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Salmonella enteritidis: AmpC Plasmid-Mediated Inducible β-Lactamase (DHA-1) with an *ampR* Gene from *Morganella morganii*

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DHA-1, a plasmid-mediated cephalosporinase from a single clinical *Salmonella enteritidis* isolate, conferred resistance to oxyimino-cephalosporins (cefotaxime and ceftazidime) and cephamycins (cefoxitin and moxalactam), and this resistance was transferable to *Escherichia coli* HB101. An antagonism was observed between cefoxitin and aztreonam by the diffusion method. Transformation of the transconjugant *E. coli* strain with plasmid pNH5 carrying the *ampD* gene (whose product decreases the level of expression of *ampC*) resulted in an eightfold decrease in the MIC of cefoxitin. A clone with the same AmpC susceptibility pattern with antagonism was obtained, clone *E. coli* JM101(pSAL2-ind), and its nucleotide sequence was determined. It contained an open reading frame with 98.7% DNA sequence identity with the *ampC* gene of *Morganella morganii*. DNA sequence analysis also identified a gene upstream of *ampC* whose sequence was 97% identical to the partial sequence of the *ampR* gene (435 bp) from *M. morganii*. The gene encoded a protein with an amino-terminal DNA-binding domain typical of transcriptional activators of the LysR family. Moreover, the intercistronic region between the *ampC* and *ampR* genes was 98% identical to the corresponding region from *M. morganii* DNA. AmpR was shown to be functional by enzyme induction and a gel mobility-shift assay. An *ampG* gene was also detected in a Southern blot of DNA from the *S. enteritidis* isolate. These findings suggest that this inducible plasmid-mediated AmpC type β-lactamase, DHA-1, probably originated from *M. morganii*.

The principal mechanism of resistance to broad-spectrum cephalosporins such as cefotaxime and ceftazidime in *Salmonella* strains involved the production of extended-spectrum class A β-lactamases such as SHV-2, TEM-3, TEM-25, and TEM-27 (derived from TEM-or SHV-type enzymes) (12, 20, 38, 41) and CTX-M-2 and PER-2 (3, 4). This mechanism has also been reported in other members of the family *Enterobacteriaceae*, especially in *Klebsiella pneumoniae*, a species which, like *Salmonella* species, lacks the chromosomal *ampC* gene. These enzymes are not active against cephamycins such as cefoxitin, and susceptibility to oxyimino-cephalosporins can be restored by clavulanic acid.

An additional form of β-lactam resistance has recently been observed. Plasmid-mediated AmpC type β-lactamases have been reported in clinical strains of *K. pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes*, and *Salmonella* spp. in nine countries. These enzymes include MIR-1, CMY-1, CMY-2, MOX-1, FOX-1, FOX-2, FOX-3, LAT-1, LAT-2, BIL-1, and ACT-1 (5–7, 11, 18, 19, 24, 29, 36, 39, 40, 45). These β-lactamases have biochemical properties typical of AmpC β-lactamases and result in a derepressed phenotype of resistance to β-lactams. Combined with impermeability, as for *K. pneumoniae* strains producing ACT-1 (11), they can confer resistance to imipenem. Some have DNA and amino acid sequences very similar to those of chromosome-mediated AmpC β-lactamases of *Citrobacter freundii* (CMY-2, LAT-1, LAT-2, BIL-1) (5, 15, 18, 29, 46) or *Enterobacter cloacae* (MIR-1, ACT-1) (11, 39), whereas the phylogenies of the other enzymes are unclear (6, 7, 19, 25, 36).

A plasmid-mediated AmpC β-lactamase, DHA-1, which confers resistance to cephamycins and oxyimino-cephalosporins and which is characterized by a probable inducible production, was identified in 1992 in Saudi Arabia in a clinical isolate of *Salmonella enteritidis* (17). In the present study, we analyzed the nucleotide sequences of the contiguous *ampC* and *ampR* genes and the phylogeny and regulation of the corresponding AmpC enzyme.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used in this study are listed in Table 1. *S. enteritidis* KF92 encoding DHA-1 (17) was isolated in November 1992 from the stool samples from a patient with lung carcinoma hospitalized at Dhahran Hospital, Dhahran, Saudi Arabia.

E. coli K-12 strain HB101 (10) was used for conjugation experiments, and *E. coli* JM101 was used for cloning and transformation. The clinical strains of *S. enteritidis*, *K. pneumoniae*, and *Klebsiella oxytoca* used in DNA-DNA hybridization experiments were isolated at Saint-Louis Hospital (Paris, France).

