Molecular Characterization of a Carbapenem-Hydrolyzing Class A β-Lactamase, SFC-1, from Serratia fonticola UTAD54

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An environmental isolate of Serratia fonticola resistant to carbapenems contains a gene encoding a class A β-lactamase with carbapenemase activity. The enzyme was designated SFC-1. The \bla_{SFC-1} gene is contained in the chromosome of S. fonticola UTAD54 and is absent from other S. fonticola strains.

The prokaryotic species Serratia fonticola, a species of the family Enterobacteriaceae, includes organisms that occur naturally in environmental waters (3); occasionally, some strains cause infections in humans (15). A recent study on the natural antimicrobial susceptibilities of strains of Serratia species (23) showed that S. fonticola expresses both a chromosomally encoded extended-spectrum class A β-lactamase and a species-specific AmpC β-lactamase. The class A enzyme corresponds to the previously characterized β-lactamase SFO-1 (13), and the homologous sequence FON-A (GenBank accession no. AJ251239) is common to S. fonticola (19).

In a previous report (18), an environmental isolate designated S. fonticola UTAD54 was shown to be resistant to carbapenems. This phenotype could be attributed to a gene encoding a class B metallo-enzyme (Shf-I) that was isolated from a genomic library (18). An additional screening of the library was done on Luria-Bertani plates supplemented with ampicillin (50 μg/ml) and kanamycin (30 μg/ml) to select for inserts and the vector, respectively. Some of the clones obtained were negative when screened by PCR using primers (18) for genes homologous to SFO-1. A recombinant plasmid containing a 1.8-kb insert was selected for study and designated pH18.

Characterization of a new β-lactamase gene. Plasmid DNA was prepared with a Qiaprep kit (Qiagen, Courtaboeuf, France), and both strands of the insert were sequenced on an ABI cycle sequencer A373 (Applied Biosystems/Perkin-Elmer, Foster City, Calif.) using the ABI Prism dye terminator kit. Analysis of sequence data revealed the presence of an open reading frame of 927 bp encoding a 33.6-kDa protein containing 309 amino acids (Fig. 1). Four nucleotides upstream of the ATG codon have the sequence AAGG, a putative ribosome-binding site (RBS). A typical

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The overall G+C content of \bla_{SFC-1} (45.3%) is characteristic of genes of Enterobacteriaceae.

A similarity search was performed with BLAST (1). SFC-1 had the highest similarity to the class A carbapenemases, in particular KPC-1 (62% identical) from Klebsiella pneumoniae (21), Sm-1 (58%), NMC-A (59%), and IMI-1 (59%) (12). Lower similarity scores were returned for the other class A β-lactamases. No putative LysR-type regulator gene was identified upstream of the \bla_{SFC-1} gene, whereas such regulators are transcribed upstream of the genes coding for NMC-A, Sm-1, and IMI-1 (7, 8).

The software SignalP (11) identified a bacterial signal peptide of 26 amino acids in the amino-terminal sequence (Fig. 1). Cleavage of this signal peptide would yield a mature protein of 30.7 kDa with a pI of 7.95.

Within the mature protein, a serine-serine-phenylalanine-lysine tetrad (S-S-F-K) was found, as was a lysine-threonine-glycine (KTG) motif. These motifs (SXXK and KTG) are characteristic of serine β-lactamases (16, 22). The nine invariant residues typical of class A enzymes (G45, S70, K73, P107, S130, D131, A134, E166, and G236) are conserved in the SFC-1 sequence. From the residues suggested to be important for class A carbapenemase activity (C69, S70, K73, H105, S130, R164, E166, N170, D179, R220, K234, S237, and C238), only S237 was not conserved in the SFC-1 sequence.

