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Molecular Characterization of the OXA-7 β-Lactamase Gene

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The OXA-7 gene, which encodes an oxacillinase, was cloned from plasmid pMG202 of *Escherichia coli* isolate 7181 (A. A. Medeiros, M. Cohenford, and G. A. Jacoby, Antimicrob. Agents Chemother. 27:715–719, 1985) and sequenced. The nucleotide sequence of the OXA-7 gene was closely related to that of the OXA-10 (PSE-2) gene, with a derived amino acid sequence of the OXA-7 enzyme showing greater than 95% homology with those of OXA-10 and OXA-11.

The oxacillinases are plasmid-mediated enzymes which belong to the class D β-lactamases (3, 3a, 8). They hydrolyze oxacillin, methicillin, and cloxacillin and are inhibited by NaCl and clavulanic acid. Several members of this group are characterized by their substrate profile, isoelectric point, and reactions with inhibitors. Eight oxacillinases have already been sequenced: the OXA-10 (PSE-2) (12) and the OXA-11 (9) sequences were found to be completely identical except for two point mutations in the coding sequence. OXA-5 (7) shows 82% identity with the enzymes described above, and OXA-2 (8) shows 40% identity. The peptide sequences of OXA-1 (16), OXA-9 (22), LCR-1 (7), and the recently identified OXA-12 (18) show that, although they have the enzymatic characteristics of a typical oxacillinase, they are distantly related to the other OXAs. All of the oxacillinase genes identified until now, except OXA-11, are located on the variable region of the integrons (11, 21). The inserted genes are flanked in the 5′ site by the motif GTTPuPu and in its 3′ end by an imperfect inverted repeat of 59 bp (6, 10). These two motifs seem to be the *cis* elements necessary for the recombination of the resistance genes, a process which is catalyzed by the integrase (6, 14). This enzyme is encoded by the 5′-conserved region of the integron itself and mediates both the insertion into and the excision from the integron of the resistance genes. This mechanism explains how plasmids have accumulated such a diversity of resistance genes.

OXA-7 β-lactamase was detected by Medeiros et al. (15) in an ampicillin-resistant isolate of *Escherichia coli*. According to its substrate profile and isoelectric point, it was suggested that this enzyme belongs to class D β-lactamases. In this paper, the nucleotide sequence of the OXA-7 β-lactamase is reported and the structure of its genetic environment is explored.

Bacterial strains and plasmids. The transconjugant *E. coli* J53-2 (*met pro* Rif^r) (pMG202) (15) was used to clone the OXA-7 gene. *E. coli* HB101 (*hsd 20 recA13 ara-14 pro-A2 lacY1*