Plasmid vector pACYC184 (13) was used for cloning and was prepared by the alkaline lysis method (9). Drigalski agar (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) containing amoxicillin (20 μg/ml) and chloramphenicol (30 μg/ml) was used for cloning.

Antibiotic susceptibility testing. MICs were determined by the agar dilution method with Mueller-Hinton agar (Sanofi Diagnostics Pasteur). Inocula of 10^4 to 10^5 CFU per spot were delivered with a multipoint inoculator. The resistance phenotype was determined by the disk diffusion method with Mueller-Hinton agar (14).

Synergy. A double-disk synergy test was performed with various cephalosporins: cefotetan, ceftazidime, cefoxitin, and cefotaxime. Disks of antibiotics were placed on agar plates inoculated with the transconjugant *E. coli* HB101 or with the clone to be tested. A disk of inhibitor was placed on the agar surface 30 mm (center to center) from the central disk. Two inhibitors were tested: clavulanate, a specific inhibitor of penicillinase (10 μg per disk), and RO48-1220, which is specific for cephalosporinase (20 μg per disk) (42) and which was obtained from

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Characteristics[a]	Source or reference
Strains		
S. enteritidis KF92	Clinical strain producing DHA-1	17
E. coli HB101	F$^-$ *l hsdS20 recA13 ara-14 proA2 leu lacY1 galK2 rpsL20 xyl-55 mtl-1 supE44*	10
E. coli JM101	*supE thi* Δ(*lac-proAB*) (F′ *tra D36 proAB lacI*^{q} ZΔM15)	Messing, 1979
E. coli J53-2 (J62.6)	Transconjugant producing BIL-1; Tc^r Nal^r	40
E. coli C600 (pMG231)	Transformant producing MIR-1; Cm^r	39
Plasmids		
pACYC184	Cloning vector; Cm^r Tc^r	13
pNH5	*Hpa*I fragment containing *ampD* gene from *E. cloacae* transferred into pBGS18 Km^r	M. H. Nicolas
pSAL1	Conjugative plasmid of 95 kb encoding DHA-1	17
Clone pSAL2-ind	Recombinant plasmid; 4.9-kb *Sau*3A-*Sau*3A fragment encoding DHA-1	This study

[a] Cm^r, chloramphenicol resistant; Nal^r, nalidixic acid resistant; Tc^r, tetracycline resistant; Km^r, kanamycin resistant.

Hoffmann-La Roche SA (Basel, Switzerland). The plates were examined after overnight incubation at 37°C.

β-Lactamase preparation and induction. Crude cell extracts were prepared by sonication of cells grown overnight in Trypticase soy broth (Sanofi Diagnostics Pasteur). Cells of *E. coli* JM101 containing pSAL2-ind were induced by dilution in fresh broth containing imipenem at 0.25, 0.5, 1, or 2 μg/ml and were incubated for 3 h before harvesting. One unit of β-lactamase activity is the enzyme activity that hydrolyzes 1 μM cephaloridine per min and is expressed per milligram of protein.

β-Lactamase assays. Microacidimetric assays of β-lactamase activity in cell-free extracts were performed as described previously with 100 μM cephalothin as the substrate (27). Protein concentrations were determined by using the Bio-Rad protein assay (Bio-Rad Laboratories, Ivry sur Seine, France) with bovine serum albumin as the standard.

IEF. Analytical isoelectric focusing (IEF) of crude extracts was performed in a polyacrylamide gel (pH 3.5 to 9.5) (37) that was subjected to electrophoresis for 18 h at 15°C. β-Lactamase activity was detected with the chromogenic compound nitrocefin. TEM-1 (pIP1100; pI 5.4), TEM-3 (pCFF04; pI 6.3), OXA-1 (RGN238; pI 7.4), and SHV-4 (pUD21; pI 7.8) were used as pI markers.

Transformation with pNH5. The *E. coli* transconjugants producing DHA-1, MIR-1, and BIL-1 were transformed with plasmid pNH5 carrying the *ampD* gene by the standard $CaCl_2$ technique (35). The plasmid carried a kanamycin resistance gene, and kanamycin was used at a concentration of 50 μg/ml to select transformants on Drigalski agar.

Enzymes. Restriction endonucleases and T4 DNA ligase were used according to the recommendations of the manufacturer (Boehringer Mannheim Biochemicals, France S.A. Meylan, France).

