ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2004, p. 1151–1158 Vol. 48, No. 4
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.4.1151–1158.2004

CFE-1, a Novel Plasmid-Encoded AmpC β-Lactamase with an *ampR* Gene Originating from *Citrobacter freundii*

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Received 13 May 2003/Returned for modification 1 September 2003/Accepted 30 December 2003

A clinical isolate of *Escherichia coli* from a patient in Japan, isolate KU6400, was found to produce a plasmid-encoded β-lactamase that conferred resistance to extended-spectrum cephalosporins and cephamycins. Resistance arising from production of a β-lactamase could be transferred by either conjugation or transformation with plasmid pKU601 into *E. coli* ML4947. The substrate and inhibition profiles of this enzyme resembled those of the AmpC β-lactamase. The resistance gene of pKU601, which was cloned and expressed in *E. coli*, proved to contain an open reading frame showing 99.8% DNA sequence identity with the *ampC* gene of *Citrobacter freundii* GC3. DNA sequence analysis also identified a gene upstream of *ampC* whose sequence was 99.0% identical to the *ampR* gene from *C. freundii* GC3. In addition, a fumarate operon (*frdABCD*) and an outer membrane lipoprotein (*blc*) surrounding the *ampR-ampC* genes in *C. freundii* were identified, and insertion sequence (IS*26*) elements were observed on both sides of the sequences identified (forming an IS*26* composite transposon); these results confirm the evidence of the translocation of a β-lactamase-associated gene region from the chromosome to a plasmid. Finally, we describe a novel plasmid-encoded AmpC β-lactamase, CFE-1, with an *ampR* gene derived from *C. freundii*.

The AmpC β-lactamase produced by gram-negative bacteria such as *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp., and *Morganella* spp. can hydrolyze several β-lactam antibiotics, including cephamycins and extended broad-spectrum cephalosporins (30).

The regulation of AmpC β-lactamase expression is intimately linked to cell wall recycling and involves at least three genes: *ampR*, which encodes a transcriptional regulator of the LysR family; *ampG*, which encodes a transmembrane permease; and *ampD*, which encodes a cytosolic *N*-acetyl-anhydromuramyl-L-alanine amidase that hydrolyzes 1,6-anhydromuropeptides (16, 21, 23, 31). AmpR has been shown to bind to a 38-bp sequence within the intercistronic region between *ampR* and *ampC*. In the absence of a β-lactam inducer, AmpR represses the synthesis of β-lactamase 2.5-fold, whereas expression is induced 10- to 200-fold in the presence of a β-lactam inducer (26). Mutations in the specific site of *ampR* work as an activator of *ampC* and result in the constitutive hyperproduction of AmpC β-lactamase (3, 4, 24). Deletion mutation of the *ampR* gene results in a slightly higher level of basal expression of the *Citrobacter freundii* β-lactamase, but enzyme synthesis can no longer be induced. Knockout mutations in the *ampD* gene result in constitutive hyperproduction of the AmpC β-lactamase even in the absence of a β-lactam inducer (6, 18).

In recent years *ampC* genes have been found on conjugative plasmids, mainly among *Klebsiella pneumoniae* isolates but also occasionally among *Escherichia coli* isolates. Some of these plasmid-encoded genes have DNA and amino acid sequences very similar to those of the chromosome-encoded AmpC β-lactamases of *C. freundii* (CMY-2, CMY-4, and LAT-1) (1, 5, 42, 43), *Enterobacter cloacae* (MIR-1 and ACT-1) (11, 33), and *Morganella morganii* (DHA-1 and DHA-2) (13, 14), although the phylogenies of the various enzymes (FOX-1, MOX-1, and CMY-9) (12, 15, 19) remain unclear (Fig. 1).

Until recently, plasmid-encoded *ampC* genes were considered noninducible because they lack the regulator gene *ampR* (35). However, this generalization is no longer valid: three inducible plasmid-encoded AmpC β-lactamases, DHA-1, DHA-2, and ACT-1, have been described; and all of these carry the *ampR* and the *ampC* genes (2, 13, 37). The mechanism by which plasmid-encoded AmpC β-lactamase was generated from the chromosomal gene has not yet been discovered.

Compared with plasmid-encoded class A extended-spectrum β-lactamases, these plasmid-encoded AmpC β-lactamases (except for ACC-1) are active against cephamycins and are also effective against oxyimino-cephalosporins, such as cefotaxime, ceftazidime, and aztreonam, a monobactam. The in vitro activities of these AmpC β-lactamases are not inhibited by clavulanic acid. Genes encoding these enzymes are now found on plasmids at increasing frequencies (34).

