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Molecular Analysis of the Sequences Surrounding bla_{OXA-45} Reveals Acquisition of This Gene by *Pseudomonas aeruginosa* via a Novel ISCR Element, ISCR5^{∇}

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The bla_{OXA-45} gene of *Pseudomonas aeruginosa* 07-406 is driven by a promoter found within a truncated IS*Pme1* insertion sequence. The gene is located between nonidentical repeats of a new IS*CR* element, IS*CR5*, which is likely responsible for its acquisition. Sequence analysis indicates that IS*CR5* is a chimera of IS*CR3* and IS*CR16*.

 bla_{OXA-45} , a gene encoding a class 2d' β -lactamase, was initially characterized from a *Pseudomonas aeruginosa* isolate 07-406 (12, 13).

A number of genetic mechanisms that enable resistance genes to be acquired by bacteria have been reported. These genetic mechanisms include plasmids, transposons, integrons, classical insertion sequences (IS), and ISCR elements (2, 4, 11).

ISCR elements are an unusual group of insertion sequences that have similarities to the IS91 family of insertion sequences (10, 11). They differ considerably from classical insertion sequences in both their structure and transposition mechanism (5, 6), and studies on the IS91 family have demonstrated that these elements can cotranspose DNA adjacent to their terminal terIS sequence, mediated by a single copy of the element (so-called one-ended transposition) (8, 9). At present, the ISCR family comprises 19 members that vary in identity between 18 and 96% (http://www.cardiff.ac.uk/medic/aboutus/departments /medicalmicrobiology/genetics/iscr/iscr_elements.html). They are found adjacent to a wide variety of antibiotic resistance genes of foreign origin and are thus implicated in their acquisition (11).

P. aeruginosa strains 07-406 and 07-408 were isolated in 2001 from two patients via the CANCER Antimicrobial Surveillance Program (12, 13). Escherichia coli strain DH5a recA1 (3) was used for library construction as described previously (13), and clones that included bla_{OXA-45} were selected by growth on plates containing ceftazidime (50 µg/ ml) plus 50 mM EDTA. EDTA was included to exclude clones containing the metallo- β -lactamase gene bla_{VIM-7} , a gene also found in this strain (12). The bla_{OXA-45} locus was constructed from multiple clones sequenced on both strands using custom-designed primers, and the 7,018-bp bla_{OXA-45} locus has been deposited in the EMBL database under the accession number AM849110. The sequence included 2,557 bp of upstream DNA and 3,671 bp of downstream DNA flanking the bla_{OXA-45} structural gene. Sequence analysis revealed that immediately upstream of the bla_{OXA-45} gene is a truncated copy of a classical insertion sequence that displays 99% identity to the insertion sequence ISPme1 from Xanthobacter autotrophicus (15). This element was located 19 bp upstream from the start codon of bla_{OXA-45} and consists of 369 bp of the right-hand end encoding the last 48



FIG. 1. Schematic of the genetic locus of bla_{OXA-45} . ORFs are depicted as boxes with arrows indicating the direction of transcription of the ORF. The truncated ISPme1 element is depicted as a checkered box indicating the truncated ORF together with vertical parallel lines representing the right-hand end inverted repeat sequence. The promoter driving the transcription of bla_{OXA-45} is drawn as a bent horizontal arrow. Solid black boxes indicate the repeated 376-bp section of DNA found upstream of both ISCR5 elements. The *ori*IS of ISCR5A and ISCR5B are depicted as dotted vertical lines found downstream of the ISCR5 ORFs.

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ISCR5A

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FIG. 2. Alignment of transposases of ISCR elements displaying the highest identity to ISCR5A and ISCR5B. Residues found in the majority of sequences are shown on a gray background. The residues that are found conserved in all IS91 family transposases are indicated by black stars above the sequences. Two key residues that differ from those in the transposases of the IS91 family are indicated by open circles above the sequences. The first residue, Q85 in the ISCR5 sequence, is a histidine residue in all IS91 family elements, and the lysine residue K333 (ISCR5) is a tyrosine in all IS91 family elements and an arginine in ISCR1, ISCR2, and ISCR4. (see reference 11 for a discussion of these differences). Gaps introduced to maximize alignment of the sequences are indicated by the dashes.

amino acids of the transposase gene together with the inverted repeat sequence. As 19 bp is too short to contain promoter sequences, the promoter driving bla_{oxa-45} expression must be located within this IS.

This IS/bla_{OXA-45} section is sandwiched between tandem repeats of approximately 2,100 bp (Fig. 1). These include two nearly identical open reading frames (ORFs), encoding putative proteins of 509 amino acids. Comparison of these two ORFs revealed 25 single base pair differences. Most of these nucleotide polymorphisms are silent, but seven account for amino acid differences between the putative encoded proteins (Fig. 2). Searches via the protein databases at EMBL-EBI (FASTA protein similarity search at http://www.ebi.ac.uk/fasta33) revealed high identity with the ISCR transposase ORFs ISCR16 (14), ISCR3, ISCR4, and ISCR1 with identities of 92%, 91%, 78%, and 51%, respectively. Alignment of these various protein sequences (Fig. 2) reveals the large sections of identity and the key motifs conserved among ISCR transposases. The DNA sequences found on either side of these putative ISCR transposase ORFs also display high identity to the corresponding *ter*IS and *ori*IS of IS*CR16*, IS*CR3*, and IS*CR4* (Fig. 3). Therefore, the bla_{OXA-45} structural gene is flanked by nearly identical copies of a new IS*CR* element, IS*CR5*, and these copies will hereafter be referred to as IS*CR5A* and IS*CR5B*.