Cloning of *ampC* and *ampR* genes. Plasmid pSAL1 DNA was isolated from the *E. coli* HB101 transconjugant by the alkaline lysis method of Birnboim and Doly (9). It was partially digested with *Sau*3A and ligated into the *Bam*HI site of pACYC184. Recombinant plasmid was introduced into *E. coli* JM101 by the standard $CaCl_2$ technique. Transformants were selected on the basis of resistance to amoxicillin (20 μg/ml) and chloramphenicol (30 μg/ml) and were further characterized by analysis of their antibiotic susceptibility patterns and determination of their pIs. The size of the insert in the recombinant plasmid was estimated by restriction enzyme digestion and electrophoresis in 1 to 3% agarose gels.

Sequencing. Double-stranded DNA was sequenced by the procedure of Sanger et al. (43) by using fluorescent dye-labeled dideoxynucleotides, thermal cycling with *Taq* polymerase, and an ABI 373A DNA sequencer (Applied Biosystems, Foster City, Calif.).

DNA and amino acid sequence analyses. Sequence analyses and comparison with other known sequences were performed with the BLAST (1) and FAST programs at the NCBI. The Clustal W program was used to produce multiple alignments of the predicted amino acid sequence of DHA-1 with the sequences of other proteins in the protein data bank.

Preparation of protein extracts. Protein extracts were prepared as described by Keegan et al. (28). *E. coli* JM101 cells containing the putative recombinant plasmid pSAL2-ind which confers inducible expression of the β-lactamase gene were grown in 250 ml of Trypticase soy broth. The cells were collected by centrifugation, washed once in 150 mM NaCl, and suspended in 2 ml of buffer A (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.5], 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride). This mixture was subjected to 10 cycles of sonication on ice, and cellular debris was removed by centrifugation at 48,000 × *g* for 30 min. The protein concentration was determined as described above.

Gel mobility-shift assays. Gel mobility-shift assays were performed as described previously (16). A 173-bp DNA fragment containing the 110-bp *ampR-ampC* intercistronic region was prepared by PCR with the oligonucleotides 5′-GGAAATCAGTGTTGCAGA and 3′-GGGTTAAGGGGGAGATAA as primers and pSAL1 as the template. Binding reactions were carried out in 10 μl of buffer B (25 mM HEPES [pH 7.5], 0.1 mM EDTA, 5 mM dithiothreitol, 10% glycerol, 50 mM KCl) containing 2 pM 173-bp DNA fragment, 2 μg of poly(dI-dC) (Pharmacia Biotech, Saclay, France), and various amounts of protein extracts (2.6 to 13 μg). The mixture was incubated at 25°C for 15 min and was subjected to electrophoresis in a 3% low-melting-point agarose gel in Tris-borate-EDTA buffer. After migration, the DNA was transferred from the agarose gel to a Hybond N$^+$ membrane (Amersham France SA, Les Ulis, France) by the Southern alkaline transfer method (35). The membrane was probed with the fluorescein-labeled 173-bp DNA fragment. The DNA labeling and detection kit was used as recommended by the manufacturer (Amersham Life Science, Les Ulis, France).

Detection of *ampG* in total *S. enteritidis* KF92 DNA. An *ampG* probe (852 bp) was prepared by PCR with *E. coli* HB101 DNA as the template and the oligonucleotide primers 5′-GCTCGCCACGCAAATCCTG-3′ and 5′-GACATAAA CTCGCCCTACA-3′ (positions 252 to 270 and 1104 to 1086, respectively [the numbering is as for the nucleotide sequence of *E. coli* JRG582 *ampG*]) (34). Before using this probe, the PCR product (*ampG*) was analyzed by PCR-restriction fragment length polymorphism analysis with *Sau*3A, *Hpa*II, and *Hha*I. The restriction endonuclease patterns obtained were consistent with the patterns generated with DNA software (DNA Strider, version 1.2). Total KF92 DNA was digested with *Eco*RI, subjected to electrophoresis, and transferred to a Hybond N$^+$ membrane as described above. The membrane was then probed with the fluorescein-labeled DNA probe. The DNA labeling and detection kit was used according to the recommendations of the manufacturer (Amersham Life Science).

Nucleotide sequence accession number. The EMBL accession number for the nucleotide sequence reported here is Y16410.

RESULTS

Inducible cephalosporinase activity. An antagonism between cefoxitin or imipenem and other β-lactams such as cefotaxime and aztreonam was originally observed by the diffusion method for both strains of *S. enteritidis* and the transconjugant *E. coli* HB101. Therefore, we hypothesized that the enzyme would be inducible, and to test this, we introduced by transformation pNH5 which carries the *ampD* gene into this transconjugant (31). The *ampD* product decreases the level of *ampC* expression. The MIC of cefoxitin for the transformant as eight times lower, and those of cefotaxime, moxalactam, and ticarcillin were half of those for the controls (Table 2). The transconjugants *E. coli* J53-2 and C600 producing the AmpC plasmid-mediated β-lactamases BIL-1 (J53-2) and MIR-1 (C600) were also transformed, but no decrease in the MICs of cefoxitin, cefotaxime, moxalactam, or ticarcillin was observed (Table 2).

