JOURNAL OF BACTERIOLOGY, May 1982, p. 528–534
0021-9193/82/050528-07$02.00/0

Vol. 150, No. 2

Common Evolutionary Origin of Chromosomal Beta-Lactamase Genes in Enterobacteria

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Received 18 September 1981/Accepted 20 December 1981

A ^{32}P-labeled fragment of DNA, encoding the major part of the chromosomal *ampC* β-lactamase gene of *Escherichia coli* K-12, was used as a hybridization probe for homologous DNA sequences in colonies of *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and different enterobacterial species. The *ampC* probe detected the presence of homologous DNA sequences in clinical isolates of *E. coli*, *Shigella flexneri*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Serratia marcescens*, and *P. aeruginosa*. No hybridization was found with *N. gonorrhoeae* colonies. In Southern blotting experiments the *ampC* probe hybridized to chromosomal DNA fragments of the same size in all enterobacterial species tested. However, the degree of hybridization differed with DNA from different species. DNA from the *Shigella* species strongly hybridized to the *ampC* probe. Furthermore, antibodies raised against purified *E. coli* K-12 *ampC* β-lactamase precipitated β-lactamases from the *Shigella* species, suggesting extensive sequence similarities between the *ampC* genes of these genera. The production of chromosomal β-lactamase in *S. sonnei* increased with increasing growth rate similar to *E. coli* K-12. This growth rate response was abolished in two β-lactamase-hyperproducing *S. sonnei* mutants, which thus seem similar to *E. coli* K-12 attenuator mutants. We propose that both the structure and regulation of the chromosomal β-lactamase genes are very similar in *E. coli* and in *S. sonnei*.

β-Lactamases are widely distributed in both gram-positive and gram-negative bacteria. Until recently their classification was based on a variety of parameters such as substrate profile, sensitivity to various inhibitors, isoelectric point, molecular weight, inducibility, and genetic localization (plasmid or chromosomally encoded) (13, 15). The protein sequences of some β-lactamases are known. Based on amino acid sequencing and enzymatic analysis, two evolutionarily distinct groups of β-lactamases, classes A and B, have been suggested (2).

The class A β-lactamases show a substrate specificity for penicillins and have been referred to as "serine" enzymes. This is because their substrates react with a serine residue in the active site region of the enzyme. The class B β-lactamases are metalloenzymes. Only one representative of this class has been studied, the *Bacillus cereus* β-lactamase II. This enzyme requires zinc ions for activity and is structurally unrelated to class A β-lactamases (2). *Escherichia coli* K-12 codes for a chromosomal β-lactamase. The complete amino acid sequence of this protein has been deduced from the DNA sequence of its structural gene *ampC* (6). No significant sequence homologies were found between the *ampC* β-lactamase and the sequenced class A enzymes. Therefore, the *ampC* β-lactamase has been assigned as a class C β-lactamase (6). Many gram-negative enterobacteria produce chromosomally encoded β-lactamases that show enzymatic similarities with the *ampC* enzyme (15). These β-lactamases, formerly referred to as class I enzymes, all exhibit a substrate specificity for cephalosporins and are considerably larger proteins than the penicillinases (15).

In this paper we show that all tested enterobacterial species carry chromosomal DNA showing extensive sequence homologies with the *ampC* gene of *E. coli* K-12. We therefore suggest that the chromosomally mediated cephalosporinases of enterobacteria and other gram-negative bacteria constitute a common class of β-lactamases, the class C β-lactamases.

The *ampC* gene of *E. coli* K-12 is poorly expressed in wild-type strains. This is partly due to the presence of an attenuator in the *ampC* leader sequence (7). Three different genetic mechanisms have been described to cause elevated production of *ampC*: (i) attenuator mutations—mutations in the *ampC* attenuator can abolish transcription termination completely and thereby cause a four- to fivefold increase in β-lactamase production (7); (ii) promoter mutations—an up-promoter mutation that causes a 15-fold increase in *ampC* β-lactamase production was recently characterized by DNA se-

quencing (7); (iii) gene amplification—From an up-promoter mutant, second-step mutants have been isolated carrying multiple copies of the *ampC* gene (12). These three mechanisms of β-lactamase hyperproduction give distinct phenotypic properties to the mutants. Mutants with multiple *ampC* copies are highly unstable in a Rec^+ background, whereas the two other groups of mutants are genetically stable. The level of *ampC* β-lactamase increases with growth rate in wild-type *E. coli* (8) and in promoter mutants (8) This growth rate-dependent regulation of *ampC* is characteristically lost in attenuator mutants (7). These phenotypic criteria were used to classify *S. sonnei* mutants with elevated production of chromosomal β-lactamase.