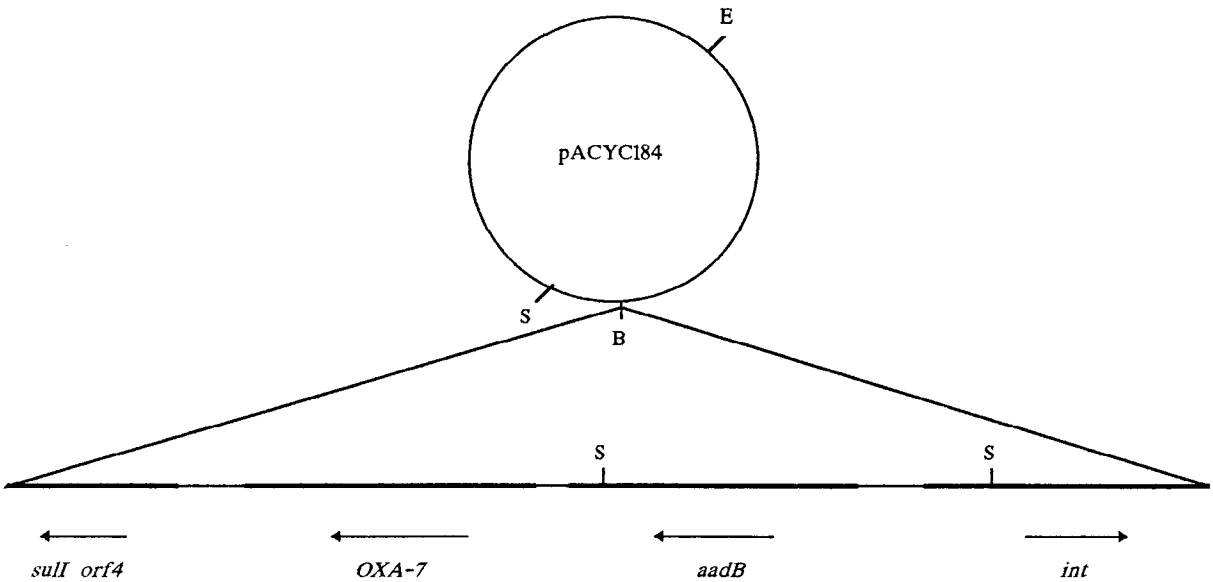

FIG. 1. Map of the clone pMLA4. The size of the insert is 4 kb. S represents the *Sph*I sites which delimit the fragments subcloned into pUC19. B and E represent the *Bam*HI and *Eco*RI sites, respectively, on the pACYC184 vector. *int* is the integrase gene. Arrows show the direction of transcription of the genes encoded by the insert.

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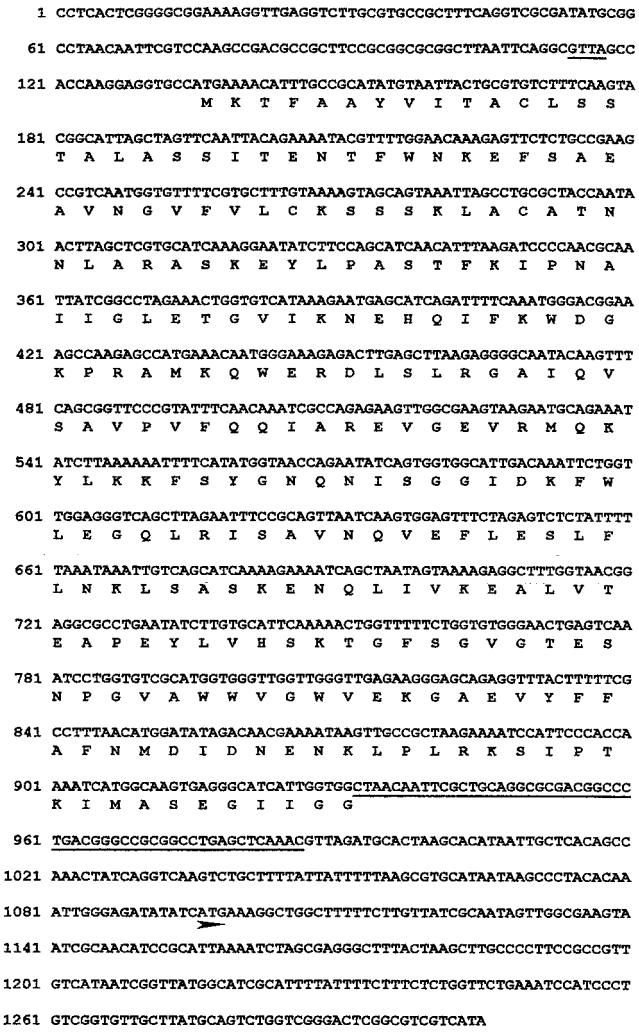

FIG. 2. Nucleotide sequence and deduced amino acid sequence of OXA-7. The GTTA motif on the 5′ end of the gene and the 59-bp element on the 3′ end of the gene are underlined. The arrowhead shows the start codon of open reading frame 4.

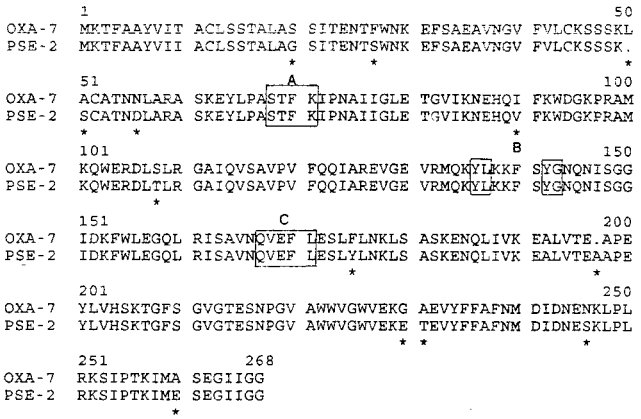

FIG. 3. Amino acid sequence alignment of OXA-7 and OXA-10. Differences in the amino acid sequences are indicated by asterisks. The underlined sequences correspond to the conserved boxes: the A box is the active serine site, and the B and C boxes indicate the sequences conserved between oxacillinases.

