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Characterization of CIA-1, an Ambler Class A Extended-Spectrum β-Lactamase from *Chryseobacterium indologenes*

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An Ambler class A β-lactamase gene, bla_{CIA-1}, was cloned from the reference strain *Chryseobacterium indologenes* ATCC 29897 and expressed in *Escherichia coli* BL21. The bla_{CIA-1} gene encodes a novel extended-spectrum β-lactamase (ESBL) that shared 68% and 60% identities with the CGA-1 and CME-1 β-lactamases, respectively. bla_{CIA-1}-like genes were detected from clinical isolates. In addition to the metallo-β-lactamase IND of Ambler class B, *C. indologenes* has a class A ESBL gene, bla_{CIA-1}, located on the chromosome.

The organism *Chryseobacterium indologenes* is the most common flavobacterium in clinical specimens and is associated with various types of infections, such as intra-abdominal infections, biliary tract infections, wound sepsis, catheter-related bacteremia, sepsis, and pneumonia (1, 11, 15, 16). However, no clonal outbreaks have been reported. *C. indologenes* is resistant to nearly all penicillins, restricted-spectrum cephalosporins, and carbapenems (3). It has been speculated that this resistance is due to metallo-β-lactamase IND (6).

Previously, two Ambler class A β-lactamases, CGA-1 and CME, were characterized in *Chryseobacterium gleum* and *Elizabethkingia meningoseptica* (formally *Chryseobacterium meningosepticum*), respectively (5, 17). CGA-1 and CME exhibit broad-spectrum profiles and are chromosomally encoded. Zeba et al. reported a single β-lactamase band at pI 9.0 in addition to IND from *C. indologenes*, using isoelectric focusing, and the band at pI 9.0 probably included an active site serine enzyme (19). However, no class A β-lactamases have been identified from *C. indologenes*. The aims of this study were to perform molecular characterization of the Ambler class A β-lactamase produced by *C. indologenes* and to investigate its distribution among other strains.

The *C. indologenes* reference strain (ATCC 29897) used in this study was purchased from the American Type Culture Collection. The MICs of the antimicrobial agents were determined using an agar dilution technique on Mueller-Hinton plates (BBL Microbiology Systems, Cockeysville, MD), with an inoculum of 10^4 CFU/spot in accordance with the performance standards for antimicrobial susceptibility testing in the guidelines published by the Clinical and Laboratory Standards Institute (8). We confirmed the bla_{IND} from *C. indologenes* ATCC 29897 by PCR (3). Conjugation experiments failed to transfer any β-lactam resistance marker from *C. indologenes* ATCC 29897 to rifampin-resistant *Escherichia coli* CSH2 (10), and extraction of plasmid DNA from *C. indologenes* ATCC 29897 was attempted; however, plasmids were not detected. Genomic DNA from *C. indologenes* ATCC 29897 was extracted, and fragments from genomic DNA, which were partially digested with PstI, were ligated into the PstI-restricted phagemid pBK-CMV (Stratagene, La Jolla, CA) by using a previously reported method (7). The recombinant clone *E. coli* BL21(pCIA-1) was obtained after selection on amoxicillin (30 μg/ml)- and kanamycin (30 μg/ml)-containing Mueller-Hinton agar plates. The recombinant plasmid was purified and sequenced on both strands. An 879-bp open reading frame, which encoded a 292-amino-acid protein, was identified. This open reading frame showed the highest similarity to other class A β-lactamases in a BLAST search. This novel CIA-1 β-lactamase had identities of 68% to CGA-1 (5), 60% to CME-1 (17), 51% to TLA-1 (18), 48% to CSP-1 (9), and 44% to VEB-1 (14) (Fig. 1).

A serine active site, characteristic of β-lactamases, was found within the mature protein sequence of CIA-1 (Fig. 1) (12). CIA-1 had 4 conserved elements of class A β-lactamases (2, 12): a Ser-X-X-Lys consensus active site serine residue at position 70, an SDN loop at position 130, a conserved Glu166, and a KTG sequence at position 234.

The G+C content of bla_{CIA-1} was 36.41%, which is typical of *Chryseobacterium* species. This result, along with the negative conjugation and plasmid isolation attempts, suggests a chromosomal location for bla_{CIA-1}.

