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Common Region CR1 for Expression of Antibiotic Resistance Genes

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The CR1 element defined by an *orf513* gene encoding a putative recombinase and a recombination crossover site has been identified upstream of several antibiotic resistance genes in *Enterobacteriaceae*. This CR1 element was shown to bring promoter sequences that play a role in the expression of unrelated antibiotic resistance genes.

The role of class 1 integrons in acquisition, dissemination, and expression of antibiotic resistance genes is now well established in *Enterobacteriaceae* (1, 3, 8). Class 1 integrons possess two conserved features consisting of an integrase gene, *intI1*, in a so-called 5′ conserved sequence (5′-CS) and *qacEΔ1* and *sul1* genes in a so-called 3′ conserved sequence (3′-CS) (1). Promoter sequences that drive the expression of downstream-located antibiotic resistance genes have been identified in the 5′ end of the integrase gene (1). The complex class 1 integrons contain the 5′-CS and part of the 3′-CS flanking one or more gene cassettes (6). Following the 3′-CS is a region known as the common region (CR) consisting of *orf513* and a recombination crossover site followed by genes that do not resemble a gene cassette and flanked by another copy of the *qacEΔ1*/*sul1* complex (6). The encoded Orf513 may play a role in the integration of genes located nearby (mostly antibiotic resistance genes) (6). The *orf513* gene and the 33-bp DNA sequence located at its right-hand boundary that may correspond to a recombination crossover site (RCS) are the common region CR1. We have recently shown that CR1 plays a role in the expression of the plasmid-mediated quinolone resistance *qnrA* gene by providing a promoter structure (5). The aim of the study was to analyze CR1 elements and their putative role in the expression of other antibiotic resistance determinants from nonrelated enterobacterial isolates. Six strains that encoded resistance determinants including QnrA and emerging extended-spectrum β-lactamases of the CTX-M type were included in this study (Table 1). Since the nucleotide sequence separating CR1 from the antibiotic resistance gene may vary, strains with different structures located upstream of identical *qnrA* and $bla_{\text{CTX-M-9}}$ genes were studied (Table 1). Primers used for PCR identification and sequencing of CR1 elements and associated resistance genes are shown in Table 2.

Mapping of the transcription start sites was performed by 5′ rapid amplification of cDNA ends (5′-RACE). Total RNA was isolated from the different strains studied using the RNeasy Midi kit (QIAGEN, Courtaboeuf, France). 5′-RACE reactions were performed using 5 μg of total RNA of each strain and the 5′-RACE System kit (version 2.0; Invitrogen Life Technologies, Cergy Pontoise, France) according the manufacturer's recommendations. After a reverse transcription step with gene-specific primer GSP1 and reverse transcriptase, the cDNA was tailed with terminal deoxynucleotidyl transferase and was subsequently amplified with another gene-specific primer, GSP2, combined with an oligo(dT) adapter primer provided with the kit (Table 2). This PCR product was used as a template for a nested PCR with another adapter primer and primer GSP3. The PCR product obtained was cloned into pCR-BluntII-Topo (Invitrogen), and the corresponding clones possessing the larger insert were sequenced. Analysis of the cloned sequence allowed the determination of the transcription initiation site(s), defined as the first nucleotide following the sequence of the adapter primer. Promoter sequences were determined subsequently. For each transcription assay, at least 10 clones were analyzed, and the entire experiment was repeated twice for all strains.

Identical CR1 elements were identified in all cases, and their 3′ ends are shown in Fig. 1. The CR1 element was associated with the *qnrA* gene in two cases, with $bla_{\text{CTX-M-9}}$ in two cases, with $bla_{\text{CTX-M-2}}$ in one case, and with the *dfrA10* gene encoding trimethoprim resistance in one case (Table 1 and Fig. 1). The distance separating the RCS of CR1 from the start codon of the antibiotic resistance genes varied. This distance upstream of the *qnrA* gene was 98 and 31 bp for *Escherichia coli* Lo and

TABLE 1. Characteristics of the clinical strains included in this study

Strain	Geographical origin	Gene	Distance separating CR1 from the resistance gene (bp)	Antibiotic resistance[a]	Source or reference
E. coli Lo	Bicêtre, France	*qnrA*	98	Quinolone	5
K. pneumoniae K149	Melbourne, Australia	*qnrA*	31	Quinolone	This study
E. coli B36	Madrid, Spain	$bla_{\text{CTX-M-9}}$	94	ESBL	This study
K. pneumoniae KP40C	Madrid, Spain	$bla_{\text{CTX-M-9}}$	94	ESBL	2
E. coli JAB	Bicêtre, France	$bla_{\text{CTX-M-2}}$	266	ESBL	4
A. baumannii AYE	Bicêtre, France	*dfrA10*	149	TMP	7

[a] ESBL, extended-spectrum β-lactamase; TMP, trimethoprim.