A plasmid-encoded AmpC β-lactamase which confers resistance to cephamycins and expanded-spectrum cephalosporins was detected in Japan in a clinical isolate of *E. coli*. In this report, we characterize a novel plasmid-encoded AmpC β-lactamase, CFE-1, and analyze the nucleotide sequences of the *ampC*, *ampR*, and surrounding genes to compare with those of chromosome-encoded and plasmid-encoded AmpC β-lactamases.

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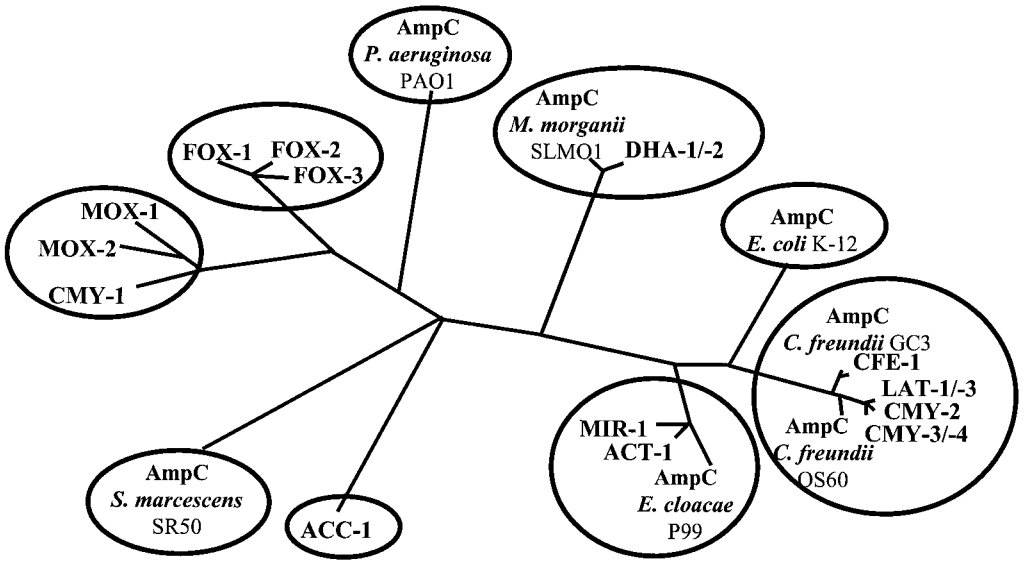

FIG. 1. Dendrogram for chromosomal and plasmid-encoded AmpC β-lactamases calculated by the Clustal V program by using the neighbor-joining method (38).

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Clinical strain *E. coli* KU6400 was isolated at a hospital in Japan in 1997 and was found to contain plasmid pKU601. *E. coli* K-12 ML4947 (AmpD wild type) and ML4953 (AmpD mutant) strains were used as recipients of the plasmid (24, 27). Plasmid pHSG398 is a vector plasmid that confers resistance to chloramphenicol.

Antibacterial agents. Reference samples of the antibacterial agents listed below were used in this study and were provided as powders of known potencies by the respective manufacturers. Piperacillin (Toyama Chemical, Toyama, Japan) was used as a representative penicillin, while cephalothin (Shionogi, Osaka, Japan), cefpodoxime (Sankyo, Tokyo, Japan), cefmetazole (Sankyo), cefotaxime (Nippon Hoechst Marion Roussel, Tokyo, Japan), and cefepime (Bristol-Myers Squibb, Tokyo, Japan) were used as representative cephems. Other β-lactam agents, including imipenem (Banyu Pharmaceutical, Tokyo, Japan) as well as chloramphenicol (Sankyo) and rifampin (Sigma Chemical, St. Louis, Mo.), were also used. Clavulanic acid (SmithKline Beecham Pharmaceuticals, Tokyo, Japan) was used as a β-lactamase inhibitor.

Drug susceptibility assay. The susceptibility profiles were determined by the agar dilution method with sensitivity disk agar (Eiken Chemical, Tokyo, Japan) according to the guidelines of NCCLS (29).

Transconjugation. Conjugation was carried out by a broth method as described previously (20). Exponential-phase Luria broth cultures of donor strain KU6400 and recipient strain ML4947 were mixed at a ratio of 1:10 (by volume). This mating mixture was incubated for 2 h at 35°C. The transconjugants were selected on sensitivity disk agar containing rifampin at 64 μg/ml and cefpodoxime at 4 μg/ml.

