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Sequence of the PSE-1 β-Lactamase Gene

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The nucleotide sequence of the PSE-1 β-lactamase gene from Tn*1403* indicates that it is contained in an integron and encodes a class A enzyme differing from PSE-4 and CARB-3 by single amino acid substitutions.

PSE-1 is the commonest plasmid-mediated β-lactamase found in β-lactam-resistant clinical isolates of *Pseudomonas aeruginosa* (17), and despite its original designation as a *Pseudomonas*-specific enzyme (7), it occurs in members of the family *Enterobacteriaceae* as well (18). It is also of interest because of its ready hydrolysis of carbenicillin (16).

The PSE-1 β-lactamase gene from Tn*1403* (14) of plasmid RPL11 has been cloned on a 3.3-kb *Bam*HI fragment in pACYC184, yielding plasmid pMON810 (15). A DNA fragment containing the PSE-1 gene from pMON810 hybridized under conditions of high stringency with cloned genes for PSE-4 or CARB-3 β-lactamases, two less common carbenicillin-hydrolyzing enzymes also found in *P. aeruginosa* (11, 15). The sequences of PSE-4 (3) and CARB-3 (13) have recently been analyzed and shown to differ by only two amino acids. We have determined the nucleotide sequence of the PSE-1 gene.

In Turku, restriction fragments within a 0.7-kb *Hin*cII segment of pMON810 were subcloned into single-stranded phage M13mp18 for sequencing. In Boston, double-stranded plasmid DNA was used. The *Bam*HI fragment from pMON810 was recloned into the *Bam*HI site of pBC SK (Stratagene, La Jolla, Calif.), a phagemid carrying the chloramphenicol acetyltransferase gene and a multiple cloning site designed for construction of nested deletions with exonuclease III and mung bean nuclease. A deletion into the PSE-1 gene was obtained after *Sac*I and *Not*I treatment using the ExoIII/Mung Bean Deletion Kit from Stratagene. DNA sequencing was performed by the dideoxy chain termination method of Sanger et al. (21) with α-^{35}S-dATP from Amersham Corp. (Arlington Heights, Ill.) and either the M13 Sequencing System (Amersham) or the Sequenase Version 2 Sequencing Kit (United States Biochemical Corp., Cleveland, Ohio). M13 sequencing primers were obtained from Amersham or New England Biolabs (Beverly, Mass.). Custom primers were constructed with phosphoramidite chemistry with a model 380A DNA synthesizer from Applied Biosystems (Foster City, Calif.). The sequence was analyzed with IBI Pustell software (IBI, New Haven, Conn.).

Figure 1 indicates segments of the gene analyzed to generate the sequence of 1,117 nucleotides shown in Fig. 2. Likely ATG initiation codons preceded by ribosome binding sites occurred at positions 98 and 146. Figure 2 shows the protein of 304 amino acids that would be produced if the first site were used. This coding sequence for PSE-1 was identical to one for PSE-4 (3) except for a C-G→A-T change at nucleotide 942 in Fig. 2 that produces an Ala→Glu substitution in PSE-4 consistent with the isoelectric point (pI) difference between PSE-1 (pI 5.7) and PSE-4 (pI 5.3). The PSE-1 sequence also differed from that for CARB-3 (pI 5.75) (13) at a single base, with a T-A→C-G transition at position 707, resulting in a Phe→Leu alteration in CARB-3. PSE-1 and PSE-4 thus resemble TEM-1 and TEM-2 in differing by only a single amino acid but are more closely related in nucleotide sequence, since TEM-1 and TEM-2 diverge at additional bases that produce no amino acid changes (5).

