Diversity of naturally occurring Ambler class B metallo-β-lactamases in Erythrobacter spp.

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Objectives: In silico analysis identified a metallo-β-lactamase (MBL) in Erythrobacter litoralis HTCC2594, sharing 55% amino acid identity with NDM-1. The aim of this work was to characterize the chromosomally encoded MBLs from several Erythrobacter spp. that may represent potential reservoirs of acquired MBLs.

Methods: Erythrobacter citreus, Erythrobacter flavus, Erythrobacter longus, Erythrobacter aquimarlis and Erythrobacter vulgaris were from the Pasteur Institute collection, France. DNA was extracted and used for shotgun cloning, and β-lactamases were expressed in Escherichia coli. MICs for resulting E. coli recombinant strains were determined by Etest. The deduced amino acid sequences were analysed and compared with BLASTP. Enzymatic activity of bacterial extracts from recombinant E. coli strains was determined by UV spectrophotometry with imipenem (100 μM) as substrate.

Results: Resulting E. coli recombinant strains harboured hypothetical MBL-encoding genes. MICs of β-lactams showed decreased susceptibility to carbapenems only for E. coli (pFLA-1) and E. coli (pLON-1), expressing the MBL from E. flavus and E. longus, respectively. MBLs from different Erythrobacter spp. shared weak amino acid identity, ranging from 45% to 75% identity. They differed greatly from that of E. litoralis HTCC2594 (and NDM-1), sharing only 11%–23% identity. Enzymatic activity against imipenem was detectable but weak in all these recombinant E. coli strains, except E. coli (pFLA-1), in which specific activity was significantly higher.

Conclusions: Several chromosomally located MBLs have been identified from Erythrobacter spp. They share weak amino acid identity and are very weakly related to other acquired MBLs (10%–23%).

Keywords: zinc, carbapenemases, carbapenems, chromosomally encoded, subclass B

Introduction

Erythrobacter spp. are strict aerobic non-spore-forming and non-motile Gram-negative species that express an anoxygenic phototroph, bacteriochlorophyll a and carotenoids, which are responsible for its smooth red-orange colour.1,2 Erythrobacter spp. are most frequently found in coastal seawaters, where they are critical components in the cycling of both organic and inorganic carbon.3 Recently, an in silico analysis identified a gene encoding a putative metallo-β-lactamase (MBL) in Erythrobacter litoralis HTCC2594, sharing a weak amino acid identity (55% identity) with the acquired carbapenemase NDM-1.4 This result clearly indicated that this species was not the reservoir of blaNDM genes. Nevertheless, this prompted us to characterize the chromosomally encoded MBLs from several Erythrobacter spp. that may represent potential ancestors of acquired MBLs.

Methods

Erythrobacter spp. strains were from the Pasteur Institute collection (France) and included Erythrobacter citreus (CIP107092), Erythrobacter flavus (CIP108097), Erythrobacter longus (CIP104268), Erythrobacter aquimarlis (CIP108636) and Erythrobacter vulgaris (CIP108956). All strains were grown aerobically on Difco Marine Agar 2216 (BD Biosciences, Le Pont de Claix, France) for 24 h at 30°C (E. flavus and E. aquimarlis), 48 h at 30°C (E. longus and E. vulgaris) or 48 h at 25°C (E. citreus). Genomic DNA was extracted and used for shotgun cloning, using Sau3AI restriction digest and a pBK-CMV plasmid, as described previously,5 and β-lactamases were expressed in E. coli DH10B (Life Technologies, Cergy-Pontoise, France). The resulting recombinant E. coli DH10B p(ECM-1), E. coli DH10B p(EFM-1), E. coli DH10B p(ELM-1), E. coli DH10B p(EAM-1) and E. coli DH10B p(EVM-1), harbouring β-lactamase genes from E. citreus, E. flavus, E. longus, E. aquimarlis and E. vulgaris, respectively, were selected on amoxicillin (50 mg/L) and kanamycin (30 mg/L) trypticase soy agar.
 MICs for the recombinant E. coli DH10B strains of various β-lactams, including piperacillin, cephalosporins, carbapenems and aztreonam, were determined by Etest (AB bioMérieux, Solna, Sweden), and by the broth microdilution method for cefalotin, cefepime and cefoxitin, and were interpreted according to CLSI guidelines.\(^6\)

Plasmids were sequenced by using combinations of universal T3 and T7 primers and a primer-walking approach on an ABI 3130 sequencer (Applied Biosystems, Les Ulis, France). The nucleotide and the deduced protein sequences were analysed with software available at the National Center of Biotechnology Information web site (http://www.ncbi.nlm.nih.gov). Multiple nucleotide and protein sequence alignments were carried out online by using the ClustalW program (http://www.genome.jp/tools/clustalw/).

Carbapenemase activity of bacterial extracts was measured by UV spectrophotometry with imipenem (100 \(\mu\)M) as substrate, as described previously,\(^7\) in 50 \(\mu\)M ZnSO\(_4\) and 0.1 M phosphate buffer (pH 7.0) at 30°C.

### Nucleotide sequence accession number

The sequences corresponding to the β-lactamase genes described in this work were assigned the GenBank accession numbers JN558587 to JN558591.