Assay for β-lactamase. Crude extraction of AmpC β-lactamase was performed as described previously (32). Cells were harvested by centrifugation (1,700 × *g*, 10 min), resuspended in 3 ml of 50 mM potassium phosphate buffer (pH 7.0), and sonicated. After centrifugation at 14,000 × *g* for 10 min at 4°C, β-lactamase activity was measured by determination of the protein content of the extract, and the protein contents of the cultures were compared. β-Lactamase activity was determined by spectrophotometry (UV2000; Shimadzu, Tokyo, Japan) at 30°C in 50 mM phosphate buffer (pH 7.0). Protein content was determined by a protein assay (Bio-Rad Laboratories, Hercules, Calif.) (10). One unit of β-lactamase activity was defined as the amount of β-lactamase that hydrolyzed 1 μmol of cephalothin in 1 min at 30°C. Cefoxitin (10 μg/ml) was used as the inducer. Induction was allowed to proceed for 60 min (4).

Cloning of the *ampC* and *ampR* genes. DNA extraction, restriction enzyme digestion, recombinant DNA manipulation, and transformations of plasmid DNA were performed as described by Sambrook et al. (40). Restriction enzymes and T4 DNA ligase were purchased from Takara Shuzo (Kyoto, Japan) and Nippon Gene (Tokyo, Japan), respectively. Plasmid pKU601 DNA was isolated from *E. coli* ML4947(pKU601) by the alkaline lysis method (8). The DNA was digested with BamHI and BglII and ligated into the BamHI site of pHSG398. The recombinant plasmid was designated pKU611 and was introduced into *E. coli* ML4947 by electroporation with a gene pulse controller unit (Bio-Rad

TABLE 1. Bacterial strains and plasmids used in this study

Bacterial strain or plasmid	Characteristics[a]
Bacterial strains	
E. coli	
ML4947	F⁻ *galK2 galT22 hsdR hsdM lacY1 metB1 relA supE*44 Rif^r (AmpD wild type)
ML4953	F⁻ *galK2 galT22 hsdR hsdM lacY1 metB1 relA supE*44 Rif^r *ampD9* (AmpD mutant)
KU6400	Clinical isolate carrying pKU601
C. freundii GC3	Clinical isolate from Japan which produces AmpC β-lactamase
Plasmids	
pKU601	Conjugative plasmid encoding CFE-1 isolated from KU6400
pKU611	14-kb fragment containing bla_{CFE-1} gene and *ampR* gene from pKU601 cloned into pHSG398
pKU612	Recombinant plasmid containing *ampC* gene of pKU611 in pHSG398
pHSG398	Cloning vector, purchased from Nippon Gene (Tokyo); Chl^r

[a] Characteristics include designation of markers, source, or derivation. Rif, rifampin; Chl, chloramphenicol.

TABLE 2. MICs of selected antibiotics for *E. coli* strains

Strain	MIC (μg/ml)[a]									
	PIP	CPD	CEF	CTX	CTX-CLA[b]	CAZ	CMZ	ATM	FEP	IPM
KU6400	>256	>256	>256	64	64	64	64	8	0.25	0.25
ML4947(pKU601)	>256	>256	>256	256	256	>256	256	64	1	0.5
ML4953(pKU601)	>256	>256	>256	256	256	>256	256	64	1	1
ML4947(pKU611)	64	>256	>256	16	16	8	16	2	0.25	0.25
ML4947(pKU612)	8	32	256	4	2	4	2	0.5	<0.063	0.25
ML4947	2	0.5	8	<0.063	<0.063	0.25	0.5	<0.063	<0.063	<0.063

[a] Antibiotics: PIP, piperacillin; CEF, cephalothin; CPD, cefpodoxime; CTX, cefotaxime; CLA, clavulanic acid; CAZ, ceftazidime; CMZ, cefmetazole; ATM, aztreonam; FEP, cefepime; IPM, imipenem.
[b] MICs were determined in the presence of clavulanic acid (5 μg/ml).

Laboratories). Transformants (containing *ampC* and *ampR*) were selected on the basis of resistance to cefpodoxime (4 μg/ml) and chloramphenicol (25 μg/ml) after overnight incubation at 37°C and were further characterized by analysis of their antibiotic susceptibility patterns. The size of the insert in the plasmid was estimated by restriction enzyme digestion and electrophoresis in 1.2% agarose gels.

