Transcriptional Analysis of the blaCTX-M-2 Gene in Salmonella enterica Serovar Infantis

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Transcriptional organization of blaCTX-M-2 present in a multiresistance plasmid of Salmonella enterica serovar Infantis suggests the presence of more than one promoter involved in the expression of the β-lactamase gene. At least two blaCTX-M-2-specific mRNAs (near to 1 kb and 5 kb) were evidenced. Two +1 signals were detected at −22 bp and −59 bp of blaCTX-M-2 defining two putative promoters.

CTX-M enzymes represent a growing family of class A extended-spectrum β-lactamases (ESBLs) that preferentially hydrolyze cefotaxime over cefazidime, both oximino cephalosporins, including more than 30 members organized in six groups (3). In Argentina, the more prevalent ESBL is CTX-M-2 (20, 22, 23), representative of the CTX-M-2 group (3).

Several reports have identified −35 and −10 promoter regions included in an ISEcp1-like element upstream to blaCTX-M-13 and blaCTX-M-14 (6), blaCTX-M-15 (2, 13), blaCTX-M-17 (4), blaCTX-M-19 (18), blaCTX-M-21 (accession number AJ416346), blaCTX-M-25 and blaCTX-M-26 (15), blaCTX-M-32 (5) and other bla genes encoding CTX-M enzymes (8, 26), involved in the expression of these enzymes.

By contrast, an open reading frame (orf513) associated to unusual class 1 integrons was found upstream to blaCTX-M-2 and blaCTX-M-9 (1, 7, 14, 19, 25, 28). Even if these genes have been suggested to be associated also to ISEcp1 (3), this association has not been confirmed up to now. To our knowledge no data have been reported related to the expression of these enzymes.

In this work we studied the transcriptional organization of blaCTX-M-2 from a Salmonella enterica serovar Infantis plasmid. Plasmid pS21 is a conjugative plasmid from a cefotaxime-resistant Salmonella serovar Infantis clinical isolate (S21). pS21 carries InS21, an unusual class 1 integron with the blaCTX-M-2 gene associated to orf513 (7).

Expression of blaCTX-M-2 in the presence of its upstream region. A fragment of 1.5 kb (including blaCTX-M-2 plus a 500-bp upstream region), obtained by PCR with Bla2 (5′-GA TACCTCGCTCATTTATTGC) and Bla-down (5′-TGTCGACCCCAAATCC) from pS21, was cloned into the pCR2.1 vector (Invitrogen), to obtain recombinant plasmid pCR. The insert of pCR was then removed by digestion with EcoRI and subcloned into the plasmid vector pPR328C1 (21). Two recombinant plasmids (pPR328C5 and pPR328C6) with the insert in both orientations were confirmed by sequence analysis of the regions flanking blaCTX-M-2. Sequence analysis of the 500-bp region upstream of blaCTX-M-2 in InS21 reveals two different regions: (i) a sequence of 266 bp 96% identical to the upstream region of the KluA-1-encoding gene (a chromosomal β-lactamase from Kluyvera ascorbata; EMBL accession number AJ272538), with identical putative ribosome-binding site sequences (AGAGG) and −10 promoter region (TTGAAAG) to those described for other enzymes of same group, but lacking a −35 promoter region (9, 10, 12); and (ii) a distal segment which is 100% identical to the 234-bp common region (CR) downstream of orf513 (CR1) described in unusual class 1 integrons (11) including the right-hand boundary of the CRs (17). A putative promoter in this region (similar to the Escherichia coli consensus promoter) has been proposed for dfrA10 of In7 (16) and could function as another promoter for blaCTX-M-2 expression (Fig. 1A). A 28-bp inverted-repeat repeat at 6 bp downstream to the blaCTX-M-2 stop codon could operate as a factor-independent transcription terminator (Fig. 1B).