galK2 rpsL20 xyl-5 mtl-1 supE44 [2]) was used as a host for different plasmids. The plasmid pACYC184 (5), from New England Biolabs, Inc., was used as cloning vector, and pUC19 (23) was used for the subclonings and the sequencing.

Culture conditions. The transconjugant *E. coli* J53-2/pMG202 was grown in Luria-Bertani medium supplemented with ampicillin at 50 μg/ml. The transformed *E. coli* HB101 cells with the pACYC184 derivatives were grown in the presence of chloramphenicol at 50 μg/ml and ampicillin at 50 μg/ml.

Extraction of DNA. Total DNA was extracted from an overnight growth of J53-2/pMG202. Cells from a 100-ml culture were washed and then resuspended in SET buffer (20% sucrose, 50 mM Tris-Cl [pH 7.6], 50 mM EDTA). They were lysed in the presence of lysozyme and sodium dodecyl sulfate (SDS) at 0.5 mg/ml and 0.5% (final concentration), respectively, and treated with 0.5 mg of RNase A per ml. The lysate was phenol-chloroform extracted and precipitated with ethanol. The DNA was recovered with a glass rod and was resuspended in TEN buffer (10 mM Tris-Cl [pH 7.6], 1 mM EDTA, 10 mM NaCl).

Restriction enzymes and cloning techniques. The restriction enzymes were purchased from New England Biolabs, Inc., and they were used according to the manufacturer's indications. The plasmid purification was done with the Qiagen plasmid purification kit. The cloning procedures were performed as described by Sambrook et al. (19). Bacterial cells were made competent by the calcium chloride method (19). Selection of the transformants was done in ampicillin at 50 μg/ml and chloramphenicol at 50 μg/ml for the pACYC184 plasmid. The chloramphenicol was omitted when the bacteria were transformed with the pUC19 derivatives.

DNA sequencing and computer analysis. Double-stranded templates were subjected to nucleotide sequencing by the method of Sanger et al. (20) with the TaqTrack sequencing system (Promega) with ^{35}S-dATP (Amersham) as a label. Both strands were sequenced with either the M13 sequencing primers or the primers deduced from the newly sequenced DNA. The nucleotide and derived peptide sequences were analyzed with the Pileup program of the Genetics Computer Group package of computer programs, version 7 (1991 [Madison, Wis.]).

Cloning and sequencing of the OXA-7 gene. Total DNA from the transconjugant J53-2/pMG202 strain was partially digested with the restriction enzyme *Sau*3AI in order to obtain fragments ranging in length from 10,000 to 15,000 bp. The digested DNA was ligated to the *Bam*HI site of the pACYC184 vector, and the recombinants were transformed into HB101 competent cells. Transformants were detected on Luria-Bertani agar supplemented with 50 μg of chloramphenicol and 50 μg of ampicillin per ml. Two clones were able to grow on ampicillin plates. The pMLA4 clone contained a 4.0-kb insert, and the pMLA2 clone contained an 8.2-kb insert. Figure 1 shows the restriction map of the pMLA4 clone. The 1.3-kb *Sph*I fragments of pMLA4 and the 2.7-kb fragment delimited by the *Sph*I site of the pACYC184 vector and one *Sph*I site on the insert were subcloned in pUC19, and the resulting recombinants were sequenced. In the 2.7-kb insert of the subclone pESC24, the nucleotide sequence revealed one open reading frame at positions 135 to 935 which presented a strong homology with the nucleotide sequence of OXA-10. The nucleotide sequence of the open reading frame and the deduced amino acid sequence are shown in Fig. 2. The first 62 nucleotides of the insert were found to be 100% homologous with the 3′ end of the *aadB* gene, which codes for aminoglycoside adenyltrans-