To construct a plasmid containing only the *ampC* gene, plasmid pKU612, pKU611 was digested with SacI and ligated into the SacI site of pHSG398. The resulting plasmid was used to transform *E. coli* ML4947, and the plasmid from which the fragment containing the *ampR* sequence was deleted was identified from the plasmid DNA size and by DNA sequencing.

DNA sequencing and sequence comparisons. Sequencing of both strands of DNA was carried out as described by Sanger et al. (41) with a BigDye terminator cycle sequencing kit and an ABI 310 DNA sequencer (Applied Biosystems, Foster City, Calif.). Sequence analysis and comparison with other known sequences were performed with the BLAST and FAST programs at the National Center for Biotechnology Information.

Nucleotide sequence accession number. The nucleotide sequence data presented in this report appear in the DDBJ, EMBL, and GenBank nucleotide databases under accession number AB107899.

RESULTS

Susceptibilities to antibiotics. The MICs of selected β-lactam antibiotics for clinical isolate *E. coli* KU6400 and the transconjugants that acquired pKU601 by conjugation at a frequency of 10^{-5} are given in Table 2. KU6400 was highly resistant to piperacillin, cefotaxime, the combination of cefotaxime and clavulanic acid, aztreonam, and all cephalosporins except cefepime. The MICs of a variety of different cephalosporins were increased for the transconjugants.

Cloning of the *ampC* and *ampR* genes and gene expression in the *E. coli* recipient. We selected seven *E. coli* transformants resistant to β-lactams in a manner similar to that of *E. coli* ML4947 that acquired pKU601 by conjugation (Table 1). These transformants were resistant to cefpodoxime, cefotaxime, and chloramphenicol.

All transformants were found to produce an AmpC β-lactamase; they harbored a recombinant plasmid (pKU611) with an insert of 14 kb from pKU601. *E. coli* isolates harboring pKU611 showed a resistance profile similar to that of pKU601 (Table 2). Addition of clavulanic acid did not modify the resistance pattern in any transformant.

Characterization of the bla_{CFE-1} gene. Both strands of the entire 14-kb insert from recombinant plasmid pKU611 were sequenced. Analysis of this insert for coding regions revealed two open reading frames (ORFs) (Fig. 2). The first consisted of 1,137 bp encoding a putative protein of 378 amino acids (Fig. 3). This ORF had an ATG start codon at position 1008 and a stop codon at position 2151. Database searches with this ORF identified similarities with several chromosome- and plasmid-encoded AmpC β-lactamases, particularly the chromosome-encoded AmpC β-lactamase of *C. freundii* GC3 (99.8% sequence identity) (17). The deduced amino acid sequence carried catalytic residues S-X-X-K, with the initial serine at position 64 (which is typical of AmpC β-lactamases); the motif Y-S-N at position 150; and the K-T-G motif at position 315.

The second ORF, which contained 876 nucleotides was transcribed in the opposite orientation and was located in the 5′ direction from the *ampC* structural gene. It began with an ATG start codon at nucleotide 876 and had a stop codon at nucleotide 3. By analogy with the *ampC* and *ampR* genes of the AmpC β-lactamase, this ORF may correspond to the regulatory gene *ampR*. A sequence corresponding to transcriptional regulators of the LysR family, particularly the AmpR proteins of the family *Enterobacteriaceae*, was deduced. The DNA sequence of the corresponding gene from *C. freundii* GC3 was 99.0% identical to the sequence of this ORF. The deduced protein sequence showed only one difference, at position 135 (Ala for Asp), compared to the sequence of *C. freundii* GC3.

The 131-bp region between the *ampR* and *ampC* start codons contained overlapping putative promoters. This region was 97% identical to the corresponding region of *C. freundii* GC3.

Sequences surrounding *ampR* and *ampC* regions. In addition to identifying the sequences in the regions surrounding the *ampR* and *ampC* sequences, sequence analysis indicated that the *frdABCD* operon of *C. freundii* (7) was located upstream from the *ampC* gene; and a part of the ORF contained the sequence for the outer membrane lipoprotein encoded by the *blc* gene of *C. freundii* (GenBank accession nos. D85910 and U21727), which was located immediately downstream of the *ampC* gene (Fig. 4). Furthermore, two IS*26* elements were observed to surround the *ampR* and *ampC* genes and were directed in the same orientation, forming an IS*26* composite transposon. One was inserted in the *frdA* gene (as detected by PCR), and another was inserted in the *blc* gene.