We investigated whether enzyme synthesis could be induced. Cephalosporinase activity was measured in extracts prepared from transconjugant *E. coli* HB101 in the absence of imipenem and after induction with various concentrations of imipenem. The activity was dependent on the concentration of imipenem. It was 8 times higher in the presence of 0.25 μg of imipenem per ml (one-quarter the MIC) than in the absence of imipenem and reached the maximum (11 times higher) at 0.5 μg of imipenem per ml (one-half the MIC), suggesting the involvement of an *ampR*-like gene.

Cloning of the *ampC* and *ampR* genes and their expression in an *E. coli* recipient. We selected several *E. coli* transfor-

TABLE 2. MICs of β-lactams for *S. enteritidis* KF92, its transconjugant, its transformant, *E. coli* JM101, *E. coli* HB101, *E. coli* J53-2 producing BIL-1, *E. coli* C600 producing MIR-1, and the transformants with pNH5[a]

Strain	Plasmid	Bla	MIC (mg/liter)										
			AMX	AMC	TIC	MEC	CF	FOX	CTX	MOX	FEP	ATM	IMP
S. enteritidis	pSAL1	DHA-1	≥512	≥512	>256	4	≥1,024	512	≥64	32	2	4	1
E. coli HB101			8	8	4	0.5	8	8	0.12	0.25	0.12	0.06	0.25
E. coli HB101	pSAL1	DHA-1	≥512	≥512	128	0.5	1,024	128	16	1	0.12	1	1
E. coli HB101	pSAL1 + pNH5	DHA-1	≥512	512	64	0.5	1,024	32	8	0.5	0.12	0.12	1
E. coli JM101			8	4	4	0.5	16	4	0.25	0.12	0.12	0.12	0.5
E. coli JM101	pSAL2-ind	DHA-1	≥512	256	≥256	1	≥1,024	256	64	4	0.5	2	1
E. coli J53-2	J62.6	BIL-1	≥512	≥512	128	4	1,024	128	32	2	0.5	ND	1
E. coli J53-2	J62.6 + pNH5	BIL-1	≥512	≥512	128	4	1,024	128	32	2	0.5	ND	1
E. coli C600	pMG231	MIR-1	≥512	≥512	128	4	≥1,024	512	32	8	0.25	ND	1
E. coli C600	pMG231 + pNH5	MIR-1	≥512	≥512	128	4	≥1,024	512	32	8	0.25	ND	1

[a] Abbreviations: Bla, β-lactamase; AMX, amoxicillin; AMC, amoxicillin plus clavulanic acid (2 mg/ml); TIC, ticarcillin; MEC, mecillinam; CF, cephalothin; FOX, cefoxitin; CTX, cefotaxime; MOX, moxalactam; FEP, cefepime; ATM, aztreonam; IMP, imipenem; ND, not done.

mants that had β-lactam susceptibility patterns (Table 2) similar to that of the *E. coli* HB101 transconjugant that produces cephalosporinase: resistance to amoxicillin, ticarcillin, cephalothin, cefoxitin, cefotaxime, and the amoxicillin-clavulanate combination. The patterns of susceptibility to other β-lactams such as mecillinam, cefepime, and imipenem were the same as that for the parent strain.

Synergy between RO48-1220 and the various cephalosporins, particularly cefotetan, was observed against these transformants, suggesting that an AmpC-type β-lactamase was produced (Fig. 1). IEF analysis of cell extracts from the transformants indicated that they mediated a β-lactamase with a pI of 7.8 which cofocused with the cephalosporinase of the wild-type strain *S. enteritidis* KF92 (data not shown).

One transformant was found to produce an inducible enzyme (antagonism between cefoxitin and cefotaxime). It harbored a recombinant plasmid (pSAL2-ind) with an insert of about 4.9 kb.

