Nucleotide Sequence of the ampC-ampR Region from the Chromosome of Yersinia enterocolitica

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The nucleotide sequence of a 3.1-kb region from the chromosome of the Yersinia enterocolitica O:5b strain IP97 containing the gene for an inducible chromosomal cephalosporinase has been determined. The cephalosporinase gene was homologous to other enterobacterial chromosomal cephalosporinase genes, and it was accompanied by a gene homologous to the regulatory ampR gene. The arrangement of genes in the Y. enterocolitica ampCR unit was identical to that in the Enterobacter cloacae and Citrobacter freundii ampCR units.

MATERIALS AND METHODS

Bacterial strains, phages, and plasmids. Plasmid pSU602 has already been described elsewhere (18). It contains a Sau3AI fragment from the chromosome of Y. enterocolitica O:5b strain IP97 cloned in the BamHI site of the vector plasmid pACYC184. In E. coli, it expressed a β-lactamase of biochemical properties similar to those described for the Bush group 1 β-lactamases. E. coli UB1637 his lys trp recA56 rpsL (5) is the nonsuppressor strain used as a host for pSU602 in Tn5seq1 insertion experiments. The donor of Tn5seq1 is λ::Tn5 seq1 b221 c1857. M13mp18 and M13mp19 propagated in E. coli JM103 were used for cloning and sequencing as described previously (16).

Transposon mutagenesis of pSU602. Tn5seq1 (14) was used for mutagenesis of pSU602. At the same time, since Tn5seq1 contains sequences homologous to SP6 and T7 primers at its ends, pSU602::Tn5seq1 derivatives can be used for sequencing. E. coli UB1637(pSU602) cells were infected with a high-titer lysate of λ::Tn5seq1 as described in reference 19 and plated on L-agar plates containing kanamycin (50 μg ml−1). The colonies obtained were replicated on L-agar plates with ampicillin (50 μg ml−1), and those that were susceptible were selected for further work.

Plasmid DNA preparation and recombinant techniques. Plasmid DNA was purified by alkaline lysis. M13 replicative form and single-stranded DNAs were prepared as described in reference 16. Restriction enzymes and DNA ligase were obtained from Boehringer Mannheim and used as recommended by the supplier. The restriction fragments indicated in Fig. 1 were cloned in the appropriate sites of M13mp18 and M13mp19 by standard methods.

DNA sequencing and analysis. The nucleotide sequence was determined by the method of Sanger, using the Sequenase sequencing kit from U.S. Biochemicals. Both M13 single-stranded and double-stranded plasmid (pSU602::Tn5seq1) DNAs were used as templates. The −40 M13 primer was used to sequence M13 clones, and SP6 and T7 sequencing primers were used to sequence pSU602::Tn5seq1 plasmid DNAs. The nucleotide sequence data were organized and analyzed by using the MicroGenie sequence analysis package from Beckman.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under the accession number X63149.

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FIG. 1. Restriction map of the insert present in recombinant plasmids pSU602 and pSU625 (partial map). Vertical arrows mark the positions of Tns5seq1 insertions used for sequencing. Horizontal bars in the lower part of the figure represent restriction fragments that were cloned in the appropriate sites of M13mp18 and M13mp19 and sequenced from both ends. Restriction maps are shown. E, EcoRI; h, HindIII; K, KpnI; P, PstI; s, Sau3AI. Only some Sau3AI sites at the ends of the pSU602 insert are shown. The S/B site at the right end of this insert is a BamHI site in pSU602 but a Sau3AI site in pSU625.

RESULTS AND DISCUSSION

DNA sequence determination. Figure 1 shows the restriction map of the insert in plasmid pSU602, the M13 subclones, and the location of Tns5seq1 insertions which were used to determine the nucleotide sequences of both DNA strands. The GATC Sau3AI site at the right end of the insert in plasmid pSU602 was next to the stop codon of an open reading frame (ORF). To extend the sequence beyond the limits of pSU602, another recombinant cephalosporinase plasmid containing a large Sau3AI fragment from the Y. enterocolitica IP97 chromosome at the same BamHI site of pACYCl84 gene, designated pSU625 (unpublished data), was used. The nucleotide sequence of a 3,143-bp EcoRI-Sau3AI fragment is shown in Fig. 2. The amino acid sequence deduced from the DNA sequence is shown along with the DNA sequence, in the one-letter code.

