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Sequence Analysis of ARI-1, a Novel OXA \( \beta \)-Lactamase, Responsible for Imipenem Resistance in *Acinetobacter baumannii* 6B92

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The sequence of the *bla*_{ARI-1} gene from imipenem-resistant *Acinetobacter baumannii* 6B92 has been determined. The structural gene encodes a 273-amino-acid protein which is most related to the OXA class D \( \beta \)-lactamases. The conserved S-T-F-K and K-T-G motifs were identified in the ARI-1 protein sequence, also named OXA-23, but significantly, a point mutation (Y→F) was identified in the Y-G-N conserved motif, also known to function in the active site.

Multiresistant *Acinetobacter baumannii* strains are now recognized as serious nosocomial pathogens (4, 5), and carbapenem-resistant strains are being reported increasingly (1, 3, 6, 11, 15, 18, 23). Imipenem-resistant *A. baumannii* 6B92 was isolated from a patient in Edinburgh, United Kingdom, in 1985 (15). Imipenem resistance was attributed to a novel serine \( \beta \)-lactamase, ARI-1 (15), and was subsequently demonstrated to be transferable to *Acinetobacter junii* (18). Imipenem resistance due to \( \beta \)-lactamases in *A. baumannii* has subsequently been reported worldwide, and two additional \( \beta \)-lactamases, ARI-2 (6) and an oxacillin-hydrolyzing enzyme (2, 11), defined by their biochemical properties, have been described. In this paper we report the nucleotide and deduced amino acid sequences of ARI-1 carried by the R plasmid pUK1356.

**Bacterial strains and plasmids.** The transconjugant *A. junii* BD413-2(pUK1356) was used as the source of ARI-1. *Escherichia coli* TG2 (**supE** hsdSΔ5 thi Δ(lac-proAB) Δ(srl-recA) 306::Tn10(ter) F* ΔlacD36 proAB + lacF* ΔlacZAM15) (17) was used as a host for recombinant plasmids prepared in the vector pUC19 (24).

**\( \beta \)-Lactamase purification and N-terminal amino acid sequencing.** Cell extracts of *A. junii* BD413-2(pUK1356) were loaded onto a Mono Q anion-exchange column (Pharmacia Biotech UK Ltd., Amersham, United Kingdom) containing X-Gal (5-bromo-4-chloro-3-indolyl-\( \beta \)-D-galactopyranoside; 32.5 mg/liter), IPTG (isopropyl-\( \beta \)-D-thiogalactopyranoside; 7.8 mg/liter), and ampicillin (50 mg/liter; Sigma Aldrich Co. Ltd., Poole, United Kingdom) containing 0.1 to 0.5 mM Tris-HCl (pH 8.8). Fractions containing \( \beta \)-lactamase activity, detected by an assay with nitrocefin, were retained. Fractions containing \( \beta \)-lactamase activity, defined only by their biochemical properties, have been described. In this study we report the nucleotide and deduced amino acid sequences of ARI-1 carried by the R plasmid pUK1356.

**Expression of the ARI-1 gene.** Enhanced chemiluminescence probe labelling, hybridization, and detection kits (Amersham Pharmacia Biotech UK Ltd., Amersham, United Kingdom) were used in accordance with the manufacturer’s instructions. Plasmid pUK1356 was extracted, restricted, and cloned with the procedures described by Sambrock et al. (17). Recombinant clones were selected on nutrient agar (IDG Ltd., Bury, United Kingdom) containing X-Gal (5-bromo-4-chloro-3-indolyl-\( \beta \)-D-galactopyranoside; 32.5 mg/liter), IPTG (isopropyl-\( \beta \)-D-thiogalactopyranoside; 7.8 mg/liter), and ampicillin (50 mg/liter; Sigma Aldrich Co. Ltd., Poole, United Kingdom).

**Hybridization and identification of the 5’ end of the ARI-1 gene.** Recombinant plasmid from a single positive clone was purified with a Qiagen (Crawley, United Kingdom) plasmid minikit and sequenced with an ABI PRISM 377 automated DNA sequencer (PE Applied Biosystems). The sequence (Fig. 1, nucleotides 1 to 1345) revealed 375 bp of the 5’ end of the *bla*_{ARI-1} gene.

**PCR amplification and complete sequencing of the ARI-1 gene.** PCR products were generated in 100-μl volumes containing 16 mM (NH₄)₂SO₄, 67 mM Tris-HCl (pH 8.8), 0.1% Tween 20 buffer, 2.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphates (Amersham Pharmacia Biotech UK Ltd.), 0.1 to 0.5 μM each primer, and 1 U of BIOTAQ polymerase (Bioline UK Ltd., London, United Kingdom) or 1.25 U of *Pfu* DNA polymerase (Promega UK Ltd., Southampton, United Kingdom). Template DNA was boiled for 10 min before being added to the reaction mixture. DNA amplification was performed in an Omn-E thermal cycler (Hybaid Ltd., Teddington, United Kingdom) under the following cycle conditions: 94°C for 5 min, 60°C for 1 min, 72°C for 2 min (one cycle); 94°C for 15 s, 60°C for 1 min, 72°C for 2 min (30 cycles); and a final extension of 72°C for 5 min (one cycle). PCR products containing the 3’ end of the ARI-1 gene were obtained by inverse PCR of HindIII or Sau3A fragments of pUK1356 by using the primer pairs P1-P2 and Invrs2-Invrs3 (Table 1). PCR products comprising the complete sequence of the *bla*_{ARI-1} gene were generated by using the primer pair P5-P6 (Table 1). The ARI-1 gene se-
sequence was determined from both strands with PCR products from three independent reactions, at least one of which was generated with *Pfu* DNA polymerase, being sequenced routinely.