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TABLE 2. Primers used in this study

Primer	Expt	Nucleotide sequence (5′–3′)	Location	Source or reference
ORF513D3	PCR	CTCACGCCCTGGCAAGGTTT	*orf513*	5
ORF513D5	PCR	CTTTTGCCCTAGCTGCGGT	*orf513*	5
QnrA	PCR	GGGTATGGATATTATTGATAAAG	*qnrA*	9
QnrB	PCR	CTAATCCGGCAGCACTATTA	*qnrA*	9
Pre-qnrA1	PCR	CGGCAGTTAAAATTGGGGCT	Upstream of *qnrA*	This study
Pre-qnrA2	PCR	GAGGGAATTTCAGGTAAGATAC	Upstream of *qnrA*	This study
CTX-MA1	PCR	SCSATGTGCAGYACCAGTAA	bla_{CTX-M}	4
CTX-MA2	PCR	CCGCRATATGRTTGGTGGTG	bla_{CTX-M}	4
dfrB	PCR	ATGCGCAGCATTTGGGTGTC	*dfr*	This study
dfrF	PCR	GTTATGGAGCAGCAACGATG	*dfr*	This study
GSP1-*qnrA*	5′-RACE	AAGTACATCTTATGGCTGACTTGA	*qnrA*	5
GSP2-*qnrA*	5′-RACE	ATGAAACTGCAATCCTCGAAACTG	*qnrA*	5
GSP3-*qnrA*	5′-RACE	TGGCTGAAGTCACACTGATAAAAG	*qnrA*	5
GSP1-$bla_{CTX-M-9}$	5′-RACE	CCAGCGCATGACCCAGCGTAAC	$bla_{CTX-M-9}$	This study
GSP2-$bla_{CTX-M-9}$	5′-RACE	GCGTCATTGTGCCGTTGACGTGT	$bla_{CTX-M-9}$	This study
GSP3-$bla_{CTX-M-9}$	5′-RACE	GCACCGCACTCGTCTGCGCATA	$bla_{CTX-M-9}$	This study
GSP1-$bla_{CTX-M-2}$	5′-RACE	CGCGAGCGGCGTGGTGGTAT	$bla_{CTX-M-2}$	This study
GSP2-$bla_{CTX-M-2}$	5′-RACE	CGTTCATCGGCACGGTAGAGAA	$bla_{CTX-M-2}$	This study
GSP3-$bla_{CTX-M-2}$	5′-RACE	CCAAGCCGACCTCCCGAACTT	$bla_{CTX-M-2}$	This study
GSP1-*dfrA10*	5′-RACE	GCACCCCAACCAGCGAAGCT	*dfrA10*	This study
GSP2-*dfrA10*	5′-RACE	GCACTTCGTGCTCTGTGATAGTT	*dfrA10*	This study
GSP3-*dfrA10*	5′-RACE	GCCTTGATTACCGAATGCTCT	*dfrA10*	This study

Klebsiella pneumoniae K149, respectively (Table 1). An identical 94-bp region was identified between the RCS of CR1 and the start codon of the $bla_{CTX-M-9}$ gene in *E. coli* B36 and *K. pneumoniae* KP40. However, a 95-bp duplication was identified at the right-hand boundary of CR1 in *E. coli* B36 (Fig. 1). A 149-bp sequence was identified between CR1 and the *dfrA10* gene in *Acinetobacter baumannii* AYE, whereas a 266-bp region was identified upstream of the $bla_{CTX-M-2}$ gene in *E. coli* JAB.