Genomic DNAs of *C. indologenes* clinical isolates (SH187, SH520, and SH3157) were investigated to clarify the distribution of the bla_{CIA-1} gene, using PCR screening with a set of designed primers, CIA-F (GCGAGAATAAACTCAGAGTA CAT) and CIA-R (AGCATGAACTTCCATAAGAGATC). Three specific amplicons were amplified by standard PCR (denaturation for 10 min at 95°C with 35 cycles of 1 min at 95°C, 1 min at 58°C,

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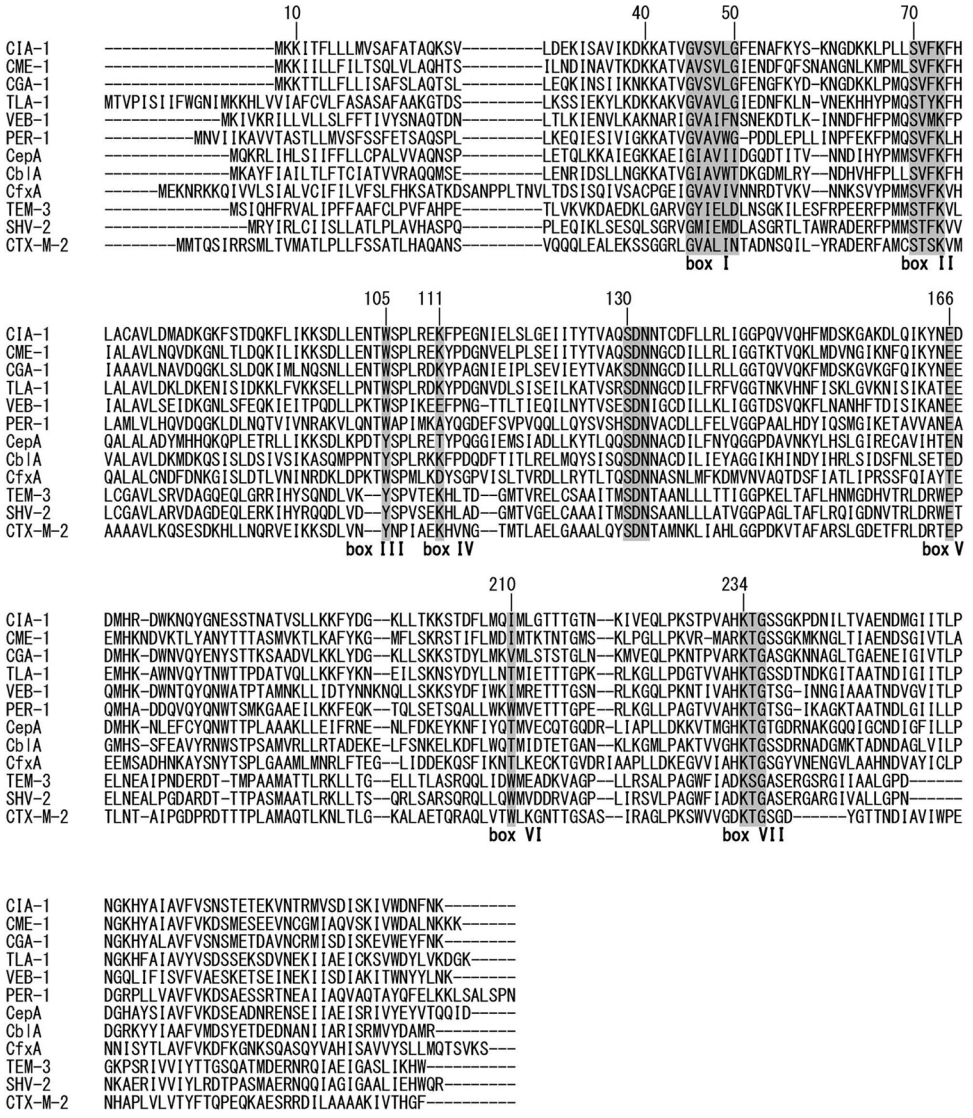

FIG 1 Amino acid sequence of β-lactamase CIA-1 with 11 class A ESBLs. Boxes I through VII correspond to amino acid sequences described by Joris et al. (12). Dashes show gaps used to optimize the alignments.

and 2 min at 72°C and a final extension for 10 min at 72°C), and analyses of predicted amino acid sequences were performed with the ClustalW program. The $bla_{\text{CIA-1}}$ gene had 98% amino acid identity to $bla_{\text{CIA-1}}$-like genes from SH187, SH520, and SH3157.

