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.

Bush group 1 β-lactamases (molecular class C) are common in gram-negative bacteria, and they are usually encoded by chromosomal genes. The enzymes from different genera share many characteristics, such as size (around 40 kDa), alkaline isoelectric point, and the ability to hydrolyze cephalosporins. In some cases, expression is inducible by β-lactam antibiotics (17). Although the mechanism of induction is not clearly understood, it requires the activity of *ampR*, *ampD* (11), and *ampG* (10). The *ampR* gene is always located at the 5' side of *ampC*, from which it transcribes in the opposite orientation. Its presence is absolutely required for *ampC* induction. AmpR binds to DNA in the *ampCR* control region, acting as a repressor of its own expression and as an activator of *ampC* transcription when the inducer is present (13). The *ampDE* and *ampG* loci are not linked to *ampCR*. Their likely function is the signaling and activation of *ampR* when the inducer is present in the culture medium.

The *ampCR* locus of three bacteria of different genera, *Escherichia coli* (9), *Citrobacter freundii* (12, 13), and *Enterobacter cloacae* (8), have been well studied. The three loci are very well conserved, although the *ampR* gene is absent in *E. coli*, probably as a result of a deletion. This makes the *E. coli* cephalosporinase noninducible by β-lactams. In the three species mentioned above, the *amp* genes are very close to the *frd* regulon encoding the fumarate reductase. In fact, the *frd* terminator overlaps with the *ampR* terminators in *E. cloacae* (8) and *C. freundii* (13) and with the *ampC* leader region in *E. coli* (6). As an exception, *Proteus vulgaris frd* genes are followed by neither *ampR* nor *ampC* genes (3).

Most *Yersinia enterocolitica* strains are known to produce two different β-lactamases (4). The genes for the two enzymes have been separately cloned and expressed in *E. coli* (18). One of the enzymes is a penicillinase. The nucleotide sequence of its gene has been determined and shows that the enzyme is a class A β-lactamase (19). The second enzyme, which is an inducible cephalosporinase, was cloned from the chromosome of *Y. enterocolitica* IP97, a strain belonging to the O:5b serotype. Here we present the nucleotide sequence of a 3-kb fragment of DNA containing the structural gene for the cephalosporinase (*ampC*) and an adjacent gene encoding a polypeptide almost identical to the regulatory AmpR proteins from *E. cloacae* and *C. freundii*.

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MATERIALS AND METHODS

Bacterial strains, phages, and plasmids. Plasmid pSU602 has already been described elsewhere (18). It contains a *Sau*3AI fragment from the chromosome of *Y. enterocolitica* O:5b strain IP97 cloned in the *Bam*HI 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 Tn*5seq*1 insertion experiments. The donor of Tn*5seq*1 is λ::Tn*5seq*1 *b*221 *c*I857. M13mp18 and M13mp19 propagated in *E. coli* JM103 were used for cloning and sequencing as described previously (16).