β-Lactamase activities. The β-lactamase activities encoded by the plasmids are shown in Table 3. *E. coli* ML4947 (pKU601) and ML4953(pKU601) produced large amounts of β-lactamase (10.9 and 14.4 U/mg of protein, respectively). When *E. coli* ML4953 (AmpD mutant) was used as the host, the β-lactamase activities encoded by pKU601 were slightly higher than those detected when *E. coli* ML4947 (AmpD wild type) was used as the host. The β-lactamase activities of these strains in the presence of cefoxitin were slightly increased com-

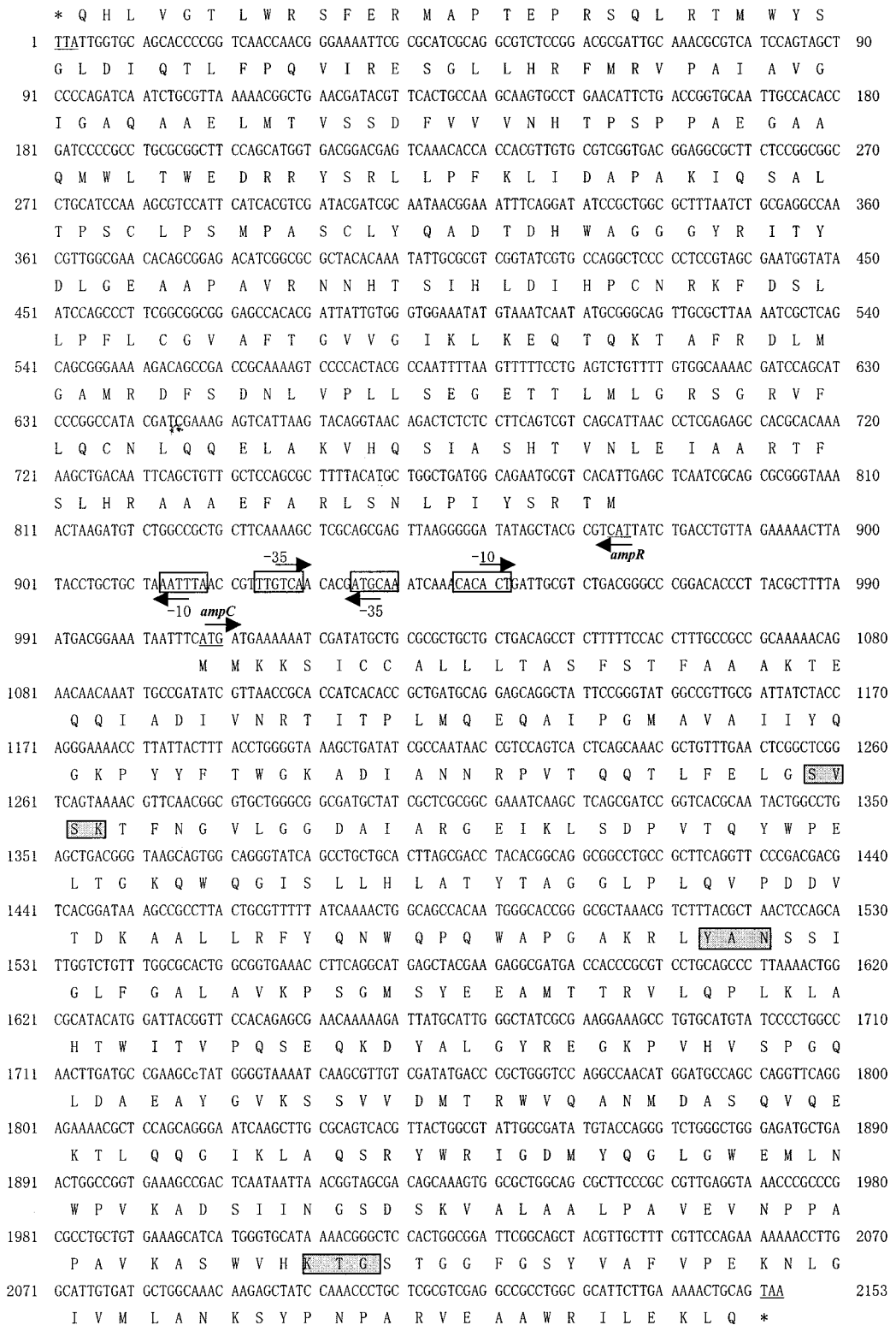

FIG. 2. Nucleotide sequence of the 2,153-bp fragment of pKU611 containing the *ampC*- and *ampR*-coding regions. Deduced amino acid sequences are designated in single-letter code. Putative promoter sequences are represented by the −35 and −10 regions (boxed). The start and stop codons of these genes are underlined. Additionally, conserved residues among class C β-lactamases are shown in shaded boxes.

```
CFE-1          MMKKSICCALLLTASFSTFAAAKTEQQIADIVNRTITPLMQEQAIPGMAVAIIYQGKPYY 60
AmpC Cit GC3   ····························································
AmpC Cit OS60  ····························································E·····
CMY-2          ·····L·····································V·····
AmpC Ent P99   ··R··L·····GI·C·AL·TPVS·K·L·EV·AN·····KA·SV·····V·····H·

CFE-1          FTWGKADIANNRPVTQQTLFELGSVSKTFNGVLGGDAIARGEIKLSDPVTQYWPELTGKQ 120
AmpC Cit GC3   ····························································
AmpC Cit OS60  ··········H·····R·····K·····
CMY-2          ··········H·····K·····
AmpC Ent P99   Y·F·····A·K···P·····I····T·····S·D·A··R···Q·····

CFE-1          WQGISLLHLATYTAGGLPLQVPDDVTDKAALLRFYQNWQPQWAPGAKRLYANSSIGLFGA 180
AmpC Cit GC3   ····························································
AmpC Cit OS60  ·R·····I·G·····E·····T·····
CMY-2          ····R·····I····R·····H·····T·····
AmpC Ent P99   ····RM·D·····E···N·S·····K··TT·····A·····

CFE-1          LAVKPSGMSYEEAMTTRVLQPLKLAHTWITVPQSEQKDYALGYREGKPVHVSPGQLDAEA 240
AmpC Cit GC3   ·····W·····
AmpC Cit OS60  ····S·····R·····N··W··L·····
CMY-2          ·····R·····N·····W·····