MATERIALS AND METHODS

Bacteria and plasmids. The bacterial strains used are listed in Table 1, and plasmid pNU5 was described by Edlund et al. (5). This plasmid contains the *E. coli* K-12 *ampC* gene cloned on a pBR322 derivative.

Media and growth conditions. In most experiments bacteria were grown in minimal medium E, described by Vogel and Bonner (16), supplemented with 1 μg of thiamine per ml. The carbon sources were as follows: 0.4% (vol/vol) glycerol, 0.2% (wt/vol) glucose, or 0.2% (wt/vol) glucose plus 1.5% Casamino Acids. LB medium of Bertani (4) was supplemented with medium E, 1 μg of thiamine per ml, and 0.2% (wt/vol) glucose. All cultures were grown aerobically on a rotary shaker in Erlenmeyer flasks with a ratio of culture volume to flask volume of 1:10. To obtain balanced growth, the cells were pregrown at 37°C in the same medium for at least 10 generations before each experiment.

Preparation of enzyme extracts. At different times, 20-ml portions of cultures were chilled, centrifuged, and sonicated as described (8). The extracts were either assayed immediately for β-lactamase amount and total protein content or frozen. The protein content was determined by the method of Lowry et al. (10), using bovine serum albumin as a standard.

Immunoelectrophoresis of β-lactamase. The immunoelectrophoresis method of Laurell (9) was used. Agarose was dissolved in a barbital-glycine-Tris buffer (pH 8.2) at a concentration of 1% (wt/vol). Ammonium sulfate-precipitated rabbit antiserum raised against purified *E. coli* K-12 β-lactamase was added at 45°C. Gels were prepared on glass plates (8 by 8 cm), and the electrophoresis was run overnight at 40 V per plate. The gels were washed, dried, stained, and destained. The area under the precipitation line was taken as a relative value of the amount of β-lactamase.

Preparation of chromosomal DNA. For isolation of chromosomal DNA, 200 ml of culture was grown to a density of 4×10^8 cells per ml. Cells were harvested, lysed with 1% (wt/vol) sodium dodecyl sarcosinate, phenol extracted, and banded in a CsCl gradient. The chromosomal band was removed and run through an A5M column (Bio-Rad Laboratories, Richmond, Calif.). Fractions containing DNA were pooled, precip-

TABLE 1. Bacterial strains

Strain	Relevant genotype characters	Comments	Source or reference
E. coli K-12			
LA5	*ampA*$^+$, *ampC*$^+$, *pyrB, leu, thr, his, rpsL*	Amp^r 1[a]	(12)
LA51	*ampA1*, *ampC*$^+$, *pyrB, leu, thr, his, rpsL*	Amp^r 15[a]	(12)
TE01	(*ampA1*, *ampC*$^+$)×10[b], *pyrB, leu, thr, his, rpsL*	Amp^r 100[a]	(12)
E. coli			
C11	Wild type	Clinical isolate[c]	(3)
C13	Wild type	Clinical isolate[c]	(3)
C14	Wild type	Clinical isolate[c]	(3)
C15	Wild type	Clinical isolate[c]	(3)
C16	Wild type	Clinical isolate[c]	(3)
C17	Wild type	Clinical isolate[c]	(3)
S. sonnei			
OS10	Wild type	Clinical isolate	This paper
OS106		Mutated to Amp^r 10[a]	This paper
OS107		Mutated to Amp^r 10[a]	This paper
OS112		Mutated to Amp^r 100[a]	This paper
OS113		Mutated to Amp^r 100[a]	This paper
S. flexneri OS20	Wild type	Clinical isolate	This paper
S. marcescens OS30	Wild type	Clinical isolate	This paper
K. pneumoniae OS40	Wild type	Clinical isolate	This paper
S. typhimurium LT2	Wild type		N. Fiil
N. gonorrhoeae Um01		Clinical isolate no. 82409/55	A. Reyn
P. aeruginosa 1822s			K. Nordström

[a] Resistance to ampicillin at 1, 10, 15, and 100 μg/ml.
[b] Denotes amplified repeat of 9.8 kilobases.
[c] Screened for as β-lactamase hyperproducer.

itated with ethanol, washed once with ethanol, suspended in water, and stored at −20°C.