Transposon mutagenesis of pSU602. Tn*5seq*1 (14) was used for mutagenesis of pSU602. At the same time, since Tn*5seq*1 contains sequences homologous to SP6 and T7 primers at its ends, pSU602::Tn*5seq*1 derivatives can be used for sequencing. *E. coli* UB1637(pSU602) cells were infected with a high-titer lysate of λ::Tn*5seq*1 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:: Tn*5seq*1) 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:: Tn*5seq*1 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.
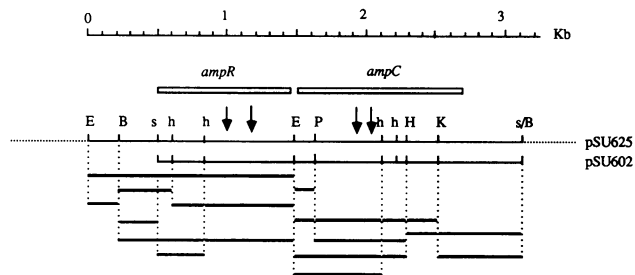
FIG. 1. Restriction map of the insert present in recombinant plasmids pSU602 and pSU625 (partial map). Vertical arrows mark the positions of Tn*5seq*1 insertions used for sequencing. Horizontal bars in the lower part of the figure represent restriction fragments that were cloned in the appropriate sites of M13mp18 and M13mp19 and sequenced from both ends. Restriction enzymes: B, *Bam*HI; E, *Eco*RI; h, *Hinc*II; H, *Hind*III; K, *Kpn*I; P, *Pst*I; s, *Sau*3AI. Only the *Sau*3AI sites at the ends of the pSU602 insert are shown. The s/B site at the right end of this insert is a *Bam*HI site in pSU602 but a *Sau*3AI 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 Tn*5seq*1 insertions which were used to determine the nucleotide sequences of both DNA strands. The GATC *Sau*3AI 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 *Sau*3AI fragment from the *Y. enterocolitica* IP97 chromosome at the same *Bam*HI site of pACYC184 gene, designated pSU625 (unpublished data), was used. The nucleotide sequence of a 3,143-bp *Eco*RI-*Sau*3AI 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 mature product 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,

```
    1 GAATTCGTCAGAATCATCAGGATGAACCACAGTATGGTCAGCAAGTCGCCCCGATAGATCAGCGTGTGCAGCGGTTGGTCATGGCACTGGTGTTTGCGGC
  101 CAAAAGTGATGGGCATATTGAGCGAAAGATGCGCAGTATTATTGAGCAGAATATGCGCGAAGCGGGTATCAGTGAGCAGGGTGAACGTCTGGTGCAACAG
  201 GCAATTGACCAGCCATTGGATCCTCAATTACTGGCGCGTGATATTCAAAATGAAGAGGAAGCGCTGGAGCTATACTTCCTGAGCTGCGCCGTCATTGATG
  301 TGGACCATTTTATGGAGCGTACGTATCTTGCCGCGCTAGGGCACGCACTGAAAATTCCGCAAGATGTTCGTGATGGAATTGAACAAGACATTCGAGAGAA
  401 AAAGCAAAGCATTACCGACTAATTTACTTGCAGGCGGCCTTTGGTATTCTCCAATAGGCCACTGTTTCAGAAGCGATGCTTCGCAGATTTTGGCGGTTCA
                           ***  E  G  E  S  K  M  E  S  L  L  W  L  A  F  D  R  M  A  P  T  E  T  R
  501 AACAGTATTATTAGACGTTAATGCGGATCTATTCTCCCTCAGACTTCATTTCAGACAATAGCCACAGAGCAAAATCCCGCATAGCCGGCGTCTCAGTGCG