The deduced amino acid sequence of SFC-1 was aligned to the sequences of 15 class A β-lactamases, using CLUSTAL W at the European Molecular Biology Laboratory website (http://www.ebi-heldelberg.de/). The enzymes and their GenBank accession numbers were the following: KPC-1 (24) from K. pneumoniae (AAG13410), IMI-1 (17) from Enterobacter cloacae (AAR93461), Sm-1 (9) from Serratia marcescens (CA882281), OXY-1 (2) from Klebsiella oxytoca (P22391), CITTD1 (14) from Citrobacter diversus (S19006), YENT (20) from Yersinia enterocolitica (Q01166), CTX-M-12 (19) from K. pneumoniae (AAG34108), CTX-M-14 (19) from Escherichia coli (CAC95170), Toho-1 (6) from E. coli (BAA07082), SFO-1 (6) from E. cloacae (BAA76882), FONA-3 from S. fonticola (CAB61639), SER_FON (13) from S. fonticola (P80545), TEM-1 (24) from E. coli (AAR25033), SHV-1 (24) from E. coli (P14557), and CARB-3 (4) from Pseudomonas aeruginosa (P37322). The dendrogram shown in Fig. 2 was derived from

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the alignment: SFC-1 clusters to the class A carbapenemases and is more closely related to a subgroup that includes the enterobacterial enzymes of extended hydrolytic spectrum.

Susceptibility to antibiotics. The MICs were determined by the E-test method (Biodisk, Solna, Sweden), and susceptibility categories were allocated according to those described in reference 10. Table 1 shows the MICs for *S. fonticola* UTAD54, *E. coli* transformed with plasmid pIH18, and untransformed *E. coli*. The DNA insert encoding SFC-1 when replicating in *E. coli* confers resistance to ampicillin, amoxicillin, piperacillin, cephalothin, and aztreonam and reduced susceptibility to meropenem and imipenem, and its activity is inhibited by the class A \( \beta \)-lactamase inhibitors. Such a resistance pattern is characteristic of a carbapenem-hydrolyzing class A \( \beta \)-lactamase.

![FIG. 1. Nucleotide and deduced amino acid sequences of the SFC-1 gene and its upstream and downstream regions. The putative -10 region and a potential RBS are in bold. The inverted repeat sequences that can act as a terminator of transcription are shaded. The putative signal peptide for protein secretion is underlined. Amino acids that correspond to conserved domains of class A \( \beta \)-lactamases are shown in bold.](image)

The probes were generated by PCR amplification in the presence of digoxigenin (Roche Molecular Biochemicals, Indianapolis, Ind.). For rRNA genes and the SFO-1 gene, the primers were previously reported (18). Specific primers were designed to amplify the *bla* 

### Table 1. MICs of antibiotics for *S. fonticola* UTAD54, *E. coli* XL2 Blue (pH18), and *E. coli* XL2 Blue (reference strain)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. fonticola UTAD54</th>
<th>E. coli XL2 Blue (pH18)</th>
<th>E. coli XL2 Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>2</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>4</td>
</tr>
<tr>
<td>Amoxicillin-CLA</td>
<td>12</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>32</td>
<td>64</td>
<td>0.75</td>
</tr>
<tr>
<td>Piperacillin-TZB</td>
<td>0.38</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>6</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.032</td>
<td>0.75</td>
<td>0.032</td>
</tr>
<tr>
<td>Ceftoxime</td>
<td>2</td>
<td>1</td>
<td>0.064</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>1.5</td>
<td>64</td>
<td>0.19</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;32</td>
<td>0.38</td>
<td>0.008</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;32</td>
<td>4</td>
<td>0.125</td>
</tr>
</tbody>
</table>

* CLA, clavulanic acid; TZB, tazobactam.
ATGTCACGCACCGTGACTG-3', and SflR (5'-GATGA ATTCTTAGAAGCCGATAGACTTTCC-3'). The probes for the SFC-1 and SFO-1 genes revealed two different I-CeuI bands, as shown in lanes 1 and 2 of Fig. 3; the probe for rRNA genes hybridized to the six I-CeuI bands (lane 3). These results thus indicate that both β-lactamase genes are chromosomally encoded and apart from each other. Hybridization of the probe for bla_{SFC-1} with DNA from \textit{S. fonticola} strains LMG 7882^T, DSM 9663, and CIP 103850 did not detect homologous sequences in these genomes.

**Concluding remarks.** \textit{S. fonticola} UTAD54 is an exceptional strain, carrying the naturally occurring β-lactamases of \textit{S. fonticola} and different classes of carbapenemases, SFC-1 and the previously reported metallo-enzyme Sfh-I. Those enzymes are not present in other \textit{S. fonticola} strains. These exceptional characteristics could be the result of the acquisition of a genetic element by horizontal gene transfer.

**Nucleotide sequence accession number.** The nucleotide sequence reported here was deposited in GenBank under accession number AY354402.

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**REFERENCES**