### Results and discussion

The recombinant E. coli DH10B strains contained short inserts (1172–2336 bp). Sequencing of the inserts of the E. coli recombinant strains identified genes encoding MBLs. When expressed in E. coli, these β-lactamases conferred resistance or reduced susceptibility to piperacillin and broad-spectrum cephalosporins (Table 1). MICs of carbapenems were low for most of them, except for E. coli DH10B p(EFM-1) and E. coli DH10B p(ELM-1), expressing the MBL from E. flavus and from E. longus, respectively. These exhibited MICs of imipenem of 1.5 and 4 mg/L, respectively (Table 1), similarly to what was observed for \(\text{bla}_{\text{IMP}}\) or \(\text{bla}_{\text{VIM}}\) genes expressed in E. coli.\(^7\,^8\)

MBLs from these different Erythrobacter spp. possessed a zinc binding motif, H(Q)XHXDH (residues 116–121), and H196 and H263, which were well conserved in MBLs belonging to the subclass B3 MBL group\(^9\) (Figure 1) and shared weak amino acid identity (45%–75% identity) (Table S1, available as Supplementary data at JAC Online). The highest amino acid identity was 75%, between EFM-1 from E. flavus and ECM-1 from E. citreus (Table S1, available as Supplementary data at JAC Online and Figure 2). However, the MBL sequences identified in this study differed significantly from the previously identified MBL of subclass B1 from E. litoralis HTCC2594 (YP_458405), with only 15% amino acid identity (Table S1, available as Supplementary data at JAC Online). Notably, these sequences also differed significantly from those of plasmid-mediated MBLs IMP-1,\(^7\) VIM-1\(^8\) and NDM-1\(^10\) (Table S1, available as Supplementary data at JAC Online and Figure 1).

A phylogenetic tree constructed with these amino acid sequences showed great diversity, and showed that none was related to acquired MBLs such as NDM-1, VIM-1 or IMP-1 (Figure 2).

Imipenem hydrolysis was detectable but at a low level for all recombinant E. coli strains, except for E. coli DH10B p(EFM-1), for which specific activity was significantly higher (90 nmol/min/mg of protein) (Table 1).

| Source, Erythrobacter sp. | MBL production\(^a\) | MIC (mg/L) of \(\beta\)-lactam | IPM | MEM | ETP | PIP | CEF | AZT | CTX | CAZ | FOX | FEP |
|--------------------------|---------------------|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| E. coli DH10B p(EAM-1)    | 3                   | 0.12                          | 11  | 0.5 | 0.19 | 0.25 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| E. aquimaris p(EFM-1)     | 90                  | 0.38                          | 10  | 0.38 | 0.38 | 0.38 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| E. flavus p(ELM-1)        | 7                   | 1.5                           | 7   | 1.5 | 1.5 | 1.5 | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  |

\(\text{AZT, aztreonam; CAZ, ceftazidime; CEF, cefalotin; CTX, cefotaxime; ETP, ertapenem; FEP, cefepime; FOX, cefoxitin; IPM, imipenem; MEM, meropenem; PIP, piperacillin.}\)

\(\text{aMBL activity is shown as nmol of imipenem hydrolysed per min per mg of protein. Data are the means of three replicate samples (the standard deviation was always <10%); in all cases, the activity was inhibited by at least 80% in the presence of EDTA (1 mM). Carbapenemase activity measured with the negative control (E. coli DH10B) yielded a value of 3 nmol/min/mg of protein, corresponding to imipenem degradation.}\)
Sequencing of the region flanking the cloned β-lactamase genes always identified an upstream gene encoding a putative 3-oxoacyl-(acyl-carrier-protein) reductase sharing 65%–85% amino acid identity with that from *Erythrobacter* sp. SD-21 (GenBank accession number EDL50470), and always identified a downstream gene encoding a putative ribosomal protein L11 methylase sharing 48%–89% amino acid identity with that from *Erythrobacter* sp. SD-21 (GenBank accession number EDL50472).

**Conclusions**

*Erythrobacter* spp. harbour chromosomally encoded MBLs showing a large diversity of amino acid sequences, and belonging to subclasses B1, B2 and B3.

**Figure 1.** Amino acid alignments of MBLs from *Erythrobacter* spp. with those of subclasses B1, B2 and B3. The sequences of naturally occurring MBLs from *Erythrobacter* spp. in this study [ELM-1 from *E. longus* (GenBank accession no. JN558589), EFM-1 from *E. flavus* (JN558587), ECM-1 from *E. citreus* (JN558590), EVM-1 from *E. vulgaris* (JN558589) and EAM-1 from *E. aquimaris* (JN558591)] were compared with MBLs from other species such as AIM-1 from *Pseudomonas aeruginosa* (CAQ53840), Bla2 from *E. litoralis* HTCC2594 (YP_458405), 3CphA from *Aeromonas hydrophila* (X57102), GOB-1 from *Elizabethkingia meningoseptica* (Q9RB00), L-1 from *Stenotrophomonas maltophilia* (Q9RBQ3), POM-1 from *Pseudomonas otitidis* (EU315252), SMB-1 from *Serratia marcescens* (AB636283), THIN-B from *Janthinobacterium lividum* (Q9AEF9) and plasmid-mediated MBLs NDM-1 (C7C422), VIM-1 (CAB46686) and IMP-1 (P52699). The residues conserved among subclass B3 are highlighted in grey, and the residues involved in zinc binding are boxed in black squares.

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to subclasses B1 or B3. Surprisingly, only few of them exhibited significant carbapenemase activity. None of these species could be considered a progenitor of any of the plasmid-mediated carbapenemases disseminating worldwide.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org).

References