The 97 bases 5′ to the first start codon for PSE-1 were identical to those preceding the coding regions for PSE-4 and CARB-3 and matched 97% with sequences upstream from genes for OXA-1 (20), OXA-2 (6), and PSE-2 (10) β-lactamases. These resistance genes, and others, are all known to be inserted into sites of DNA elements called integrons that also include the gene for an enzyme involved in such integration (22). Mercier et al. have previously shown that sequences homologous to the *tnpI* recombinase locus of Tn*21* are present in Tn*1403* adjacent to the PSE-1 gene (19). Indeed, bases 1 to 79 in Fig. 2 are identical to part of the Tn*21* *tnpI* locus and end in an AAAGTTA hot spot for recombination (19). There are potential ribosome binding sites at positions 89 to 94 and 132 to 138 in Fig. 2, each followed by an initiation codon. Which of these sites is used to initiate translation is not yet known, although Boissinot and Levesque have argued that the second site is more likely in the PSE-4 gene (3). Downstream from the PSE-1 gene is a sequence common to PSE-4 and CARB-3 with the potential secondary structure as shown in Fig. 2.

The deduced amino acid sequence of PSE-1 indicated that it belongs among class A β-lactamases. If the PSE-1 residues designated ABL30 to ABL285 according to the common numbering scheme for class A β-lactamases (1) are used for

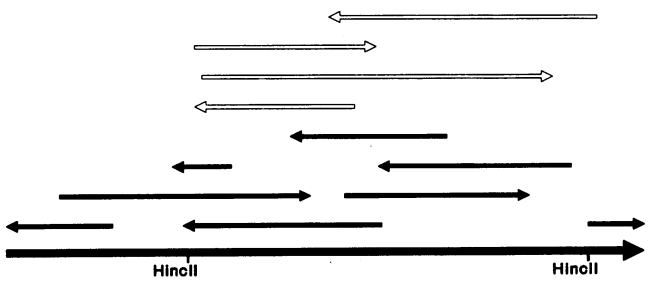

FIG. 1. Sequencing strategy for PSE-1 β-lactamase gene. The final sequence is represented by the bottom arrow. Fragments above it that are shown as solid arrows were sequenced from double-stranded plasmid DNA, while sequences for fragments shown as unfilled arrows were determined by using single-stranded M13 phage derivatives.

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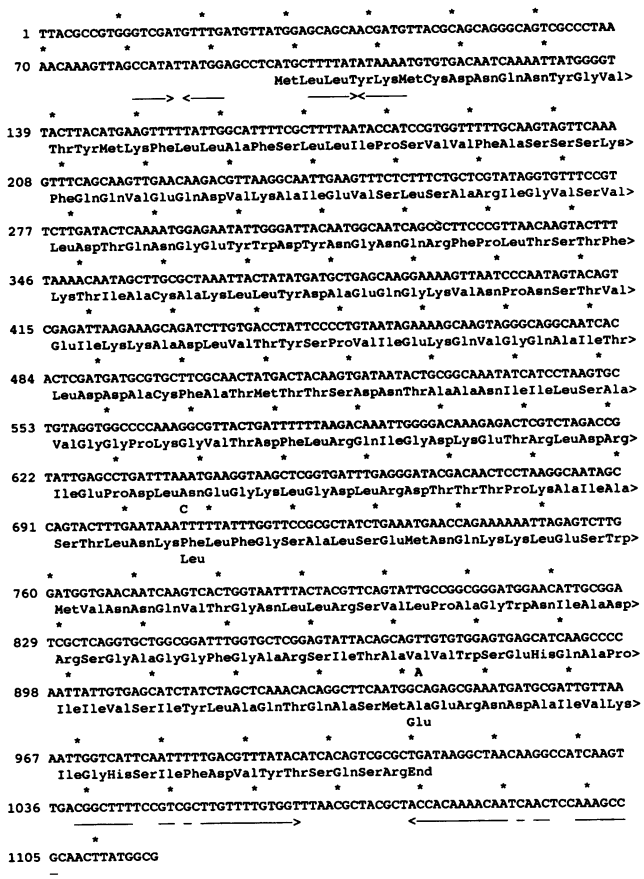

