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Biochemical Characterization of CPS-1, a Subclass B3 Metallo-β-Lactamase from a Chryseobacterium piscium Soil Isolate

Dereje Dadi Gudeta,a Simona Pollini,b Jean-Denis Docquier,b Valeria Bortolaia,a Gian Maria Rossolini,a,c,d Luca Guardabassia,a,b,c

Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark; Department of Medical Biotechnologies, University of Siena, Siena, Italy; Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy; Department of Biomedical Sciences, Ross University School of Veterinary Medicine, St. Kitts, West Indies

CPS-1 is a subclass B3 metallo-β-lactamase from a Chryseobacterium piscium isolate collected from soil, showing 68% amino acid identity to the GOB-1 enzyme. CPS-1 was overproduced in Escherichia coli Rosetta (DE3), purified by chromatography, and biochemically characterized. This enzyme exhibits a broad-spectrum substrate profile, including penicillins, cephalosporins, and carbapenems, which overall resembles those of L1, GOB-1, and acquired subclass B3 enzymes AIM-1 and SMB-1.

Metallo-β-lactamases (MBLs) are among the most clinically relevant β-lactamases because of their broad-spectrum activity against most β-lactams, including carbapenems, and lack of susceptibility to β-lactamase inhibitors available for clinical use (e.g., clavulanate, sulbactam, tazobactam, and avibactam) (1). MBLs require a metal cofactor for β-lactam hydrolysis and are inhibited by EDTA (2, 3). They are classified functionally as group /H9252

-streptococcus, and biochemical properties (11, 12). Genus Chryseobacterium comprises species living in the environment that can occasionally behave as opportunistic pathogens (9). Some species of this genus, such as Chryseobacterium gleum and Chryseobacterium indologenes, have been shown to produce MBLs as resident enzymes. For instance, C. gleum produces CGB-1, a subclass B1 MBL presenting low affinity for carbapenems (10), while C. indologenes produces IND-type (IND-1 to IND-15) subclass B1 MBLs exhibiting heterogeneous structural and biochemical properties (11, 12).

We recently discovered CPS-1 (GenBank accession number AJP77054.1), a new subclass B3 MBL from a Chryseobacterium piscium strain (Stok-1) isolated from soil in Warwickshire, United Kingdom (13). In this article, we report the structural features and biochemical properties of CPS-1 compared to those of previously described MBLs and of putative MBLs encoded by genomes of Chryseobacterium species available in the Integrated Microbial Genomes database.

CPS-1 shared the highest amino acid (aa) identity with putative MBLs detected in Chryseobacterium caeni (81%) (here referred to as CPS-2; GenBank accession number WP_027382699.1) and Chryseobacterium formosense (80%) (here referred to as CPS-3; GenBank accession number KF00120.1) and with the GOB-1 MBL from Elizabethkingia meningoseptica, formerly Chryseobacterium meningosepticum (68%) (14). CPS-1 appeared to be more distantly related to other subclass B3 enzymes, including FEZ-1 (35% aa identity) from Legionella (Fluoribacter) gormanii (15), BJP-1 (31% aa identity) from Bradyrhizobium japonicum (16), and L1 (25% aa identity) from Stenotrophomonas maltophilia (17), although it could be aligned with these enzymes without introducing major gaps (Fig. 1). Compared to GOB-1, 92-aa substitutions were detected in the CPS-1 enzyme, including Glu165Lys, His228Lys, and Met221Leu (BBL numbering scheme) (4). Amino acid residues spanning positions 156 to 166 (loop 1) and 220 to 230 (loop 2) are considered to cover the active site groove of subclass B3 enzymes (17, 18). Position 221 is critical for MBL structure and catalysis (19), and the Ser221Met substitution observed in GOB enzymes with respect to nearly all other subclass B3 enzymes has been shown to contribute to enzyme stability due to the hydrophobic nature of Met (19, 20). We hypothesize a similar role for the Leu residue at position 221 in CPS-1, being a Leu hydrophobic amino acid. Similar to CPS-1, CPS-2 and CPS-3 also displayed Met and Leu, respectively, at position 221, indicating that both substitutions can occur among CPS-like enzymes.

The blaCPS-1 open reading frame (ORF) was amplified from C. piscium Stok-1 genomic DNA with primers containing Ndel (CPS-1F, 5′-GGGATATGAGAAACCTGACACTTTT-3′) and BamHI (CPS-1R, 5′-GGGATCTTATTTTTGCCTGAACTCT-3′) restriction sites (underlined). The Ndel-BamHI-digested blaCPS-1 ORF was cloned into the corresponding sites in the pET-9a expression vector (Merck Millipore, Germany) to produce the recombinant plasmid pET-CPS-1. The cloned insert was subjected to confirmatory sequencing (Macrogen, Republic of Korea) to exclude the presence of mutations introduced during the PCR. Escherichia coli Rosetta (DE3) cells (Merck Millipore, Germany) were transformed with pET-CPS-1 by electroporation.

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Address correspondence to Luca Guardabassi, lg@sund.ku.dk.