The $bla_{\text{CIA-1}}$ open reading frame encodes a putative protein comprising 292 amino acids with a molecular mass of ~32.5 kDa (Fig. 1). The mature peptide has a theoretical pI of 9.0, which corresponded to the pI of CIA-1 by isoelectric focusing (data not shown). It is probably the same β-lactamase band observed by Zeba et al. (19). The susceptibility of *C. indologenes* ATCC 29897 to β-lactams was similar to that reported for *C. indologenes* (13). The MICs of several β-lactams reported previously (5) are shown in Table 1. *C. indologenes* ATCC 29897 was resistant to amino- and carboxypenicillins, narrow-spectrum cephalosporins, cefotaxime, cefoperazone, carbapenems, and aztreonam; however, it was susceptible to ceftazidime and cefepime (Table 1). The antibiotic effects of piperacillin, ceftazidime, and cefepime were decreased 2- to 32-fold in the presence of clavulanic acid (Table 1). *E. coli* BL21(pCIA-1) was resistant to amoxicillin, ticarcillin, carbenicillin, narrow-spectrum cephalosporins, and ceftazidime and had reduced susceptibility to cefotaxime, cefoperazone, cefepime, and aztreonam. Clavulanic acid reduced the MICs of β-lactams for *E. coli* BL21(pCIA-1) (Table 1). The MIC of *C. indologenes* ATCC 29897 decreased to below 1/8 by the addition of piperacillin or cefepime with clavulanic acid. The antibiotic susceptibility pattern of the *C. indologenes* clinical isolate SH187 was similar to that of *C. indologenes* ATCC 29897. The expression of class B β-lactamase IND can explain the resistance of *C. indologenes* ATCC 29897 to cefoxitin and carbapenems (3).

In this study, we demonstrated the presence of the bla_{CIA} gene in the *C. indologenes* chromosome by the absence of conjugation transfer, determined the nucleotide sequence of $bla_{\text{CIA-1}}$ (G+C content, 36%) and detected $bla_{\text{CIA-1}}$-like genes in clinical isolates. *C. indologenes* was shown to possess chromosomally encoded class

TABLE 1 Antimicrobial susceptibilities of *C. indologenes* ATCC 29897, *C. indologenes* SH187, *E. coli* BL21(pCIA-1), and reference strain *E. coli* BL21

	MIC (μg/ml)			
β-Lactam	*C. indologenes* ATCC 29897	*E. coli* BL21 (pCIA-1)	*C. indologenes* SH187	*E. coli* BL21
Penicillin G	64	128	16	4
Amoxicillin	256	256	64	1
Carbenicillin	>256	>256	256	2
Ticarcillin	>256	>256	>256	1
Piperacillin	256	16	32	0.5
Cefazolin	256	16	256	1
Cephalothin	256	32	128	1
Cephalexin	256	64	>256	4
Cefuroxime	>256	32	128	0.5
Cefotaxime	64	1	16	≤0.125
Ceftazidime	8	32	8	≤0.125
Ceftriaxone	128	1	64	≤0.125
Cefoperazone	32	1	16	≤0.125
Cefepime	4	0.5	4	≤0.125
Cefoxitin	16	1	8	1
Moxalactam	128	≤0.125	128	≤0.125
Imipenem	128	≤0.125	2	≤0.125
Aztreonam	256	8	256	≤0.125
Amoxicillin + CLA[a]	256	2	4	1
Piperacillin + CLA	8	0.5	0.25	0.5
Ceftazidime + CLA	4	≤0.125	4	≤0.125
Cefepime + CLA	0.5	≤0.125	0.5	≤0.125

[a] CLA, clavulanic acid at 2 μg/ml.

A extended-spectrum β-lactamase (ESBL) CIA, in addition to class B β-lactamase IND, and the class A β-lactamase of CIA-1 shared functional and structural similarities with CME-1, CME-2, and CGA-1.

Nucleotide sequence accession number. The nucleotide sequences of the complete bla_{CIA} genes from *C. indologenes* ATCC 29897, SH187, SH520, and SH3157 that have been reported in this study have been submitted to the GenBank and EMBL databases under accession no. AB639753, AB674566, AB674567, and AB674568, respectively.

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