AmpC Ent P99   ·····P··Q·····K····D····N··KA·EAH··W···D··A·R····M···Q·

CFE-1          YGVKSSVVDMTRWVQANMDASQVQEKTLQQGIKLAQSRYWRIGDMYQGLGWEMLNWPVKA 300
AmpC Cit GC3   ····························L··
AmpC Cit OS60  ·····I··A·····H·····E·····L··
CMY-2          ·····I··A·····H·····A·····L··
AmpC Ent P99   ····TN·Q··AN··M···APEN·ADAS·K···A·····S·····E·

CFE-1          DSIINGSDSKVALAALPAVEVNPPAPAVKASWVHKTGSTGGFGSYVAFVPEKNLGIVMLA 360
AmpC Cit GC3   ····························································
AmpC Cit OS60  ····························································
CMY-2          ····························································
AmpC Ent P99   NTVVE·····P··VA·····P·····I···QI·····

CFE-1          NKSYPNPARVEAAWRILEKLQ.                                        381
AmpC Cit GC3   ····················.
AmpC Cit OS60  ····················.
CMY-2          ·····V·····.
AmpC Ent P99   ·T·····YH···A··.
```

FIG. 3. Derived amino acid sequence of the CFE-1 β-lactamase compared to the sequences of other selected class C β-lactamases. The alignments of the deduced amino acid sequence of the CFE-1 β-lactamase with the AmpC β-lactamases of *C. freundii* GC3 (AmpC Cit GC3), *C. freundii* OS60 (AmpC Cit OS60), and *E. cloacae* P99 (AmpC Ent P99) and with the CMY-2 β-lactamase are shown. Dots indicate identical amino acids at that residue.

pared with the basal level, but the increases were not significantly different. This suggests that the bla_{CFE-1} gene may produce the enzyme constitutively.

The activity of CFE-1 was not inhibited by clavulanic acid, a characteristic confirming the close resemblance of CFE-1 to the AmpC β-lactamase, as mentioned in the description of the nucleotide sequence.

pKU611, which encoded the *ampR* and *ampC* genes, and recombinant plasmid pKU612, which encoded only the *ampC* gene (i.e., it lacked the *ampR* gene), were introduced into *E. coli* ML4947; and the β-lactamase activities of each construct were analyzed (Table 3). The specific enzyme activity of *E. coli* ML4947(pKU611) was 1.4 U/mg of protein, while that of *E. coli* ML4947(pKU612) was 0.2 U/mg of protein, a markedly lower level of expression.