       S  Q  L  R  T  L  W  Y  G  G  L  S  V  T  T  S  F  P  Q  I  R  E  S  R  L  L  H  M  F  M  A  P
  601 GGACTGTAGGCGAGTTAACCAATATCCCCCGAGACTGACTGTCGTGCTAAAAGGTTGGATTATGCGTTCACTCCGTAGCAGGTGCATAAACATAGCGGGT
       P  A  L  A  I  G  I  E  A  Q  A  A  E  L  M  S  V  S  S  D  F  M  M  I  Q  Q  S  P  S  P  V  S  G
  701 GGAGCCAGCGCGATACCTATTTCTGCCTGCGCCGCTTCCAGCATACTGACTGACGAGTCGAACATCATTATTTGCTGGGAAGGTGAAGGTACAGACCCCC
       G  A  A  A  F  W  A  S  W  N  M  A  R  Y  S  G  L  L  M  F  R  Q  L  D  T  P  P  T  F  R  K  S  I  S
  801 CCGCAGCAGCAAACCAAGCCGACCAATTCATCGCACGGTAGGAACCGAGCAGCATAAACCTTTGAAGATCCGTAGGTGGCGTAAATCTTTTGCTAATTGA
       P  A  C  L  Q  A  L  P  P  A  Y  M  F  H  S  E  H  W  A  L  A  G  N  G  Y  R  I  A  Y  D  L  G  E
  901 GGGGGCGCACAGTTGGGCCAAAGGTGGAGCATACATAAAATGTGACTCGTGCCATGCCAGGGCTCCATTGCCGTAACGAATGGCATAATCCAGTCCTTCA
       A  V  V  D  V  R  N  N  H  T  S  L  L  I  D  V  H  P  S  H  Q  Q  F  D  R  L  R  S  L  L  Y  G  T
 1001 GCCACAACATCGACACGATTATTATGTGTTGAGAGTAAAATATCCACATGTGGGGAATGTTGTTGAAAATCCCTCAATCGTGACAATAAATAGCCGGTAG
       A  F  T  G  V  V  G  V  R  V  K  E  R  I  I  G  T  S  F  R  D  L  T  D  A  I  R  D  F  S  D  N  L  I
 1101 CAAAGGTCCCAACAACACCCACCCTGACTTTTTCACGTATTATTCCAGTGGAGAAACGGTCTAGCGTATCCGCGATACGATCAAAAGAATCGTTCAGGAT
       P  L  L  N  E  G  E  T  T  L  V  L  G  R  S  I  R  I  F  L  R  C  N  L  R  Q  E  L  A  K  V  Q  Q
 1201 GGGGAGCAGATTCTCCCCTTCGGTTGTGAGCACTAATCCGCGTGAAATACGAATAAAGAGGCGACAGTTGAGGCGTTGTTCCAGCGCTTTGACCTGCTGA
       S  I  A  A  H  T  V  N  L  E  I  A  A  K  T  F  S  L  Q  R  A  A  A  E  F  A  R  L  S  N  L  P  I
 1301 CTGATTGCTGCATGGGTAACATTTAATTCGATAGCAGCCTTAGTAAAACTTAACTGTCTGGCCGCAGCCTCGAAAGCGCGAAGTGAATTTAACGGAATAT
       Y  S  R  V  M
 1401 AGCTGCGAACCATAGATTGACTTGTTAGATTTTCTATTATCAAGTGCTAAATATAATCGATTGTTATCCATAGTCAATCATTGCAGAATTCTTCACGCAA
                                                M  K  K  S  I  I  N  T  L  I  F  T  S  I  A  T  F
 1501 AAGGAGCCACGCTGCATACCATTTATCAGTCTATGGAAGATTTACTAATGATGAAAAAGTCTATAATCAATACCTTGATCTTTACTTCTATCGCAACTTT
       P  L  Y  T  L  A  Q  T  K  L  T  E  L  Q  V  A  T  I  V  N  N  T  L  T  P  L  L  E  K  Q  G  I  P
 1601 CCCACTTTATACCTTGGCACAAACCAAACTTACGGAGCTGCAGGTTGCGACTATCGTCAATAACACCCTGACGCCGTTGCTCGAAAAACAGGGTATTCCC
       G  M  A  V  A  V  F  Y  D  G  K  P  Q  F  F  N  Y  G  M  A  D  I  K  A  G  R  P  V  T  E  N  T  L  F
 1701 GGTATGGCCGTCGCAGTATTTTATGACGGAAAGCCGCAATTCTTTAACTATGGTATGGCGGATATAAAAGCAGGTCGTCCGGTAACCGAAAATACACTGT