Preparation of plasmid DNA. Plasmid DNA was prepared as follows: 500 ml of culture was grown to 4 × 10^8 cells/ml, plasmid content was amplified by chloramphenicol addition, and the culture was further incubated for at least 6 h. Cells were harvested and suspended in 6.6 ml of Tris (50 mM)–sucrose (25% [wt/vol], pH 8.0) and kept on ice. Solid lysozyme (1 mg) was added together with 1.5 ml of EDTA (0.5 M, pH 8.0), and the cells were incubated on ice for 5 min. After this, 10.6 ml of "lytic mix" (50 mM Tris-hydrochloride–62.5 mM EDTA–1% sodium dodecyl sarcosinate [pH 8.0]) was added carefully. Chromosomal DNA was precipitated by adding 5.5 ml of 5 M NaCl. After 20 min of incubation on ice, the lysates were cleared by centrifugation at 20,000 × *g* for 20 min. The supernatants containing the plasmid DNA were mixed with 0.6 volume of ice-cold isopropanol and incubated at −20°C for 10 min. The DNA was collected by centrifugation at 17,000 × *g* for 10 min and dissolved in 1.3 ml of TE buffer (10 mM Tris-hydrochloride–1 mM EDTA [pH 7.4]). Nondissolvable material was removed by centrifugation in an Eppendorf centrifuge. To 1.1 ml of the final supernatant, 4.1 ml of a CsCl-ethidium bromide solution (80 g of CsCl in 52 ml of TE buffer and 8 ml of ethidium bromide [5 mg/ml]) was underlaid, and the DNA was banded in a Beckman VTi-65 rotor at 55,000 rpm for 180 min. The centrifugation step was repeated once. The plasmid band was collected and run through an A5M column. DNA was stored at −20°C.

Preparation of ^{32}P-labeled *ampC* fragment. Plasmid pNU5 was digested with restriction enzymes *Xho*I and *Kpn*I, and the products were separated on a 5% (wt/vol) polyacrylamide gel. The restriction enzyme fragments were isolated from polyacrylamide gels as previously described (1). The *ampC* DNA fragment was labeled by nick translation as described by the manufacturer of the nick-translation kit (New England Nuclear Corp., Boston, Mass.).

Restriction enzyme digestion of bacterial DNA and agarose gel electrophoresis. The conditions for using restriction endonucleases were those suggested by the manufacturer. The reaction mixture routinely contained 4 to 5 μg of bacterial DNA. The DNA was loaded on 0.7% (wt/vol) horizontal agarose gels, (19 by 14 by 0.4 cm) in 20 mM sodium acetate, 2 mM EDTA, 33 mM Tris-hydrochloride (pH 7.8) and subjected to electrophoresis at 50 V for 16 h.

Blotting and hybridization procedures. Denaturated digests of bacterial DNAs were transferred to nitrocellulose filters after electrophoresis in agarose gels and baked essentially as described by Southern (14). The hybridization procedure was as described by Wahl et al. (17). The hybridization mixture contained 0.05 μg of the 1,060-base-pair (bp) *ampC* fragment per ml. Filters were exposed to Dupont Cronex 4 X-ray film with an intensifying screen. The colony hybridization procedure was, with minor modifications, as described by Moseley et al. (11).

Materials. All chemicals were of the highest grade commercially available. α-Aminobenzylpenicillin (*d*-ampicillin) and benzylpenicillin (penicillin G) were kindly provided by AB Astra, Södertälje, Sweden.