DISCUSSION

In addition to previous reports concerning the MOX-1 and CMY-9 plasmid-encoded AmpC β-lactamases, which were detected in an *E. coli* clinical isolate in Japan (12, 19), in the present study we characterized a novel AmpC β-lactamase gene, bla_{CFE-1}, in a Japanese *E. coli* clinical isolate. This is the first report in East Asia of a plasmid-encoded AmpC β-lactamase, CFE-1, carrying an *ampR* gene derived from the *C. freundii* chromosome.

Our findings depict the organization of sequences surrounding the *ampR-ampC* region, including bla_{CFE-1}, which is seen in various enterobacterial species. Most plasmid-encoded AmpC β-lactamases, like CMY-2, CMY-4, and LAT-1, lack the *ampR* gene. *Citrobacter* spp. and *Enterobacter* spp. possess *ampR* and *ampC* genes, the fumarate operon *frdABCD* immediately

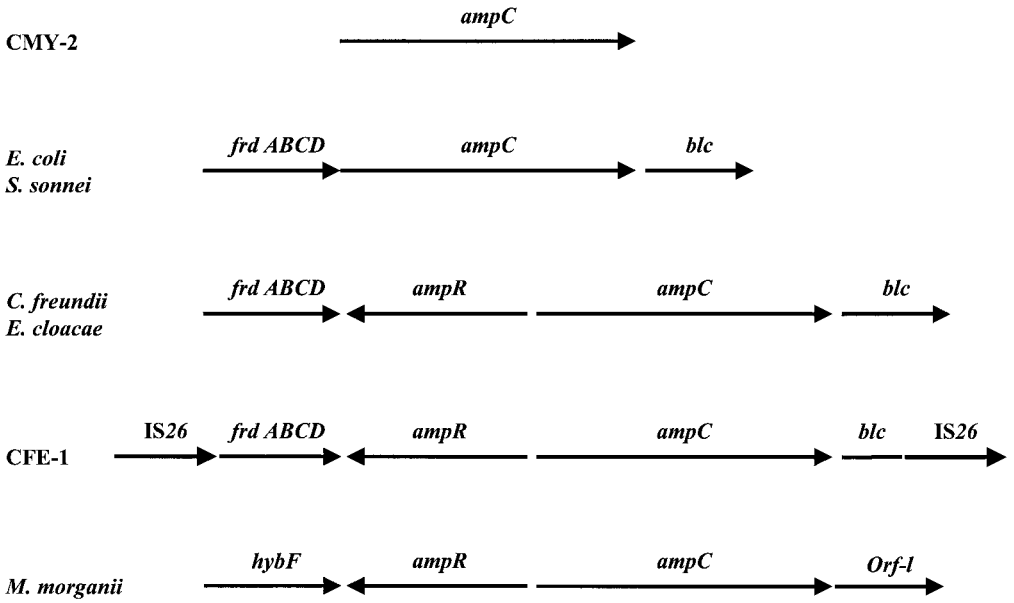

FIG. 4. Organization of the sequences surrounding *ampC* in various enterobacterial species. The positions of the fumarate operon (*frdABCD*), *blc*, *hybF*, *orf-1*, IS*26*, *ampC*, and *ampR* genes are shown, with directions indicated by arrows.

downstream of the *ampR* gene, and also outer membrane lipoprotein *blc* immediately downstream of the *ampC* gene (7, 9). In contrast, *M. morganii* possesses the *ampR* and *ampC* genes but not the fumarate operon (36); *hybF* is substituted for the fumarate operon upstream from the *ampC* gene (Fig. 4).

Analysis of the bla_{CFE-1} gene revealed that it has a very close relationship to the chromosomal gene that encodes the AmpC β-lactamase in *C. freundii* (1) (Table 4). The amino acid sequence of CFE-1 showed 99.5% identity with that of *C. freundii* GC3 isolated from clinical specimens in Japan (17), differing only at position 221 (Leu for Trp) and position 298 (Val for Leu) (Fig. 3). This identity was greater than that with *C. freundii* OS60 (95.0%) (26). The amino acid sequence of AmpR showed 99.0% identity with that of *C. freundii* GC3, differing only at position 135 (Ala for Asp). This similarity strongly suggests that the bla_{CFE-1} gene is derived from the chromosomal *ampC* gene of *C. freundii* GC3 (1). This hypothesis is supported by the finding that bla_{CFE-1} has both the *ampR* gene and the *ampC* gene, as well as the *frdABCD* gene operon and the *blc* gene, which were found to surround the *ampR* and *ampC* genes, as in *C. freundii*.

