 218, which has been shown to be critical for the transposase activity of IS*91* and speculated to be also critical for those of the IS*CR* elements, was present in motif IV of the IS*CR20* transposase.

The $bla_{\text{TLA-1}}$ gene was followed by part of a gene encoding a methyl-accepting chemotaxis protein that shares 58% amino acid identity with that found in *Desulfovibrio magneticus* RS-1 (GenBank accession number YP_002952376). A group II intron, containing an *orf458* gene, was identified at the left extremity of the IS*CR20* element (Fig. 1). The *orf458* gene encoded a putative reverse transcriptase and maturase sharing 69% amino acid identity with those of *Vibrio cholerae* (GenBank accession number ABV21790). Analysis of the nucleotide sequence surrounding the $bla_{\text{TLA-1}}$ gene did not yield other insertion sequences that could be part of a transposon or any class 1 integron.

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▽ Published ahead of print on 28 June 2010.

TABLE 1. Sequences of primers used for detection of the bla_{TLA-1} gene and its circular form with IS*CR20* and for mapping the genetic environment

Primer name	Primer sequence	Position[a]
TLA-1F	TGTGTGCTTTTTGCTTCTGC	1
TLA-1R	GCTTCCGGTTTTATGAGCAA	2
TLA-1-5′-TR1	TACTGCTTTTAAGCGAATCCG	3
TLA-1-5′-TR2	TTAGCCGCAAAAGCAGAAGC	4
TLA-1-5′-TR3	AAGCAAAAAGCACACAAAATGC	5
IS91-5′-TR1	ACAACCGACATTGACCGTGG	6
IS91-5′-TR4	CTAAAACATGCCAGTGCGGG	7
IS91-5′-TR5	AATGCTCTAGGCGGTCAAGG	8
IS91-5′-TR6	GACATGGCTTTTTGCTGGCG	9
IS91-5′-TR7	GATATTGCTTCGATGCAGCAC	10
TLA1-3′-TR1	CATTGCTGTTTATGTGTCGG	11
TLA1-3′-TR4	CATCGCGTCATTAATCCTGC	12
TLA1-3′-TR5	GATCGAACAGATTTCCGCCT	13
TLA1-3′-TR6	ACGAACAACAGAAAAGCGCC	14
IS91-3′extR	CTTTTGTAACAACCTTGCCC	15
TLA1-3′extF	GTATTATTACTCTGCCGAACGG	16
IS91-5′extR	TCTGAAGTACGAATGGCCAC	17
IS91-3′extF	ACCAAGATTATTGACTGGGC	18
ICR20F	CAAAGCTGATCGTGAAGCCC	19
Maturase F	AACATCATGCTCGACCCAC	20
Methyl R	CATCGAATTGAGCGAGTCG	21

[a] Positions of the primers indicated in Fig. 1. All the primers were designed in this study.

A collection of 11 TLA-1-producing *Enterobacteriaceae* clinical strains (3 *E. coli* strains [including *E. coli* R170], 1 *K. pneumoniae* strain, and 7 *Enterobacter cloacae* strains) isolated from different samples (urine, blood, and tracheal fluid samples) from 11 patients hospitalized over a 10-year period since 1991 from several hospitals in Mexico City, Mexico, were retrospectively analyzed. These isolates were selected among clinical ESBL-producing enterobacterial isolates on the basis of two criteria: their ESBL profile and their production of a β-lactamase with an isoelectric point of 9.0. By DNA restriction with XbaI endonuclease, followed by pulsed-field gel electrophoresis (PFGE) analysis, we showed that most of the isolates were not clonally related, with four distinct *E. cloacae* PFGE types and three distinct *E. coli* PFGE types, suggesting a diffusion of bla_{TLA-1}-positive plasmids. The bla_{TLA-1} gene was transferred from seven of those distinct PFGE types to *E. coli* J53 by conjugation performed as described previously (6). Together with the expression of an ESBL phenotype, the *E. coli* transconjugants were resistant to trimethoprim, sulfamethoxazole, and tetracycline, and some of these transconjugants were resistant to amikacin ($n = 5$) or gentamicin ($n = 1$). Plasmid analysis performed by the Kieser technique (4) allowed us to visualize several plasmids in all the clinical isolates but only a single plasmid from each *E. coli* transconjugant. Southern blot hybridization with a bla_{TLA-1}-specific probe indicated that this ESBL gene was located on a single plasmid, being of either ca. 110 or 150 kb. PCR-based replicon typing (PBRT) analysis performed on the *E. coli* transconjugants (2) showed that the plasmids carrying the bla_{TLA-1} gene belonged to the IncA/C, IncL/M, or IncN incompatibility group, corresponding to the 150-, 150-, and 110-kb plasmids, respectively, highlighting that three different plasmid types were at the origin of the diffusion of the bla_{TLA-1} gene.