Analysis of the 5′-RACE PCR products obtained from

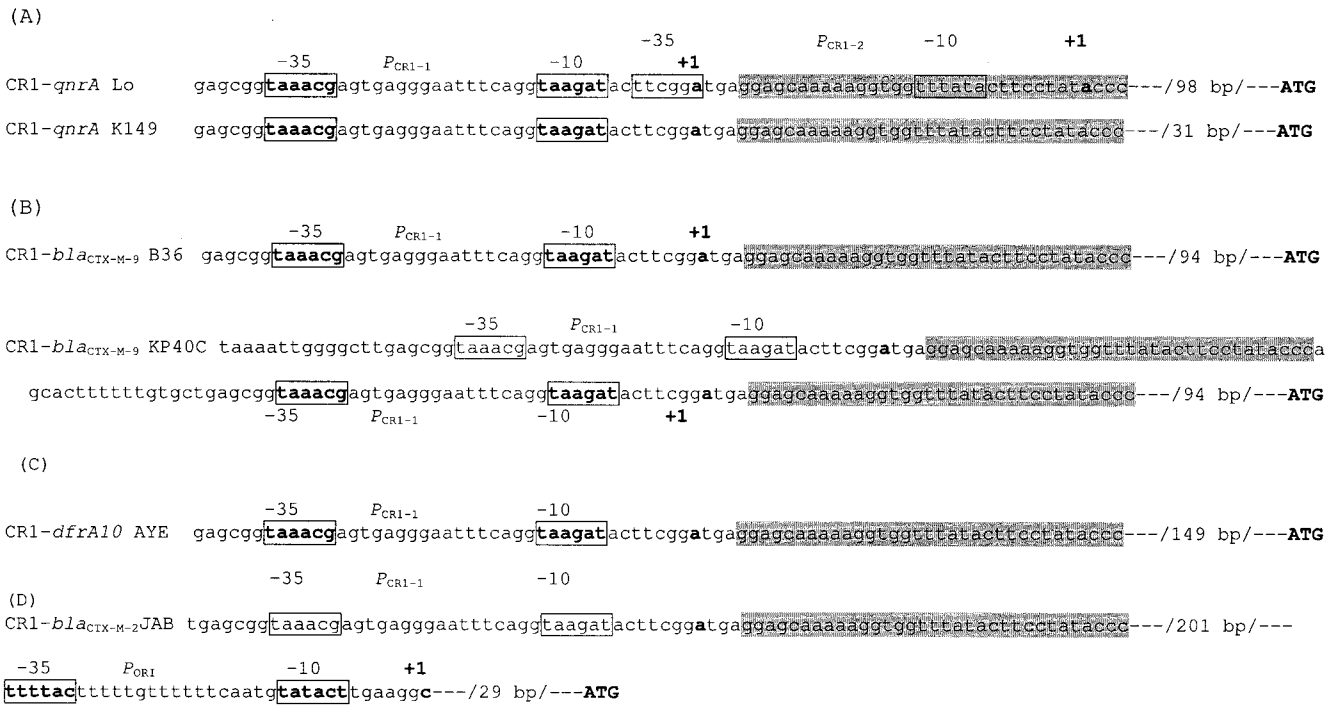

FIG. 1. Promoter structures for the expression of antibiotic resistance genes as determined by 5′-RACE experiments for the *qnrA* gene from *E. coli* Lo and *K. pneumoniae* K149 (A), $bla_{CTX-M-9}$ from *E. coli* B36 and *K. pneumoniae* KP40C (B), *dfrA10* from *A. baumannii* AYE (C), and $bla_{CTX-M-2}$ from *E. coli* JAB (D). The +1 initiation sites of transcription and the active promoter sequences are in boldface type. The −35 and −10 motifs of other promoters are boxed. Promoter P_{CR1-2}, which was identified previously for the *qnrA* gene in *E. coli* Lo, is indicated for the sake of consistency (5). The ATG start codons are capitalized. The right-hand boundary of the CR1 element is shaded in gray.

QnrA-positive *E. coli* Lo revealed a single type of transcription product that was different from that previously reported (5). Although promoter P_{CR1-2} has been identified previously (5), a +1 transcription site located 136 bp upstream of the *qnrA* gene led to the identification of another promoter, termed P_{CR1-1} (Fig. 1). In the previous study, identification of the P_{CR1-2} promoter was likely the result of selection of truncated transcripts during the 5′-RACE experiment. The same promoter, P_{CR1-1}, controlled the expression of the *qnrA* gene in *K. pneumoniae* K149, the $bla_{CTX-M-9}$ gene in *E. coli* strain B36 and in *K. pneumoniae* KP40C, and the *dfr10* gene in *A. baumannii* AYE (Fig. 1). These results also indicated that promoter P_{CR1-1}, provided by the CR1 element, was active in *Enterobacteriaceae* and *A. baumannii*. The expression of the $bla_{CTX-M-2}$ gene in *E. coli* JAB depended on another promoter, P_{ORI}, located outside the CR1 element between the CR1 and the initiation codon of the resistance gene (Fig. 1).

Our results showed that CR1-mediated promoter sequences were involved in the expression of the *qnrA*, *dfrA10*, and $bla_{CTX-M-9}$ genes (Fig. 1) and that P_{CR1-1} plays a major role in antibiotic resistance gene expression when CR1 is present. This study emphasizes that CR1 plays a significant role by providing promoter sequences for the expression of unrelated antibiotic resistance genes.

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