In addition, two IS*26* elements were detected on plasmid pKU601; one was located immediately upstream from the *frdA* gene, while the other was located immediately downstream of the *ampC* gene and was inserted in the *blc* gene (Fig. 4). These were directed in the same orientation as that seen for the IS*26* composite transposon (25). Some plasmid-encoded β-lactamase genes form part of transposons frequently flanked by insertion sequence elements, such as IS*26* (SHV-2a and ACC-1) (22, 28) and IS*Ecp1* (CTX-M5) (39). These results present direct evidence that the bla_{CFE-1} gene translocated to a plasmid from the chromosome of *C. freundii* strain GC3 by using the IS*26* function(s). Further studies are continuing to determine whether the bla_{CFE-1} gene is capable of translocation.

As shown in Tables 2 and 3, an *E. coli* strain harboring pKU601 encoding bla_{CFE-1} expressed β-lactamase constitutively in the presence or absence of a β-lactam inducer and in the AmpD wild type (ML4947) or AmpD mutant (ML4953). *E. coli* ML4947(pKU612), which lacks the *ampR* gene, showed a decrease in β-lactamase activity compared with that of *E. coli* ML4947(pKU611); nevertheless, the AmpC β-lactamases of *C. freundii*, *E. cloacae*, and *M. morganii* with an *ampR* deletion showed increased levels of β-lactamase expression. This result

TABLE 3. β-Lactamase activities of *E. coli* strains

Strain	Relative β-lactamase activity (U/mg of protein)[a]	
	Noninduced	Induced[b]
KU6400	7.7	7.9
ML4947(pKU601)	10.9	11.1
ML4953(pKU601)	14.4	16.3
ML4947(pKU611)	1.4	1.7
ML4947(pKU612)	0.2	ND[c]

[a] β-Lactamase activities are the geometric mean determinations for three independent cultures. The standard deviations were within 10%.
[b] Cefoxitin (10 μg/ml); was used as the inducer.
[c] ND, not done.

TABLE 4. Identity of the CFE-1 amino acid sequence to those of other AmpC β-lactamases

β-Lactamase	% Identity with:				
	CFE-1	GC3	OS60	CMY-2	P99
CFE-1	100	99.5	95.0	95.3	74.5
C. freundii GC3 AmpC		100	95.5	95.8	74.5
C. freundii OS60 AmpC			100	95.8	73.2
CMY-2				100	75.1
E. cloacae P99 AmpC					100

indicates that AmpR of pKU601 seems to function as regulator of the constitutive expression of bla_{CFE-1}. Bartowsky and Normark (3, 4) have reported that the activation of *ampC* transcription in *C. freundii* is dependent on the conversion of AmpR into a transcriptional activator. The AmpR mutants of *C. freundii*, which have Glu instead of Gly at position 102 or Tyl instead of Asp at position 135, express β-lactamase at high levels. Increased levels of β-lactamase expression have been reported (24) when the Arg-86 and Asp-135 mutations are present in *E. cloacae* AmpR. In the *ampR* gene of pKU601, the amino acid at position 135 was Ala, whereas it was Asp in the wild type. These results may indicate that overexpression of β-lactamase is dependent on mutation of the *ampR* gene.

In summary, *E. coli* plasmid pKU601 was characterized as harboring a novel plasmid-encoded AmpC β-lactamase, CFE-1, with an *ampR* gene. The high level of constitutive CFE-1 expression in *E. coli* is presumably caused by the mutation in the *ampR* gene, in which the Asp at position 135 is changed to Ala. These results indicate the dissemination of a resistance gene to different enterobacterial species through mobilization of a plasmid and transposable event-mediated events.

ACKNOWLEDGMENTS

This work was supported in part by grants (12670264 and 10008336) from the Japanese Ministry of Education, Culture, Sports, Science and Technology; the COE program of the Japanese Ministry of Education, Culture, Sports, Science and Technology; the Japanese Ministry of Health, Labor, and Welfare (the Research Project of Emerging and Establishment of Rapid Identification Methods); and the Charitable Trust Clinical Pathology Research Foundation of Japan.

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