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FIG 1 Amino acid alignment of CPS-1 (GenBank accession number AJP77054.1), CPS-2 (GenBank accession number WP_027382699.1), CPS-3 (GenBank accession number KFF00120.1), GOB-1 (GenBank accession number AAF04458), BJP-1 (NP_772870), AIM-1 (GenBank accession number AM998375), and SMB-1 (GenBank accession number AB636283) with the secondary structure of FEZ-1 (GenBank accession number CAB96921). Stars, metal binding residues; triangle, position 221; boxes, residue differences between CPS-1 and GOB-1; broken lines, loops spanning the active site groove of subclass B3 MBLs. The figure was made by using ESPrint (29).
TABLE 1 Kinetic parameters of purified CPS-1 enzyme for the hydrolysis of different β-lactams, in comparison with those reported in the scientific literature for other subclass B3 metallo-β-lactamases (MBLs)

<table>
<thead>
<tr>
<th>β-Lactam substrate</th>
<th>CPS-1</th>
<th>k&lt;sub&gt;cat/K&lt;sub&gt;M&lt;/sub&gt; (M&lt;sup&gt;-1&lt;/sup&gt;·s&lt;sup&gt;-1&lt;/sup&gt;) for subclass B3 metallo-β-lactamase&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzylpenicillin</td>
<td>1,400</td>
<td>1.3 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>3,000</td>
<td>2.5 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ticaricillin</td>
<td>&gt;700</td>
<td>5.2 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Temocillin</td>
<td>&gt;8</td>
<td>9.0 × 10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>63 ± 2</td>
<td>1.3 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>20 ± 0.8</td>
<td>2.5 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>46 ± 1</td>
<td>9.8 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>170 ± 5</td>
<td>1.5 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>&gt;80</td>
<td>7.6 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>130 ± 10</td>
<td>8.5 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cefepime</td>
<td>151 ± 1</td>
<td>2.0 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Imipenem</td>
<td>184 ± 32</td>
<td>2.0 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Meropenem</td>
<td>180 ± 7</td>
<td>5.3 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>62 ± 2</td>
<td>8.6 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Doripenem</td>
<td>300 ± 8</td>
<td>6.7 × 10&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&lt;0.08</td>
<td>ND</td>
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</tbody>
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<tr>
<th>k&lt;sub&gt;cat/K&lt;sub&gt;M&lt;/sub&gt; (M&lt;sup&gt;-1&lt;/sup&gt;·s&lt;sup&gt;-1&lt;/sup&gt;) for subclass B3 metallo-β-lactamase&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOB-1</td>
</tr>
<tr>
<td>FEZ-1</td>
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<td>BIP-1</td>
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<tr>
<td>L1</td>
</tr>
<tr>
<td>AIM-1</td>
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<tr>
<td>SMB-1</td>
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</tbody>
</table>

<sup>a</sup> GOB-1, FEZ-1, BIP-1, and L1 are resident MBLs produced by *Pseudomonas aeruginosa* (14), *Legionella gormanii* (15), *Braundizium japonicum* (16), and *Stenotrophomonas maltophilia* (23, 24), respectively. AIM-1 and SMB-1 are acquired subclass B3 metallo-β-lactamases produced by *Pseudomonas acruginosa* (25) and *Serratia marcescens* (26) clinical isolates, respectively. ND, data not determined.

<sup>b</sup> data not available.

<sup>c</sup> K<sub>M</sub> was determined as an inhibition constant (K<sub>i</sub>) by using 145 μM imipenem as reporter substrate.
taint type of β-lactam substrate. For example, GOB-1 hydrolyzes 0.14 μg/ml of cefotaxime and meropenem better than imipenem (14), FEZ-1 hydrolyzes ceph- alosporins better than penicillins (15), and BIP-1 prefers narrow-spectrum cephalosporins over penicillins (16). A broad-spectrum substrate profile is a characteristic feature of the subclass B3 MBLs known to date, namely, AIM-1 detected in Pseudomonas aeruginosa isolates (25) and SMB-1 detected in a Serratia marcescens isolate (26). In these enzymes, recognition of β-lactam substrates is likely mediated by the presence of Gln157 in loop 2 (18, 27). AIM-1 hydrolyzes benzylpenicillin, most cephalosporins (cephalothin, cefotaxime, cefturoxime), and imipenem with a catalytic efficiency 1 order of magnitude higher than that of CPS-1, while SMB-1 has catalytic efficiencies comparable to that of CPS-1 for most substrates except for ceftazidime and cefepime, which are hydrolyzed less efficiently by SMB-1 than by CPS-1 (26). The high catalytic efficiency of CPS-1 for ampicillin, cefoxitin, and ceftazidime may account for the high MIC values observed in recombinant E. coli TOP10 expressing blaCPS-1 from the pZ21MCS vector (64, 64, and 4 μg/ml, respectively) (13). However, the MICs of cefotaxime and meropenem were low (0.5 and 0.094 μg/ml, respectively) despite the high catalytic efficiency observed for these substrates. Apparent discordance between catalytic efficiency and MIC values of different β-lactams has been observed for other subclass B3 MBLs in E. coli laboratory strains (28).

In conclusion, CPS-1 is a new member of subclass B3 MBLs with broad substrate specificity, as it is able to efficiently hydrolyze penicillins, cephalosporins, and carbapenems of clinical importance. The broad-spectrum profile of CPS-1 resembles the catalytic efficiencies of AIM-1 and SMB-1, even though CPS-1 is distantly related to these subclass B3 MBLs based on sequence homology.

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