Nucleotide sequence. The 4.9-kb DNA insert of pSAL2-ind was sequenced. Two long open reading frames (ORFs) were found (Fig. 2). The first was 1.137 kb long and mediated a putative protein of 378 amino acids. This ORF had an ATG start codon at position 987 and a stop codon at position 2124 (Fig. 2). Database searches with this ORF identified similarities with several chromosome- and plasmid-mediated class C β-lactamases (Fig. 3). The product of this ORF was most similar to the chromosome-mediated AmpC β-lactamase of *M. morganii* (98.7% sequence identity) (2). The product of the ORF had 53 to 58% sequence identity with AmpC β-lactamases of *C. freundii*, *E. cloacae*, *Yersinia enterocolitica*, and *E. coli*, and five plasmid-mediated enzymes (CMY-2, ACT-1, LAT-1, BIL-1, and LAT-2). There was more divergence from the AmpC β-lactamases of *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Aeromonas sobria* and from FOX-1 and MOX-1 (37 to 45% sequence identity). There were several conserved serine β-lactamase motifs: the SXSK motif from the active site of the serine β-lactamase, the typical class C motif YXN, and the KTG domain.

The second ORF containing 873 nucleotides (nt) was transcribed in the opposite orientation and was located 5′ from the *ampC* structural gene (Fig. 2). It began with an ATG codon at nt 876 and ended with a stop codon at nt 3. By analogy with the *ampC-ampR* genes in members of the family *Enterobacteriaceae* which produce inducible AmpC β-lactamases, this ORF may correspond to the regulatory gene *ampR*. The deduced sequence of the product of this ORF was very similar to the sequence of the transcriptional regulators of the LysR family, particularly to those of the AmpR proteins of the members of the family *Enterobacteriaceae*. The partial DNA sequence (435 bp) (2) of the corresponding gene from *M. morganii* was 97% identical to the sequence of this ORF. The deduced protein sequence of this ORF was only 60 to 62% identical to the sequences of the known AmpR proteins from *C. freundii*, *E. cloacae*, and *Y. enterocolitica*. The predicted sequence of 280 amino acids had the characteristics of a typical LysR transcriptional regulator protein (21), with a helix-turn-helix DNA-binding motif in the N-terminal region (data not shown), as previously observed in the *ampR* gene from *M. morganii* (2).

The 111-bp region between the *ampR* and *ampC* start codons contained putative overlapping promoters (Fig. 2). This region was 98% identical to the corresponding region of *M. morganii* DNA (2) and was smaller than the corresponding regions of *C. freundii*, *E. cloacae*, and *Y. enterocolitica* DNA (111 versus 140 bp) (23, 33, 44).

Binding of AmpR to DNA in the *ampR-ampC* intercistronic region. Because we have detected antagonism between antibiotics and positive induction of the gene, we tested whether the *ampR* gene was functional. To test the binding of AmpR to the 111-bp intercistronic region, we performed gel mobility-shift assays. The 173-bp fragment encompassing the intercistronic region was retarded when it was mixed with the cell extracts containing the AmpR protein prepared from pSAL2-ind (Fig. 4).

Detection of *ampG* in *S. enteritidis* KF92. The *ampG* gene is required for activation of the *ampR* gene in members of the family *Enterobacteriaceae* with an inducible cephalosporinase. We detected this gene in *S. enteritidis* chromosomal DNA by hybridization to an *ampG* probe from *E. coli* HB101. We found that *S. enteritidis* KF92 had in its genome an *ampG* gene which

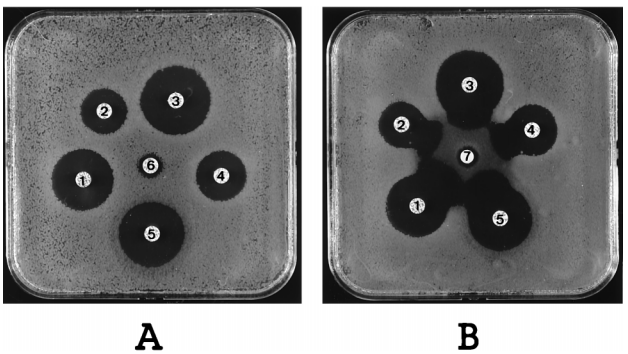

FIG. 1. Double-disk synergy test with the transformant *E. coli* JM101 (pSAL2-ind). (A) Synergy test with 10 μg of clavulanate (disk 6); (B) synergy test with 20 μg of RO48-1220 (disk 7). Disks: 1, cefotaxime; 2, ceftazidime; 3, moxalactam; 4, cefotetan; 5, aztreonam.