Except for *E. coli* isolate R2915 and its transconjugant, PCR amplification identified in all cases the same IS*CR20* element upstream of the bla_{TLA-1} gene. It has been suggested that the promoter of the bla_{TLA-1} gene was located 71 bp from the translational start site by researchers using a computer-based promoter analysis (8). However, by using a 5′ rapid amplification of cDNA end PCR system (Invitrogen, Cergy-Pontoise, France) and primers TLA-1-5′-TR1 to TLA-1-5′-TR3 (Table 1), the initiation site of transcription of the bla_{TLA-1} gene was mapped from *E. coli* R170. Promoter sequences were located 144 bp upstream of the translational start codon, corresponding to the −35 promoter sequence TTGACA separated by the 17-bp distance from the −10 promoter sequence TTAAAG. This result showed that the bla_{TLA-1} gene expression was driven by the IS*CR20* element. One pair of outward primers chosen at each end of the IS*CR20* insertion sequence was used to detect the circular form of this novel IS*CR* associated with the bla_{TLA-1} gene. The amplifications performed using strain R170 and its transconjugant as templates failed.

Nineteen IS*CR* elements with weak structural relationship (18 to 96%) have been found to be associated with different families of antibiotic resistance genes. As demonstrated with the transposition model of the IS*1294* elements (9), it is proposed that a transposase encoded by the IS*CR* has the ability to cotranspose DNA adjacent to its terminal ter*IS* sequence through a rolling-circle (RC) transposition mechanism (10). It has been suggested that the mobilization by IS*CR* elements of adjacent DNA sequences likely involving sequences located

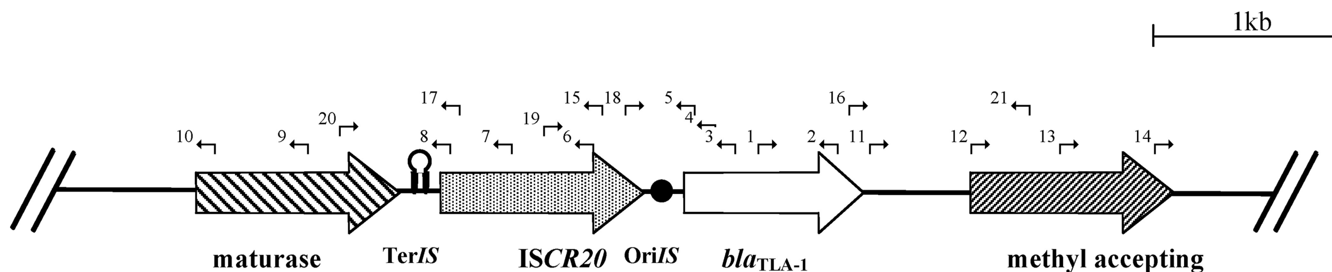

FIG. 1. Schematic representation of the sequences surrounding the bla_{TLA-1} gene in *E. coli* X170. The positions of primers shown in Table 1 are indicated by arrows; primers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 are primers TLA-1F, TLA-1R, TLA-1-5′-TR1, TLA-1-5′-TR2, TLA-1-5′-TR3, IS91-5′-TR1, IS91-5′-TR4, IS91-5′-TR5, IS91-5′-TR6, IS91-5′-TR7, TLA-1-3′-TR1, TLA-1-3′-TR4, TLA-1-3′-TR5, TLA-1-3′-TR6, IS91-3′-extR, TLA-1-3′extF, IS91-5′-extR, IS91-3′extF, ISCR20F, maturase F, and methyl R, respectively. Genes are shown by large arrows, and the transcription orientations of the genes are indicated. The putative origin of replication, ori*IS*, and termination of transposition, ter*IS*, of the IS*CR20* element are indicated by a solid black circle and a black hairpin, respectively.

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                                 MOTIF I                                                        MOTIF II
IS91      38   IMACGTTLMGYTQWCCSSPDCCHTKKVCFRCKSR-SCPHCGVK   80      96   PWQHIVFTLPCQYWSL    112
ISCR20    40   FTDCADWSKGVARIRCED--CGHSYFRPFSCKVFHLCPSCDQK   80      98   PHRQFVFTIIPKILRPY   114
ISCR1     62   LLQCGRLEYGFMRVRCED--CHHERLVAFSCKRRGFCPSCGAR  102     119   PIRQWVLSFPFQLRFL    135
ISCR2     61   FLQCGRLEHGFLRVRCES--CHAEHLVAFSCKRRGFCPSCGAR  101     118   PMRQWVLSFPFQLRFL    134
ISCR3     53   YLRCGVLEHGFLRVVCEH--CRAERLVAFSCKKRGFCPSCGAR   93     110   PVRQWVLSFPYPLRFL    126
ISCR5     53   YLRCGVLEHGFLRVVCEH--CRAERLVAFSCKKRGFCPSCGAR   93     110   PVRQWVLSFPYPLRFL    126
ISCR7-tr       -------------------------------------------             PVRQWVLAVPKRLRYF
ISCR8     60   YLECGIFAHGFARARCGD--CGHDYFVAFSCKGRGVCPSCTTR  100     118   PVRQWVLSVPKRLRYF    134
ISCR19    69   YLRCGVLEHGFLRVVCEH--CRAERLVAYSCKKRGLCPSCGAR  109     127   PVRQWVLSFPYPLRFL    143