A 376-bp section of unidentified DNA is found 5' adjacent to both copies of ISCR5 (Fig. 1). This is different from ISCR3, ISCR4, and ISCR16, which all have groEL sequences in the same position (14). Curiously, the oriIS end of the ISCR5 elements displays higher identity (98%) to the respective end of ISCR16 than to the same section of ISCR3 (80%) (Fig. 3). This same difference is seen in the ISCR5 transposase, in that the sequence coding for the N-terminal section of the ISCR5 transposase displays higher identity to ISCR3 than to ISCR16 (Fig. 2), and the sequence coding for the carboxy terminus of the protein displays the opposite, i.e., higher identity to ISCR16 than to ISCR3. This observation is consistent with a homologous recombination event between ISCR3 and ISCR16-like elements resulting in the chimeric element ISCR5. Further upstream of ISCR5B is an additional 1,400 bp of sequence that displays identity to



FIG. 3. (A) Alignment of the 5' DNA sequences of ISCR elements displaying the highest identity to ISCR5. Nucleotides that are identical to those in the sequence of ISCR3 are shown on a gray background. The GTG start codons of the various ISCR element transposase genes are indicated by asterisks, and nucleotides are numbered upstream starting at this codon. Sequences were collected from the following data banks and accession numbers: GenBank AF261825 (ISCR3), CP000604 (ISCR16), GenBank AY341249 (ISCR4), and AM849110 (ISCR5B [this study]). The ISCR5 5'-terminal sequence displays 100% identity to the respective sequence from ISCR3 until 98 bp upstream of the start codon where identity to ISCR3 and other elements is abruptly lost. The same sequence displays 86% identity to the equivalent sequence from ISCR16. A short 4-bp inverted repeat sequence at this position is indicated by inverted arrows above the nucleotide sequence which is similar to that found in the *ter*IS of IS91. (B) Alignment of the 3'-terminal DNA sequences of ISCR elements displaying the highest identity to ISCR5. Nucleotides that are identical to those in the sequence of ISCR5 are shown on a gray background. The stop codons of ISCR5, ISCR16, and ISCR3 transposase genes are indicated by black stars, and the *ori*IS sequence from ISCR 1. The ISCR5 3'-terminal sequence displays the highest identity to the extreme terminus of the various ISCR 4. The various ISCR 4. The vertical arrow indicates the junction between the ISCR 4. The store of ISCR5 3'-terminal sequence displays the highest identity (98%) to ISCR16 but only 80% identity to the respective sequence from ISCR3.

hypothetical protein-encoding regions found in several genome-sequencing projects (for example, 68% and 77% identity over ~500 bp to two hypothetical proteins found in *Stenotrophomonas maltophilia* genome sequence CP00111 and 67% identity over ~500 bp to a hypothetical protein from *Cellvibrio japonicus* Ueda 107 genome sequence CP000934).

The copy number and genomic location of bla_{OXA-45} were determined by preparation and digestion of genomic DNA in agar plugs and separated using pulsed-field gel electrophoresis as described previously (7). The plugs were digested with enzymes SpeI, I-Ceu1, and S1. S1 is known to nick single-stranded DNA and is used to unwind closed circular DNA so that plasmids run true to size in agarose gels (1). Hybridization of the bla_{OXA-45} probe to DNA of high molecular weight (>1 Mb) from S1- and I-Ceu1-digested DNA indicates that bla_{OXA-45} is chromosomally en-

coded (Fig. 4). SpeI digests show that the two isolates (isolates 07-406 and 07-408) are identical. However, probing of these digests indicated that the copy number of bla_{OXA-45} is greater in isolate 07-408 than in 07-406 and also that their chromosomal positions are different (Fig. 4). This is interesting because isolates 07-406 and 07-408 were collected in 2001 from separate patients in the same ward of the M. D. Anderson Cancer Center in Texas (12). This observation is consistent with replicative transposition events of ISCR5A comobilizing bla_{OXA-45} . It is also likely that these events are of recent origin, presumably occurring on transfer from one patient to another.

In summary, two ISCR5 elements are found flanking the bla_{OXA-45} gene in *P. aeruginosa* isolate 07-406. ISCR5A found upstream of the bla_{OXA-45} gene is implicated in the acquisition of this gene by *P. aeruginosa* by virtue both of its position and



FIG. 4. (A) Pulsed-field gel of genomic DNA from *P. aeruginosa* strains 07-406 and 07-408. Lane 1, lambda ladder pulsed-field gel markers; lanes 2, 4, and 6, strain 07-406 DNA digested with SpeI, I-Ceu1, and S1, respectively; lanes 3, 5, and 7, strain 07-408 DNA digested with SpeI, I-Ceu1, and S1, respectively. (B) Autoradiograph of the gel shown in panel A probed with bla_{OXA-45} .

structure. There are now 19 ISCR elements with distinct sequences, and the evidence that these elements are of central importance in the acquisition of resistance mechanisms by pathogenic bacteria is steadily increasing.

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