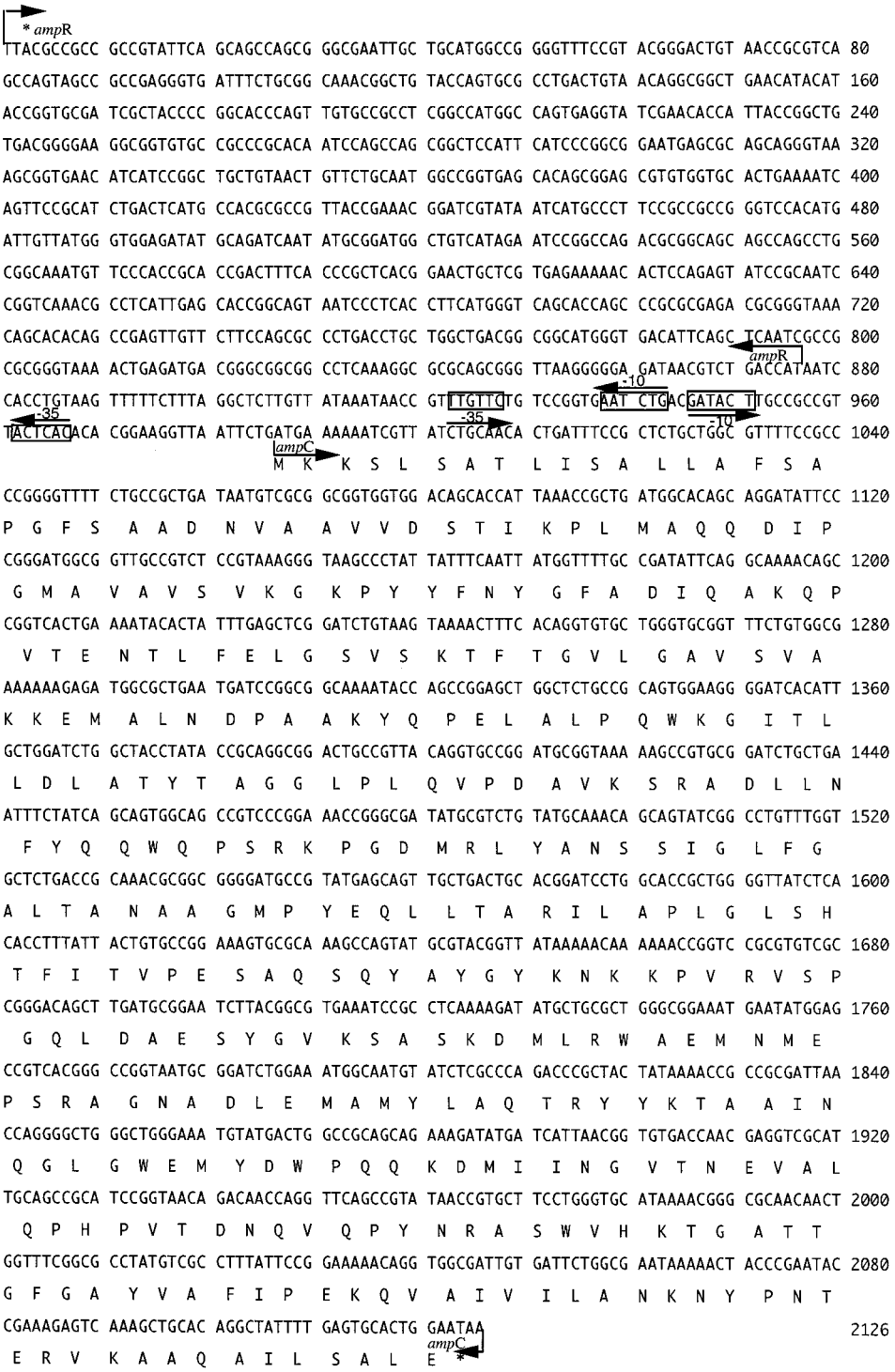

FIG. 2. Nucleotide sequences of the *ampC* and *ampR* genes and of the intercistronic region from pSAL2-ind and the deduced amino acid sequence of DHA-1. Putative −35 and −10 regions of the promoters of the two genes are boxed.

was involved in the inducible synthesis of this plasmid-mediated β-lactamase (Fig. 5).

DISCUSSION

We report here on the nucleotide sequence, phylogeny, and regulation of an inducible AmpC plasmid-mediated β-lactamase. This enzyme, previously called DHA-1 (17), has a pI of 7.8 and confers on clinical strain *S. enteritidis* KF92 and on transconjugant *E. coli* HB101 a pattern of β-lactam resistance similar to those of strains of members of the family *Enterobacteriaceae* that overproduce their chromosome-mediated cephalosporinase and strains with plasmid-mediated AmpC β-lactamase, especially clinical isolates of *K. pneumoniae*. Recently,