Transcriptional characterization of blaCTX-M-2. Total RNA was extracted using the FastRNA kit BLUE (BIO 101) according to the manufacturer’s recommendations. Samples were dissolved in 0.2% diethyl pyrocarbonate-treated water. The probe used was a 902-bp PCR ampiclon produced from pS21 DNA with the Bla1 and Bla2 primers (7), labeled by random primer synthesis employing [α-32P]dCTP (27). Hybridization was performed under high-stringency conditions with an incubation temperature of 42°C for 16 h. At least two blaCTX-M-2 transcripts were detected by Northern blot in S21 (Fig. 2). Considering blaCTX-M-2 gene size, the 0.95- to 1.38-kb blaCTX-M-2-specific transcripts may correspond to monocistronic transcripts.

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However, larger (5-kb) \( \text{bla}_{\text{CTX-M-2}} \)-positive transcripts were also detected, which may correspond to polycistronic mRNAs.

The organization of \( \text{bla}_{\text{CTX-M-2}} \)-specific mRNA was explored by reverse transcription-PCR (RT-PCR) strategies (Fig. 3). RNA (3 \( \mu \)g) was reverse transcribed in a final volume of 100 \( \mu \)l using 2 \( \mu \)l hexanucleotide (Promega) or 10 \( \mu \)l reverse primer, 10 \( \mu \)l M (Bla2 or Bla-up), according to the Reverse Transcription System (Promega). Ten microliters of RT product was used for specific amplification by PCR experiments in a final volume of 100 \( \mu \)l. RNA samples without RT were used as negative controls. Each RT-PCR was performed in triplicate. Every PCR (I, II, III, and IV) done on S21 RNA subjected to random RT (with hexanucleotides) and directed RT (with Bla2 primer) was positive, and the predicted amplicons were obtained in each case. These results and the presence of \( \text{bla}_{\text{CTX-M-2}} \)-positive transcripts of 5 kb suggest that at least one promoter located in CR1 (or even upstream of CR1) could mediate \( \text{bla}_{\text{CTX-M-2}} \) expression.

Transcription System (Promega). Ten microliters of RT product was used for specific amplification by PCR experiments in a final volume of 100 \( \mu \)l. RNA samples without RT were used as negative controls. Each RT-PCR was performed in triplicate. Every PCR (I, II, III, and IV) done on S21 RNA subjected to random RT (with hexanucleotides) and directed RT (with Bla2 primer) was positive, and the predicted amplicons were obtained in each case. These results and the presence of \( \text{bla}_{\text{CTX-M-2}} \)-positive transcripts of ~5 kb suggest that at least one promoter located in CR1 (or even upstream of CR1) could mediate \( \text{bla}_{\text{CTX-M-2}} \) expression.

Primer extension was done using the 5'-labeled Bla-up oligo-
putative promoters with a score cutoff at 0.90, all located prox-
 journalistic program (http://www.fruitfly.org/seq_tools/promoter
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 putative promoters defined in this upstream region (P1 and
 sequences were searched for [26]) is identical to that analyzed
 sites that support the promoter prediction program results.
 Prokaryotic prediction on the 500-bp sequence upstream of
 blaCTX-M-2 was carried out by Neural Network Promoter Predic-
 tion program (http://www.fruitfly.org/seq_tools/promoter.html). Three sequences were detected by this program as
 putative promoters with a score cutoff at 0.90, all located prox-
 imal to the start codon of blaCTX-M-2. One of them included the
 –10 and –35 regions of the proposed P1 promoter, and the
 other two sequences contained only the –10 region of the
 P1 and P2 putative promoters.

 Although several putative promoters have been proposed for
 blaCTX-M-2 group genes by sequence analysis, this is the first
 experimental characterization of two transcriptional starting
 sites that support the promoter prediction program results.
 Putative –35 and –10 promoter sequences were proposed by
 Saladin et al. (26) in two Proteus mirabilis isolates producing
 CTX-M-2-type β-lactamases. They employed an internal
 ISεcp1 forward primer (ISεcp1 U1) containing the typical
 –35 promoter region, for PCR amplification. However, imme-
diately downstream to this primer, a 17-bp sequence identical
to the 3′ end of CR1 (where is included orf513) was described.
 Considering the 3′ end sequence of CR1, a nonspecific hybrid-
 ization of the ISεcp1 U1 primer could not be disregarded.
The 266-bp sequence present immediately upstream to
 blaCTX-M-2-type genes in those isolates (where no promoter
 sequences were searched for [26]) is identical to that analyzed
 here.

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