                                 MOTIF III                            MOTIF IV                MOTIF V
IS91     139   VEPGIFTVIHTWGRDQQWHPHIHLSTTAGGVT   171     249   YFGSYLKK   257    310   RMVRYYGFLS   320
ISCR20   144   LLCASVVSYQSFGEFARFHPHWHVLVLEGGFT   176     241   ALGQYVVR   249    301   QLVRRYGVYA   311
ISCR1    168   AQTGSVTLIQRFGSALNLNVHYHMLFLDGVYA   200     323   RLCRYISR   331    382   NLTRFHGVFA   392
ISCR2    167   AKTGAVTLIQRFGSALNLNVHFHMLFLDGVYV   199     320   RLCRYISR   328    379   NLTRFHGVFA   389
ISCR3    159   AQQGAVTLIQRFGSALNLNIHFHMLWLDGVYV   191     315   KLCRYITR   323    374   HLTRFHGVFA   384
ISCR5    159   AQQGAVTLIQRFGSALDLNVHFHMLWLDGVYD   191     315   KLCRYITR   323    374   HLTRFHGVFA   384
ISCR7-tr       AHSGAVAFIHRFGSSLNEHVHFHCCVIDGVFE               RLLRYCAR                HRHRYYGVLA
ISCR8    171   LHIGAIAFIHRFGSSLNEHVHFHVCVVDGVFE   203     289   RLLRYCAR   297    355   HRHRYFGVLA   365
ISCR19   176   AQQGAVTLIQRFGSALNLNVHFHMLWLDGVYD   208     333   KLCRYITR   341    392   HLTRFHGVFA   402
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FIG. 2. Comparison of the sequence motifs of IS*CR20* and those of the IS*91* family transposases. The sequences (with their GenBank accession numbers shown in parentheses) shown are as follows: IS*CR1* (FJ187822), IS*CR2* (AY055428), IS*CR3* group, including IS*CR3*, IS*CR4*, IS*CR6*, IS*CR14*, and IS*CR16* (FJ183463), IS*CR7* (AJ250371), IS*CR8* (AF028594), IS*CR15* (AM998375), and IS*CR19* (EU503121). The five motifs found within IS*91* group elements are indicated. The amino acid residues conserved in all IS*91* group elements are shown boxed. Gaps introduced to maximize alignment are indicated by dashes.

only at their left extremities and the frequent observation of resistance genes bracketed by two copies of IS*CR* may be the consequence of secondary recombination events (10). This has been recently shown with the identification of the $bla_{\text{VEB-1a}}$ ESBL gene flanked by two copies of IS*CR2* in *Acinetobacter baumannii* (7). Here, no IS*CR20* element was identified downstream of the $bla_{\text{TLA-1}}$ gene, casting doubt on the involvement of that element in the mobilization process of the $bla_{\text{TLA-1}}$ gene. Further work is needed to understand the process that gave rise to the mobilization of the $bla_{\text{TLA-1}}$ gene.

Besides describing a novel IS*CR* element, our study indicates its involvement in the expression of the $bla_{\text{TLA-1}}$ gene and its likely role in its acquisition. In addition, we showed here that the dissemination of the $bla_{\text{TLA-1}}$ gene may have already occurred in Mexico and that it was related to different plasmid backbones. Further screening of TLA-1-positive isolates should be performed at least in neighboring countries, such as countries in Central America and in the southern United States. We showed a heterogeneity of plasmid backgrounds, supporting the hypothesis that the $bla_{\text{TLA-1}}$ gene was very likely harbored by a mobile genetic structure.

Nucleotide sequence accession number. The nucleotide sequence reported in this study has been deposited in the GenBank database under accession number GU441460.

This work was funded mostly by a grant from the INSERM (U914) and by grants from the European Community (TROCAR, HEALTH-F3-2008-223031) and from the Ministère de l'Education Nationale et de la Recherche (UPRES-EA3539), Université Paris XI, France.

We thank M. Toleman for providing us with an IS*CR* number.

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