The ampC gene. An ORF was found between the ATG start codon at position 1548 and the TGA stop codon at position 2714. This ORF could translate into a 389-amino-acid polypeptide which perfectly matched the sequence of other known group 1 β-lactamases. The estimated molecular mass of the polypeptide was 43,082 Da. The amino-terminal residues presumably constitute a signal peptide involved in the periplasmic localization of the enzyme. According to the von Heijne prediction method (20), the cleavage site might be located between A-24 and Q-25. The removal of this signal peptide would result in a protein of 365 amino acids, with a molecular mass of 40,384 Da. Comparison of the amino acid sequences of mature forms of known Bush group 1 β-lactamases, using the MicroGenie program Align,
showed that the *Y. enterocolitica* cephalosporinase was equally distant (57% of identity) from the other three known enterobacterial cephalosporinases, which formed a compact group with a degree of identity of about 70% among them (Table 1).

The *ampR* gene. A second large ORF has been found, with transcription oriented opposite to that of *ampC*. It started with the ATG at position 1413 and ended at the TAG stop codon at position 529 and was identified as the regulatory *ampR* gene. Some Tn5seg1 insertions in pSU602, selected because they inactivated the resistance to ampicillin, were found to map within the *ampR* gene. This indicated that *ampC* was poorly expressed in the absence of a functional *ampR* gene product.

The predicted translation product of this ORF was a polypeptide of 294 amino acids. Its deduced amino acid sequence was aligned with the sequences of AmpR proteins from *E. cloacae* and *C. freundii*, which are identical in 237 of 292 residues (81.2%). AmpR from *Y. enterocolitica* showed greater divergence than its relatives (60.9 and 65.3% identity with *C. freundii* and *E. cloacae* AmpR, respectively). This divergence will allow better delimitation of conserved regions in AmpR. A recent report (1) described four amino acid positions whose substitutions abolished AmpR biological function. G-102, D-135, and Y-264 are conserved in *Y. enterocolitica* AmpR. The A-for-S substitution at position 35 of *Y. enterocolitica* AmpR does not seem to alter the function of the protein. The S-35 residue is conserved in most members of the LysR family of activator proteins (7) and has been located to the second helix of a helix-turn-helix motif involved in binding DNA. In principle, alanine could probably retain the α-helix structure of the motif, and consequently the DNA binding ability would not be affected by the change.

The degree of conservation between the *ampC* genes is higher than that between the accompanying *ampR* genes. This probably reflects the different functions of the two polypeptides. Fewer conservative changes are allowed in AmpR than in AmpC, since AmpR is a 32-kDa polypeptide which binds DNA and perhaps small ligands and also oligomerizes, whereas AmpC is a 40-kDa protein whose only known function is to hydrolyze the β-lactam ring.

The control region. The 135-bp region between the *ampR* and *ampC* start codons, containing the promoters for both genes as well as the binding site for AmpR, is known as the control region. Its functions have been well studied in *C. freundii* (13). The *Y. enterocolitica* control region is very similar to its *C. freundii* counterpart, and we presume that the same functions reside in the homologous regions (Fig. 3).

The surroundings of the *ampRC* unit. A total of 528 bp beyond the *ampR* stop codon and 429 bp after the *ampC* stop codon were sequenced. We have not found evidence of transcription terminators in these sequences, nor was there similarity to the corresponding regions from *E. coli*, *C. freundii*, or *E. cloacae*. Similarity comparison of the DNA sequences flanking the *ampRC* unit as well as the putative translation products against the GenBank and GenPept data bases by using the FASTA program (15) did not yield any significant similarity. This was surprising, since the high degree of identity between the *amp* units predicted the finding of the *frd* genes in front of *ampR*. In addition, it had been reported previously that identical EcoRI bands from the chromosomes of *E. coli* and *Y. enterocolitica* hybridized with a frdA probe (2). It would be interesting to study whether the gene orders are similar in *Y. enterocolitica* and *P. vulgaris* ampC or *Y. enterocolitica* frd loci would be necessary to test this hypothesis.

In conclusion, this report suggests that the inductive *ampC* genes and their underlying regulatory mechanism are very well conserved in enterobacteria. *Y. enterocolitica* ampCR genes should be useful as a third system for the study of the induction of *ampC* expression. At the same time, the finding that the *Y. enterocolitica* *ampC* gene is not linked to *frd* could be exploited to study the gene rearrangements which led to species segregation during enterobacterial evolution.

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### REFERENCES