RESULTS

Detection of β-lactamase genes in different bacterial species by colony hybridization. The physical map of *E. coli* K-12 chromosomal DNA in the *ampC* region is shown in Fig. 1. The *ampC* gene of *E. coli* K-12 is 1,193-bp long with an *Xho*I site located 248 bp downstream, and a *Kpn*I site 1,344 bp downstream from the first base in the *ampC* coding sequence (Fig. 1). The *Xho*I/*Kpn*I fragment, 1,060 bp long, therefore carries the major coding part of the *ampC* gene. This fragment was purified from plasmid pNU5 carrying the entire *ampC* gene. The fragment was ^{32}P-labeled by nick translation and used as a probe for homologous DNA sequences using the colony hybridization technique (11). The inoculum was adjusted so the same amount of cells was obtained on the nitrocellulose filter after 24 h of growth. The gram-negative organisms examined in this way were strains or clinical isolates of *N. gonorrhoeae*, *P. aeruginosa*, *E. coli*, *S. flexneri*, *S. sonnei*, *S. typhimurium*, *S. marcescens*, and *K. pneumoniae* (Fig. 2). A strong positive response was found as expected

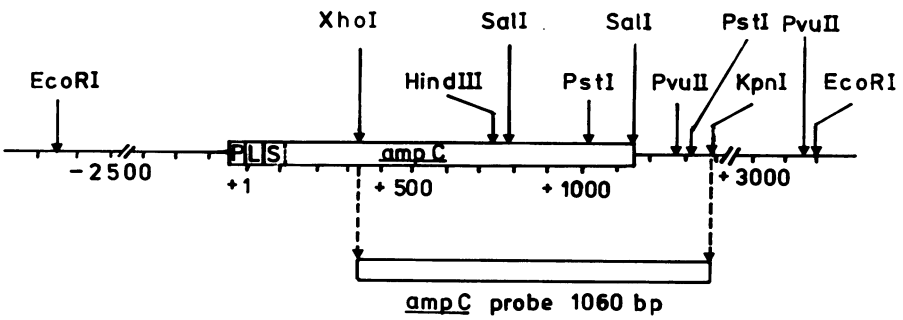

FIG. 1. Physical map of the 6.1-kbp *Eco*RI fragment of the *E. coli* chromosomal DNA surrounding the *ampC* gene. P, L, and S indicate promoter region, leader region, and signal peptide, respectively. The 1,060-bp *ampC* probe, used in the hybridization experiments, is indicated in the figure.

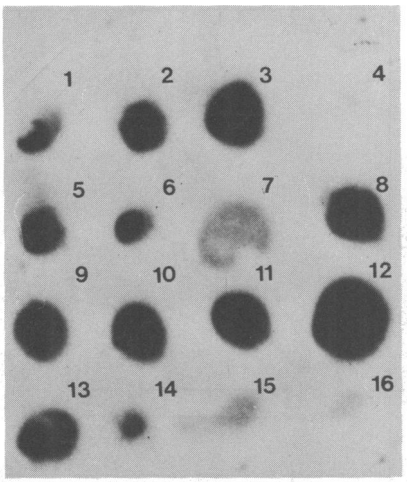

FIG. 2. Colony hybridization using DNA print from different gram-negative bacteria with the 1,060-bp ^{32}P-labeled *ampC* fragment as a probe. The numbers in the figures indicate the following strains: (1) *E. coli* K-12 LA5, (2) *E. coli* K-12 LA51, (3) *E. coli* K-12 TE01; (4) *N. gonorrhoeae* Um01, (5) *S. sonnei* OS10, (6) *S. flexneri* OS20, (7) *S. typhimurium* LT2, (8) *E. coli* C11, (9) *E. coli* C13, (10) *E. coli* C14, (11) *E. coli* C15, (12) *E. coli* C16, (13) *E. coli* C17, (14) *K. pneumoniae* OS40, (15) *S. marcescens* OS30, and (16) *P. aeruginosa* 1822s.

with DNA prints from *E. coli* K-12 strains and clinical isolates of *E. coli*. However, a positive response was also found for *S. flexneri*, *S. sonnei*, and *K. pneumoniae*. A much weaker response was found with DNA prints from *S. typhimurium*, *S. marcescens*, and *P. aeruginosa* colonies. No response was found for *N. gonorrhoeae*. These data suggested that a DNA sequence homologous to the *ampC* gene was present in all enterobacterial species tested as well as in *P. aeruginosa*.