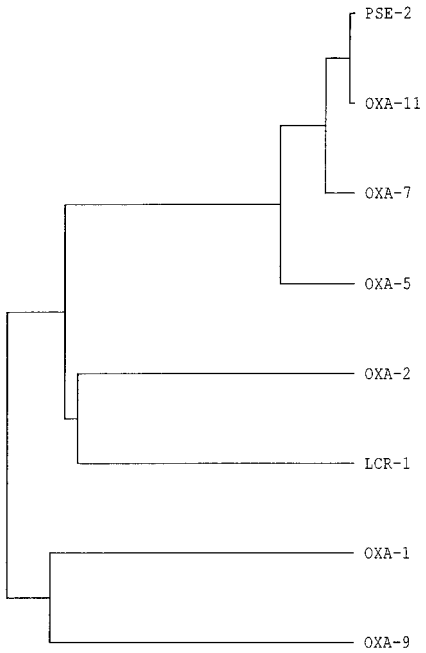

FIG. 4. Dendrogram obtained for the class D β-lactamases by the Pileup program of the Genetics Computer Group package, version 7 (1991).

ferase, an enzyme that confers resistance to gentamicin (4). The 3′ constant region of the integron containing open reading frame 4 (17) and the 5′ end of the *sul*I gene (not shown in Fig. 2) (1) start at position 987. The two clones analyzed here did not permit us to map the *sul*I gene further downstream. The 1.3-kb *Sph*I of the clone pMLA4 fragment was partially sequenced and shown to contain the 5′ portion of the *aadB* gene and the 3′ end of the integrase gene (Fig. 1).

Nucleotide and peptide sequence alignment. The nucleotide sequence and the peptide sequence of OXA-7 were compared with those of known oxacillinases. The alignment between OXA-7 (protein) and the other oxacillinases showed 95% homology with OXA-10 (Fig. 3). The same level of homology was found with OXA-11, but with OXA-5, the homology was 81%. Amino acid identity was 45% with OXA-2 and only 27% with the OXA-1, OXA-9, and LCR-1 β-lactamases. In Fig. 4, the evolutionary distances between the class D β-lactamases, determined on the basis of their peptide sequence, are shown.

The OXA-7 gene is located on an insert unit. The sequence from position 932 to position 985 is homologous with the 59-bp consensus sequence of the imperfect inverted repeat, which provides the active recombination site for the gene's integration into integrons (6, 10). Twenty bases upstream from the first ATG, at positions 114 to 117, is located the GTTA motif, which marks the insertion point for the resistance genes. This configuration designates the insert unit. At the 3′ end of the *aadB* gene, the presence of a 59-bp element indicates that the gene forms a cassette and is inserted via the integrase mechanism.

The OXA-7 gene is an oxacillinase first studied by Medeiros et al. (15). Its enzyme characteristics were determined and indicated that OXA-7 indeed belongs to the class D β-lactamases. The OXA-7 gene, residing on plasmid pMG202, is integrated as a cassette in an integron like the other known oxacillinases, except OXA-11. The gene is flanked by the 59-bp element at the 3′ end and by the motif GTTPuPu at the 5′ end, indicating that it can be inserted in or excised from the integron in a cassette-like manner by the integrase enzyme. On the variable region of the same integron upstream from the OXA-7 gene there is found another antibiotic resistance gene, *aadB*. This gene is also flanked by the 59-bp element, indicating that the two genes are inserted independently in the integron. The deduced peptide from OXA-7 was compared with known oxacillinases. The conserved sequence STFK, which is the active site in both the class A and class D β-lactamases (13), is also present in the OXA-7 sequence, as were the conserved sequences (boxes B and C in Fig. 3) which were identified as specific to oxacillinases (7). From the peptide and the nucleotide sequence comparisons, we can conclude that there is a subgroup of highly homologous enzymes within the oxacillinase group: the OXA-10, OXA-11, OXA-7, and OXA-5 enzymes. The OXA-2 enzyme seems to have diverged early from this subgroup. The most distantly related sequences are those of OXA-1, OXA-9, OXA-12, and LCR-1.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been assigned EMBL accession number X75562.

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