       E  L  G  S  V  S  K  T  F  T  G  V  A  G  E  Y  A  M  Q  T  G  I  M  N  L  N  D  P  V  T  E  Y  A
 1801 TTGAACTCGGTTCAGTGAGTAAGACCTTTACCGGCGTCGCCGGAGAATATGCCATGCAGACCGGTATTATGAACCTTAATGATCCCGTGACGGAATATGC
       P  E  L  T  G  S  Q  W  K  D  V  K  M  L  H  L  A  T  Y  T  A  G  G  L  P  L  Q  L  P  D  S  V  T
 1901 TCCCGAACTAACGGGCAGCCAGTGGAAAGATGTGAAAATGCTGCATCTGGCGACGTACACTGCGGGTGGACTTCCTCTGCAACTCCCCGATTCGGTGACT
       D  Q  K  S  L  W  Q  Y  Y  Q  Q  W  Q  P  Q  W  A  P  G  V  M  R  N  Y  S  N  A  S  I  G  L  F  G  A
 2001 GACCAGAAATCATTGTGGCAATATTATCAACAATGGCAGCCACAGTGGGCACCTGGGGTGATGCGTAATTATTCTAATGCCAGCATTGGGCTATTCGGTG
       L  A  V  K  R  S  Q  L  T  F  E  N  Y  M  K  E  Y  V  F  Q  P  L  K  L  D  H  T  F  I  T  I  P  E
 2101 CGCTGGCGGTGAAAAGAAGCCCAGTTAACATTTGAAAACTATATGAAGGAATATGTGTTCCAACCTTTAAAACTGGATCATACCTTTATCACTATCCCCGA
       S  M  Q  S  N  Y  A  W  G  Y  K  D  G  Q  P  V  R  V  T  L  G  M  L  G  E  E  A  Y  G  V  K  S  T
 2201 ATCCATGCAGTCAAATTATGCGTGGGGATATAAAGATGGTCAACCAGTGCGCGTTACCTTGGGTATGCTGGGTGAGGAAGCCTATGGAGTGAAATCCACC
       S  Q  D  M  V  R  F  M  Q  A  N  M  D  P  E  S  L  P  A  G  N  D  K  L  K  E  A  I  I  A  S  Q  S  R
 2301 TCGCAAGATATGGTGAGATTTATGCAGGCCAATATGGACCCAGAAAGCTTACCTGCCGGTAACGATAAGCTAAAAGAAGCGATTATCGCATCTCAATCAC
       Y  F  Q  A  G  D  M  F  Q  G  L  G  W  E  M  Y  S  W  P  I  N  P  Q  G  V  I  A  D  S  G  N  D  I
 2401 GTTATTTCCAGGCAGGAGATATGTTCCAGGGCTTGGGATGGGAAATGTATAGCTGGCCTATCAATCCACAGGGGGTGATTGCCGATAGTGGCAATGATAT
       A  L  K  P  R  K  V  E  A  L  V  P  A  Q  P  A  V  R  A  S  W  V  H  K  T  G  A  T  N  G  F  G  A
 2501 TGCGTTAAAACCACGTAAGGTTGAGGCATTGGTACCTGCACAACCTGCTGTGCGAGCATCCTGGGTTCATAAGACCGGTGCAACCAATGGCTTTGGCGCT
       Y  I  V  F  I  P  E  E  K  V  G  I  V  M  L  A  N  K  N  Y  P  N  P  V  R  V  Q  A  A  Y  D  I  L  Q
 2601 TATATTGTATTCATCCCAGAGGAGGAAGGTTGGCATAGTGATGTTAGCGAACAAGAATTACCCTAATCCAGTCCGCGTACAAGCAGCATACGATATTCTTC
       A  L  R  ***
 2701 AAGCATTGCGTTGAGATGACAGACGGCTAAACATTTCTGCTGCGCTTCTATGAAATAAGTTATAATATTGTGAAAAAAAATCCACGCAATTGCGATTTTA
 2801 TAGCATTTTCGATTCTTATGAGATTCAGCGAAACCTCATGAGATAACGAATTTTATTTAGACGTTGAACAAGAAGTTCATCACATCGCCATCTTTTACGA
 2901 TGTAATCTTTACCTTCTGAGCGCATCTTCCCAGCTTCTTTTGCACCTTGCTCACCTTTGTAGGTAATAAAGTCGTCAAAAGCGATAGTTTGGGCGCGGAT
 3001 AAAGCCTTTTTCAAAGTCGGTGTGAATTTTACCGGCAGCTTGTGGCGCTGTTGCACCTACAGGGATGGTCCAGGCACGAACTTCTTTAACACCGGCGGTG
 3101 AAGTAAGTTTGCAGATTCAACAATTCATAACCGGCACGGATCC
```