Southern blotting and hybridization of chromosomal digests from different bacterial species with an *ampC* probe. The ^{32}P-labeled *ampC* probe was hybridized to *Xho*I/*Kpn*I-digested chromosomal DNA of *E. coli*, *S. sonnei*, and *S. flexneri*. The probe hybridized to a DNA fragment of the same size in the tested strains (data not shown). To further establish that the hybridized chromosomal fragments really correspond to the *ampC* gene, the chromosomal DNA from the same strains was cleaved with *Xho*I and *Pvu*II. In *E. coli* K-12 a *Pvu*II site is situated 90 bp closer to the *Xho*I site than is the *Kpn*I site. As expected, the *Xho*I/*Kpn*I *ampC* probe hybridized to a *Xho*I/*Pvu*II fragment in *E. coli* K-12, *E. coli* (clinical isolate), *S. sonnei*, and *S. flexneri* that was slightly smaller than the probe (Fig. 3). The same *ampC* probe was used in hybridization with *Xho*I/*Pvu*II-cleaved chromosomal DNA of *S. marcescens*, *K. pneumoniae*, *S. typhimurium*, and *N. gonorrhoeae* (Fig. 3). Hybridization was obtained with a DNA fragment approximately 90 bp shorter than the *ampC* probe in the three enterobacterial strains too. The different intensities of the bands in the autoradiograms could indicate sequence differences between *ampC* of *E. coli* K-12 and the corresponding DNA of the tested species, because the same amount of DNA was used and strains were only compared when run on the same gel. The *ampC* probe did not hybridize to chromosomal DNA of *N. gonorrhoeae*.

Expression of β-lactamase in *S. sonnei*. The β-

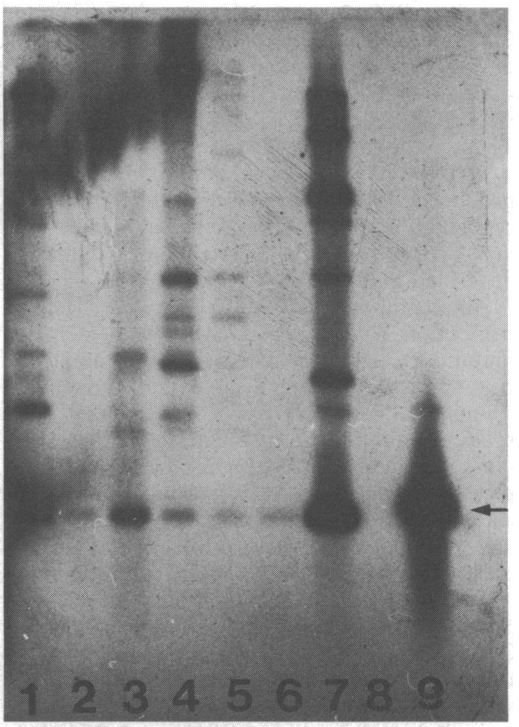

FIG. 3. Southern blotting analysis of *Xho*I/*Pvu*II chromosomal digests from different gram-negative bacteria, using the ^{32}P-labeled *ampC* probe. The numbers in the figures indicate the following strains: (1) *E. coli* K-12 LA51, (2) *S. typhimurium* LT2, (3) *S. sonnei* OS10, (4) *S. flexneri* OS20, (5) *K. pneumoniae* OS40, (6) *S. marcescens* OS30, (7) *E. coli* C13, and (8) *N. gonorrhoeae* Um01. Track 9 contains the ^{32}P-labeled 1,060-bp *Xho*I/*Kpn*I *ampC* probe. The arrow indicates the position for the *Xho*I/*Kpn*I fragment. A number of fragments resulting from partial digestion are also hybridizing with the probe, due to difficulties in obtaining optimal conditions for the restriction enzymes when cutting chromosomal DNA.

lactam resistance was below 2 μg of ampicillin per ml in the *Shigella*, *Salmonella*, *Klebsiella*, and *Serratia* species, and the β-lactamase production in these strains was as low as in the *E. coli* K-12 wild-type situation (data not shown). In this context we would like to emphasize the linear relationship that exists between ampicillin resistance and amount of β-lactamase produced (12). In *E. coli* K-12, this low β-lactamase production is due, in part, to the presence of an attenuator structure in the *ampC* leader region. Since a functional attenuator is required for growth rate-dependent regulation (7), the regulatory response for the chromosomally encoded β-lactamase of *S. sonnei* strain OS10 was analyzed. Cultures of *S. sonnei* were harvested during steady-state growth in various media, and the relative amount of chromosomal β-lactamase was determined. This determination could be done because antibodies raised against purified *E. coli* K-12 *ampC* β-lactamase precipitated β-lactamase from *S. sonnei*. The relative amount of β-lactamase increased as a function of growth rate in a similar way to the *ampC* β-lactamase of *E. coli* K-12 wild-type strain LA5 (Fig. 4B). Thus, within a range of $k = 0.6$ to 2.0, there was a sixfold increase in amount of β-lactamase produced. Therefore, it seems likely that the regulation of β-lactamase expression in *S. sonnei* also is mediated by an attenuator structure as described in *E. coli* K-12 (7).