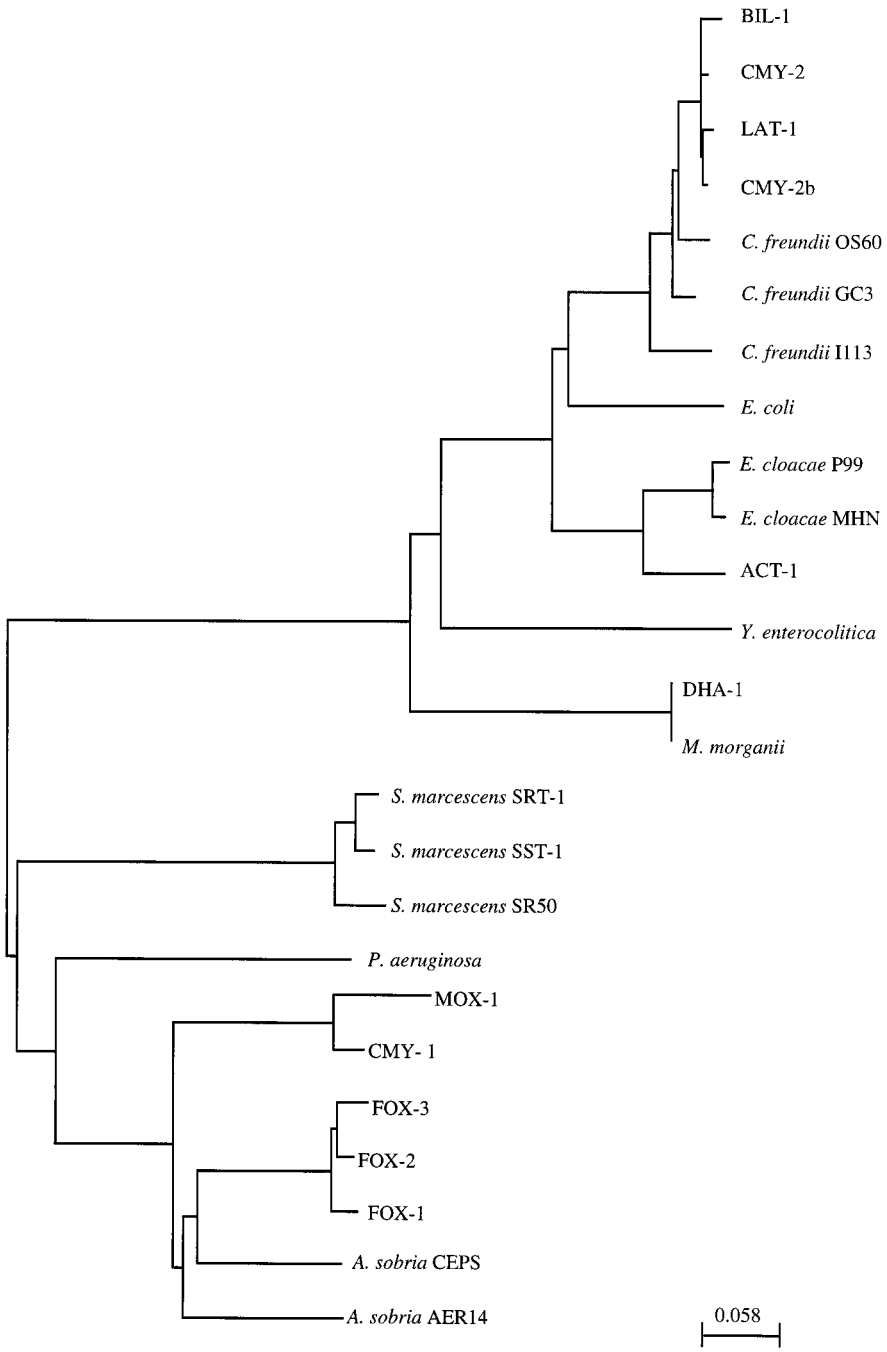

FIG. 3. Schematic representation of multiple-sequence alignments of 25 class C β-lactamases calculated with Clustal W. Amino acids sequences were directly obtained from the GenBank, EMBL, or SWISS-PROT database.

another plasmid-mediated class C β-lactamase (CMY-2b) was identified in a single clinical isolate of *Salmonella senftenberg* in Algeria (29). The incidence of these enzymes has increased since 1989. In *K. pneumoniae*, *E. coli*, and *Salmonella* spp., they are easy to detect on the basis of the isolate's phenotype. Inhibitors such as RO48-1220 that are active at least against class C β-lactamases have been described (42) and may make it easier to detect these enzymes by the disk synergy test with cephamycins such as cefotetan in particular. Detection of such β-lactamases is more difficult in *Enterobacter* spp. and *Citrobacter* spp. because these species have inducible chromosome-mediated cephalosporinases, and mutations in the *ampD* gene lead to overproduction of the enzyme.