FIG. 2. Nucleotide sequence of the PSE-1 β-lactamase gene and flanking DNA. The deduced amino acid sequence commencing with ATG at positions 98 to 100 is shown although initiation at positions 146 to 148 is at least as likely. In the sequence of CARB-3, a C rather than a T at position 707 causes a Phe→Leu substitution, while in PSE-4, an A rather than a C at position 942 results in an Ala→Glu change. Arrows indicate potential inverted repeat sequences.

comparison, there were 119 of 251 (47.4%) residues identical with PSE-3, 111 of 248 (44.8%) identical with TEM-1, and 85 of 251 (34.7%) identical with PC1 β-lactamase of *Staphylococcus aureus*, all class A enzymes. However, there was some overlap with class D β-lactamases as well, with 35 of 219 (16%) residues identical to those of OXA-2, 14 of 64 (21.9%) residues identical to those of OXA-1, and 11 of 52 (21.2%) residues identical to those of PSE-2. An alignment of PSE-1, TEM-1 (2), PSE-3 (4), and PSE-2 (10) plus PC1 (8, 9) with known secondary structural features indicated is shown in Fig. 3. The amino acids that vary between PSE-1, PSE-4, and CARB-3 correspond to sites in the PC1 structure that are not directly involved in the active site depression (8). The question of which attributes of the PSE group promote carbenicillin hydrolysis deserves additional study. There are 15 amino acids common to PSE-1 and PSE-3 that are absent from PC1 and TEM-1, including four that are unique to PSE-1, PSE-2, PSE-3, PSE-4, and CARB-3, located in or near the β4 strand close to the active site in the tertiary structure of PC1, and hence are prime candidates for further evaluation.

Nucleotide sequence accession number. The PSE-1 gene sequence analyzed in this study has been deposited in GenBank with accession no. M69058.

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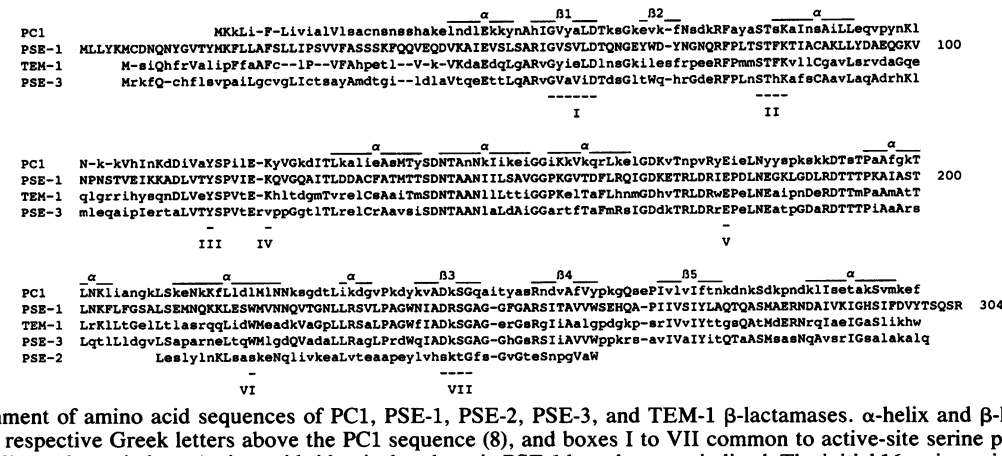

FIG. 3. Alignment of amino acid sequences of PC1, PSE-1, PSE-2, PSE-3, and TEM-1 β-lactamases. α-helix and β-ladder structure is indicated by the respective Greek letters above the PC1 sequence (8), and boxes I to VII common to active-site serine proteins interacting with penicillin (12) are shown below. Amino acids identical to those in PSE-1 have been capitalized. The initial 16 amino acids would be absent if the PSE-1 sequence were initiated at positions 146 to 148. Only a portion of the PSE-2 sequence is shown.

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