Ampicillin-resistant mutants were isolated from the *S. sonnei* strain OS10. The incidence of mutants resistant to at least 5 μg of ampicillin per ml was 10^{-9} per viable cell. Four such *Shigella* mutants were further analyzed. The relative amounts of β-lactamase in these mutants were considerably higher than found in the parent strain (Fig. 4A). It could thereby be established that the increase in β-lactam resistance was due to elevated production of β-lactamase. The effect of different growth rates on β-lactamase production was studied in the four *Shigella* mutants. In the *S. sonnei* mutants OS112 and OS113 the relative amounts of β-lactamase increased with growth rates as in the wild-type strain OS10, whereas this response was abolished in strains OS106 and OS107. In these two *Shigella* mutants the level of β-lactamase was invariant within a k range of 0.6 to 1.5 and therefore showed the same regulatory behavior as *E. coli* K-12 attenuator mutants (7).

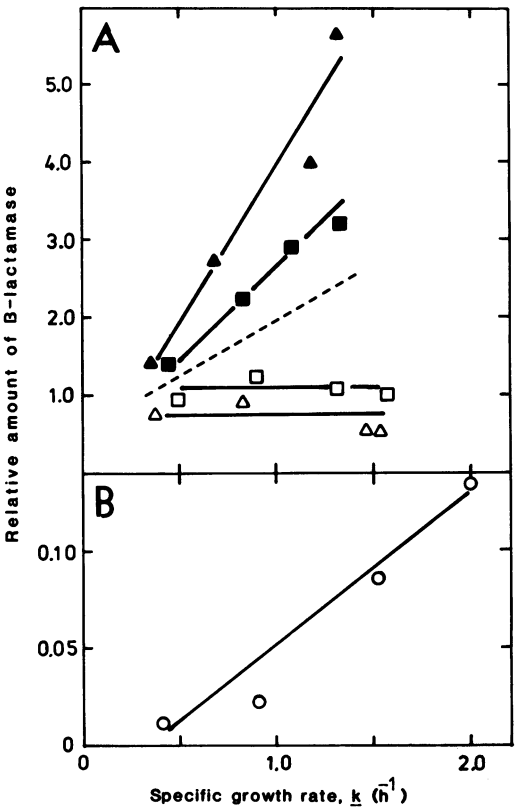

FIG. 4. Levels of β-lactamase at different growth rates in (A) *S. sonnei* OS106 (△), OS107 (□), OS112 (▲), and OS113 (■), and (B) *S. sonnei* OS10 (○). The relative amount of β-lactamase is plotted as a function of the first order constant for growth (k), as calculated from the expression $k = \ln 2/\text{mass doubling time}$ in hours. The media used were as follows: medium E + glycerol, medium E + glucose, medium E + glucose + Casamino Acids, and LB medium. The dotted line in (A) shows the level of β-lactamase at different growth rates in *E. coli* K-12 up-promoter mutant LA51 as a comparison.

DISCUSSION

In this article we demonstrate that an *ampC* probe from *E. coli* K-12 hybridizes to DNA fragments of the same size in strains of *E. coli*, *S. flexneri*, *S. typhimurium*, *K. pneumoniae*, and *S. marcescens*. It seems likely that they all contain a DNA segment that shows sequence homology to that of the chromosomal *ampC* β-lactamase gene of *E. coli* K-12. The hybridization was consistently more pronounced between the *E. coli ampC* probe and the *Shigella* strains than with representatives from the other enterobacterial genera. In addition, antibodies raised against purified *E. coli ampC* β-lactamase precipitated the *ampC* β-lactamase of *S. sonnei*. *S. flexneri* responded in the same way (data not shown). Thus, the chromosomal *ampC* genes and their gene products are probably very similar in *E coli* and in *Shigella*. In the Southern blotting experiments, DNA from *S. marcescens*, *K. pneumoniae*, and *S. typhimurium* hybridized

weakly to the *ampC* fragment. Furthermore, no immunological reaction occurred between *E. coli ampC* β-lactamase antibodies and enzyme extracts from these species (data not shown). These facts suggest that their *ampC* β-lactamases have undergone considerable structural changes through evolution.