Some plasmid-mediated class C β-lactamases probably originate from the chromosomal *ampC* genes of *E. cloacae* (MIR-1 and ACT-1) or *C. freundii* (BIL-1, CMY-2, LAT-1, and LAT-2), whereas the origins of the other enzymes are unknown (CMY-1 and MOX-1) or uncertain (FOX-1, FOX-2, and FOX-3). We demonstrated by sequencing that this AmpC type β-lactamase (DHA-1) has a sequence very similar to that of the chromosome-mediated cephalosporinase of *M. morganii*. Unlike other plasmid-mediated AmpC-type β-lactamases, DHA-1

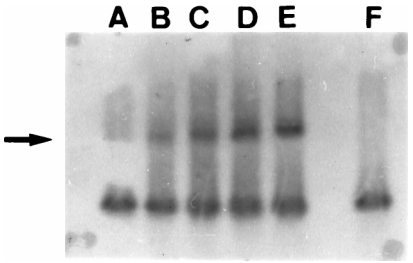

FIG. 4. Gel mobility-shift assay of the *S. enteritidis ampR* and *ampC* intercistronic region with cell protein extracts containing the AmpR regulator protein. The intercistronic sequence, an end-labeled 173-bp PCR product, was retarded with various concentrations of protein extracts from the transformant pSAL2-ind. Lane A, 2.6 μg; lane B, 5.2 μg; lane C, 7.8 μg; lane D, 10.4 μg; lane E, 13 μg; lane F, 0 μg. The position of the retarded complexes containing the intercistronic region is indicated by the arrow.

was inducible, as demonstrated by transformation with the *ampD* gene and the results of an induction test with imipenem as the inducer. These results strongly suggest the involvement of an *ampR*-like gene in pSAL1.

The nucleotide sequence of the upstream region of the *ampC* gene was very similar to that of the *ampR* gene and the intercistronic *ampC-ampR* region of *M. morganii* (97 to 98% identity) (2). The protein sequence deduced from *ampR* gene was very similar to that of the transcriptional regulators of the LysR family with a helix-turn-helix motif in the N-terminal region. This protein appeared to be functional, as shown by the gel mobility-shift assay.

Induction of the chromosome-mediated cephalosporinase AmpC of some members of the family *Enterobacteriaceae* (*C. freundii*, *E. cloacae*) is under the control of three genes, *ampR*, *ampD*, and *ampG* (8, 30, 32). The *ampC* and *ampR* genes are species specific and are present only in members of the family *Enterobacteriaceae* with inducible β-lactamases. The *ampD* and *ampG* genes are probably very common and highly conserved in all members of the family *Enterobacteriaceae* because they encode enzymes involved in cell wall metabolism. AmpG is a permease and AmpD is a cytosolic amidase (22, 26, 34). The presence of the *ampD* gene, which is not closely linked to the *ampR-ampC* region, negatively regulates *ampC* expression (32). A homologous gene was detected in chromosomal DNA of *Salmonella* spp. by hybridization with an intragenic *ampD* probe from *E. cloacae* (38a). The *ampG* gene is

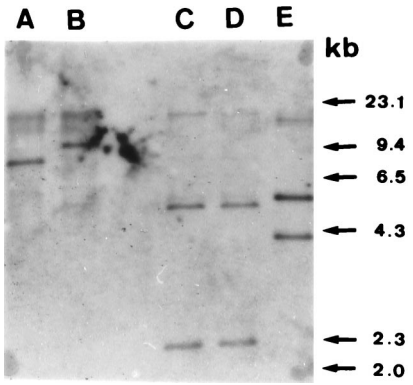

FIG. 5. Southern blot analysis of DNA from *S. enteritidis* KF92 probed with *ampG* from *E. coli* HB101. Lane A, clinical isolate of *K. oxytoca*; lane B, clinical isolate of *K. pneumoniae*; lane C, clinical isolate of *S. enteritidis*; lane D, *S. enteritidis* KF92; lane E, *E. coli* HB101.

required for activation of *ampC* by AmpR (30). We demonstrated the presence of the *ampG* gene in *S. enteritidis* KF92, so this strain has all the genes required for the inducible synthesis of DHA-1.

Thus, DHA-1 is highly related to the chromosome-mediated β-lactamase of *M. morganii* and is inducible. The gene encoding the class C enzyme may have migrated from the chromosome of *M. morganii* to a plasmid, with this gene migration being mediated by transposable elements. Analyses of the sequences flanking the *ampC* and *ampR* genes in pSAL1 are in progress in our laboratory.

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