FIG. 2. Nucleotide sequence of the *Eco*RI-*Sau*3AI 3,143-bp DNA fragment from the chromosome of *Y. enterocolitica* containing the *ampC* and *ampR* genes. The deduced amino acid sequence in the one-letter code is shown above the DNA sequence. The stop codons are indicated (***).
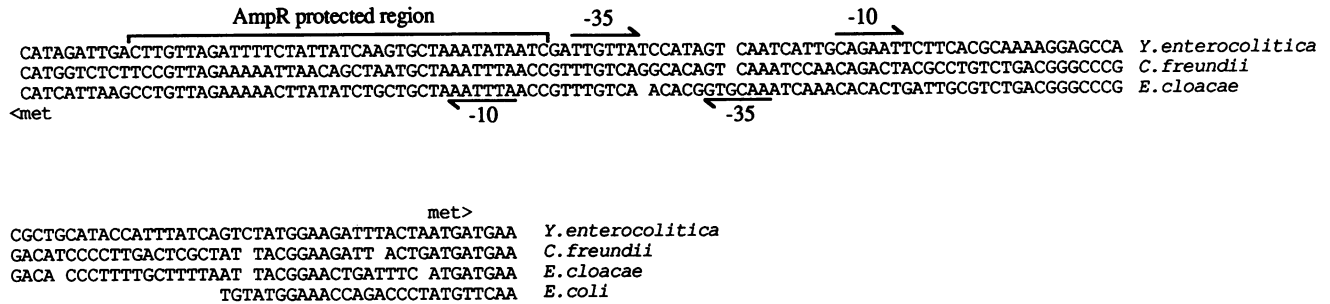
```
                  AmpR protected region                    -35                    -10
          ┌─────────────────────────────────────────┐      ╲                       ╲
CATAGATTGACTTGTTAGATTTTCTATTATCAAGTGCTAAATATAATCGATTGTTATCCATAGT CAATCATTGCAGAATTCTTCACGCAAAAGGAGCCA Y.enterocolitica
CATGGTCTCTTCCGTTAGAAAAATTAACAGCTAATGCTAAATTTAACCGTTTGTCAGGCACAGT CAAATCCAACAGACTACGCCTGTCTGACGGGCCCG C.freundii
CATCATTAAGCCTGTTAGAAAAACTTATATCTGCTGCTAAATTTAACCGTTTGTCA ACACGGTGCAAATCAAACACACTGATTGCGTCTGACGGGCCCG E.cloacae
<met                                          ╲                 ╲
                                              -10               -35
```

```
                                met>
CGCTGCATACCATTTATCAGTCTATGGAAGATTTACTAATGATGAA   Y.enterocolitica
GACATCCCCTTGACTCGCTAT TACGGAAGATT ACTGATGATGAA    C.freundii
GACA CCCTTTTGCTTTTAAT TACGGAACTGATTTC ATGATGAA    E.cloacae
        TGTATGGAAACCAGACCCTATGTTCAA             E.coli
```

FIG. 3. Alignment of the intercistronic ampR-ampC region from Y. enterocolitica, C. freundii, and E. cloacae. The start codons and −10 and −35 regions of the promoters are shown below the DNA sequence for ampR and above it for ampC. The fourth sequence in the final part of the alignment is the 5' end of the E. coli ampC gene.

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 Tn*5seq*1 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 *ampR* genes is higher than that between the accompanying *ampC* 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 *Eco*RI 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*, in which the *frd* genes are not followed by *ampRC*. The characterization of *P. vulgaris ampC* or *Y. enterocolitica frd* loci would be necessary to test this hypothesis.

In conclusion, this report suggests that the inducible *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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TABLE 1. Percent amino acid sequence identity between the AmpC cephalosporinases from different enterobacteria

Source of AmpC	% Identity to AmpC of:		
	E. cloacae P99	C. freundii OS60	E. coli K-12
Y. enterocolitica IP97	56.7	56.2	58.3
E. cloacae P99		73.2	69.3
C. freundii OS60			75.4

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