The *ampC* gene of *E. coli* K-12 has been DNA sequenced. The deduced amino acid sequence shows no homologies with previously sequenced β-lactamases (6). Therefore, it could be concluded that the *ampC* enzyme is distinct from the class A β-lactamases. These enzymes constitute a group of internally related "serine" β-lactamases with a preference for penicillins as substrates. The only class B metallo-requiring β-lactamase is the *B. cereus* β-lactamase II (2). Preliminary partial sequence analysis suggests it is structurally unrelated to the class A enzymes (2). The *ampC* β-lactamase of *E. coli* K-12 shows no special requirement for metal ions. The active site of *E. coli ampC* β-lactamase has recently been localized to a region around serine 80 of the precursor form of the enzyme (V. Knott-Hunziker, S. Waley, B. Jaurin, and T. Grundström, Biochem. J., in press). Interestingly, this region shows an 11 out of 14 homology with the active site peptide of the chromosomal cephalosporinase of *P. aeruginosa* (6). Each of the three differences could be explained by a single base substitution. Clearly, this shows that the *E. coli ampC* β-lactamase is a "serine" enzyme evolutionary related to the cephalosporinase of *P. aeruginosa*.

The *E. coli* K-12 β-lactamase is a constitutive enzyme in the sense that β-lactam antibiotics or other external effectors do not affect expression from *ampC*. However, *ampC* is not an unregulated gene, since the expression from *ampC* increases with growth rate. This so-called metabolic regulation of *ampC* requires the presence of a functional attenuator in the noncoding *ampC* leader region. The current model for metabolic regulation of *ampC* implies that the degree of transcription termination at the *ampC* attenuator decreases with increasing growth rate due to an increased availability of an antitermination factor (7).

In this paper we show that the *ampC* β-lactamase of *S. sonnei* also is metabolically controlled. The ampicillin-resistant β-lactamase-hyperproducing *S. sonnei* mutants fall into two classes with respect to this regulation. Two of the mutants, OS112 and OS113 showed an intact metabolic control on *ampC* expression. These two mutants are similar in behavior to an up-promoter mutant of *E. coli* K-12. The two *S. sonnei* mutants with a low increase in β-lactamase production, OS106 and OS107, showed no growth rate-dependent control of β-lactamase production. These mutants are thus similar to *E. coli* K-12 mutants which are altered in the terminator stem of the *ampC* attenuator. The analogies between *E. coli* and *S. sonnei* are so striking that we postulate that the *S. sonnei ampC* β-lactamase is also controlled by attenuation of transcription. Cloning, DNA sequencing, and in vitro transcription from wild-type and regulatory *S. sonnei* mutants will test our hypothesis. Since the chromosomal β-lactamase is poorly expressed in most wild-type enterobacterial species, the possibility exists that not only the chromosomal structural genes but also their regulatory regions are very similar.

In conclusion, we have demonstrated that the chromosomal β-lactamase genes of several enterobacterial genera show extensive sequence homologies with the *ampC* gene of *E. coli* K-12. Compared to the *E. coli* K-12 *ampC* gene, the *S. sonnei ampC* gene showed a similar regulation, and its expression could also be mutated to higher levels. These data together with the DNA sequencing data of Jaurin and Grundström (6) and the active site study of Knott-Hunziker et al. (in press) lead us to suggest that the chromosomal cephalosporinase genes of enterobacteria and *P. aeruginosa* code for a structurally related class of "serine" β-lactamases (class C β-lactamases). These β-lactamases probably have an evolutionary origin which is different from the previously sequenced "serine"-penicillinases (2). The constitutive cephalosporinase genes probably have a regulatory behavior similar to that of *E. coli* and *S. sonnei*. However, other types of regulatory patterns must also exist since it is known that the cephalosporinases of *S. marcescens*, *P. aeruginosa*, and of some other gram-negative genera are induced by β-lactams (15).

ACKNOWLEDGMENTS

We thank Monica Persson and Stina Olofsson for valuable technical assistance.

This work was supported by grants from the Swedish Natural Science Research Council (Dnr 3373), and the Swedish Medical Research Council (Dnr 5428).

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