**Table 1. Oligonucleotides used in this study**

<table>
<thead>
<tr>
<th>Oligonucleotide primer</th>
<th>Application</th>
<th>Sequence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Positions in nucleotide sequence shown in Fig. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARI-N</td>
<td>DNA hybridization</td>
<td>5'-ACG CTG ACG CTA GAA AAT TAA AAT AAT GA-3'</td>
<td>1026-1051</td>
</tr>
<tr>
<td>P1</td>
<td>PCR and sequencing</td>
<td>5'-TGG ACA ATC TGA CTC GGC CT-3'</td>
<td>1072-1053 (complement)</td>
</tr>
<tr>
<td>P2</td>
<td>PCR and sequencing</td>
<td>5'-TGG AGA ACC AGA AAA CGG AT-3'</td>
<td>1240-1259</td>
</tr>
<tr>
<td>Invrs2</td>
<td>PCR and sequencing</td>
<td>5'-TTT TGG AAA GAC TGG TTT TG-3'</td>
<td>1610-1629</td>
</tr>
<tr>
<td>Invrs3</td>
<td>PCR and sequencing</td>
<td>5'-CTG CTG TCG AAT TAC AGC AT-3'</td>
<td>1451-1432 (complement)</td>
</tr>
<tr>
<td>Invrs1</td>
<td>Sequencing</td>
<td>5'-TGG TGA GAT CAA GAC CG-3'</td>
<td>1404-1385 (complement)</td>
</tr>
<tr>
<td>P5</td>
<td>PCR and sequencing</td>
<td>5'-AAG CAT GAT GAG CGC AAA A-3'</td>
<td>785-803</td>
</tr>
<tr>
<td>P6</td>
<td>PCR and sequencing</td>
<td>5'-AAA AGG CCC ATT TAC ATG AACA-3'</td>
<td>1850-1830 (complement)</td>
</tr>
</tbody>
</table>

<sup>a</sup> All oligonucleotides were purchased from Life Technologies Ltd.

<sup>b</sup> I, inosine; R, any purine (A or G); Y, any pyrimidine (T or C).

Sequence data analysis, alignment, and phylogeny. For computer analysis of sequence data, the software from the BCM Search Launcher was used (21). The *bla*~ARI-1~ gene comprises an 822-bp open reading frame, between the initiation

![FIG. 1. Nucleotide and deduced amino acid sequences of the ARI-1 gene and the ARI-1 protein from A. baumannii 6B92. The boldface ATG and TAA represent the initiation and termination codons, respectively. The β-lactamase active site S-T-F-K and the conserved motifs F-G-N and K-T-G are shown in boldface type. A proposed cleavage site generating a possible signal sequence is indicated with a vertical arrow. Nucleotide sequence in italics shows 90% homology with the *phaB*~Ac~ upstream region (19). ● indicates the transcription start site identified by Schembri et al. (20). An imperfect inverted repeat sequence at the 3' end of the gene is shown by a broken line, and possible cassette junctions are shown boxed.](image-url)
FIG. 2. Cladogram relating ARI-1 to 18 other class D β-lactamas.

Analysis of the genetic environment of blalarii. The majority of oxa genes are contained within mobile cassettes (13, 14, 16) normally found inserted within integrons (10). The sequence immediately upstream of the blalarii structural gene did not reveal cassette characteristics (i.e., consensus core sequence GTTRRRY). Nevertheless, a large region of 944 bases showing more than 90% homology with the phaBAc upstream region previously identified in the chromosome and plasmids of Acinetobacter strains isolated from sludge (17) was found. Schembri et al. (20) have shown a transcription start point within this region (position 920 in Fig. 1). This may indicate that the blalarii gene has been specifically inserted downstream of an active promoter, enabling low-level constitutive expression of the gene in A. baumannii. Analysis of the region immediately downstream of blalarii revealed an imperfect inverted repeat sequence with some of the characteristics of the 59-bp recombination sites found in cassette structures (16). Although characteristic of gene cassettes, it is possible that this sequence is functioning as a transcription terminator sequence since there was no evidence of an inverse core sequence (consensus sequence RYYYAAC). Furthermore, continuation of the published pha operon sequence could not be identified. Despite the apparent lack of evidence of an integron or cassette location for the blalarii gene, two GTTA sequences (boxed in Fig. 1) were identified and may define the ends of a potential novel and unusual cassette structure.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the EMBL nucleotide database under accession no. AJ132105.

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REFERENCES


