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Sequences of the NPS-1 and TLE-1 β-Lactamase Genes

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The NPS-1 and TLE-1 β-lactamase genes were cloned and sequenced. NPS-1 differed from LCR-1 β-lactamase in 8 of 260 amino acids. TLE-1 differed from TEM-1 by a single Asp(115)→Gly substitution and has been renamed TEM-90.

β-Lactamases can be classified by function or by structure, with, in general, a good correlation between the two approaches (3). Newly discovered enzymes usually have their genes sequenced, but for some older enzymes, only a functional characterization is available. We have cloned and sequenced the genes for NPS-1 and TLE-1 β-lactamases, enzymes discovered in the 1980s and previously characterized only in biochemical terms. NPS-1 is a plasmid-mediated β-lactamase reported in two isolates of *Pseudomonas aeruginosa* from a hospital in the United Kingdom in 1986 (7). Based on its biochemical characteristics, the enzyme was assigned to group 2a, penicillin-hydrolyzing enzymes inhibited by clavulanic acid, in the Bush-Jacoby-Medeiros classification (3). From plasmid pMLH50 in *P. aeruginosa* strain M302 (7), the NPS-1 gene was cloned (11) with *Eco*RI as a 7-kb insert into vector plasmid pBC SK (Stratagene, La Jolla, Calif.) encoding chloramphenicol resistance to produce plasmid pMG264. For sequencing, a Tn*7*-based transposon carrying a kanamycin resistance gene was inserted into purified pMG264 by using the GPS-1 Genome Priming System (New England BioLabs, Beverly, Mass.), and the resulting derivative was introduced into electrocompetent *Escherichia coli* strain DH10B (Gibco BRL, Rockville, Md.) by electroporation. After selection with 50 μg of kanamycin per ml and 30 μg of chloramphenicol per ml, colonies were screened for loss of resistance to ampicillin at 100 μg/ml. In ampicillin-susceptible colonies, the transposon was assumed to have inserted into the NPS-1 β-lactamase gene. With primers (primerN and primerS) that matched nucleotides at the extremities of the inserted transposon, cycle sequencing (Perkin-Elmer Cetus, Norwalk, Conn.) of the bla_{NPS-1} gene was initiated and continued by primer walking until both DNA strands were analyzed. An open reading frame of 783 bp encoded a 260-amino-acid protein, which differed by 8 amino acids from the sequence of LCR-1 β-lactamase (4) (Fig. 1). The amino acid differences were located in regions outside the STFK tetrad at amino acids 63 to 66 and outside the KTG motif at amino acids 201 to 203: 26 (Lys→Gln), 29 (Leu→Gln), 45 (Gly→Arg), 168 (Gln→Arg), 208 (Met→Ile), 222 (Lys→Gln), 258 (Pro→His), and 259 (Thr→Ala).

LCR-1 β-lactamase is a plasmid-mediated enzyme also found in a strain of *P. aeruginosa* in the United Kingdom in the 1980s (12). Based on its activity against methicillin and oxacillin, LCR-1 was classified among the cloxacillin-hydrolyzing β-lactamases of group 2d in the Bush-Jacoby-Medeiros classification. The structural similarity of NPS-1 and LCR-1 implies that NPS-1 was misclassified as a group 2a enzyme. Indeed, although NPS-1 was reported to hydrolyze methicillin at <0.1% the rate of benzylpenicillin, activity with oxacillin was 40% that with benzylpenicillin (7), unlike other group 2a enzymes. A Blast search (1) indicated that NPS-1 has 30 to 35% amino acid identity to OXA-2, OXA-3, OXA-5, OXA-7, OXA-15, or OXA-20 and thus structurally belongs in class D, a group that includes enzymes with even less homology, such as that between OXA-1 and OXA-2 (23% identity) or OXA-1 and OXA-3 (22% identity). LCR-1 is encoded by transposon Tn*1412* (6), which has been sequenced (GenBank accession no. L36547). The homology between sequence downstream from bla_{NPS-1} and Tn*1412*, however, ends 40 nucleotides after the bla_{LCR-1} gene, indicating that bla_{NPS-1} is found in a different genetic environment.

We also cloned and sequenced the TLE-1 β-lactamase gene. TLE-1 was reported in a clinical isolate of *Escherichia coli* from Brazil in 1985 (10). TLE-1 resembles TEM-1 in substrate profile, but has a pI of 5.55, unlike the pI of 5.4 for TEM-1. From pMG204b of *E. coli* strain 7604 (10), the TLE-1 gene was cloned by using *Eco*RI as a 10-kb insert into vector plasmid pBC SK to produce plasmid pMG265. The gene was amplified by PCR with TEM primers 1 and 2 as described by Mabilat and Goussard (9) from positions −5 to18 and 1074 to1054 in the Sutcliffe numbering of bla_{TEM} (13) and sequenced with these primers and primers T3 (5′-GTA TTA TCC CGT GTT GAC [positions 440 to 557]) and T4 (5′-GGC TTC ATT CAG CTC CGG [positions 718 to 701]). From the nucleotide sequence (GenBank accession no. AF351241), the deduced amino acid sequence of TLE-1 differed from that of TEM-1 in a single amino acid (Asp [GAT]→Gly [GGT]) at position 115, a change consistent with the observed difference from TEM-1 in pI occurring at a site on the surface of the enzyme far from the active site, which has been reported to be tolerant of amino acid substitutions without a decrement in function (5). To the known, naturally occurring, functionally silent polymorphisms in the TEM gene (TEM-2, TEM-13, TEM-57) (2, 8) can be added TEM-90, the new alternate name for TLE-1.

Nucleotide sequence accession number. The nucleotide sequence of bla_{NPS-1} has been deposited in GenBank under accession no. AY027589.

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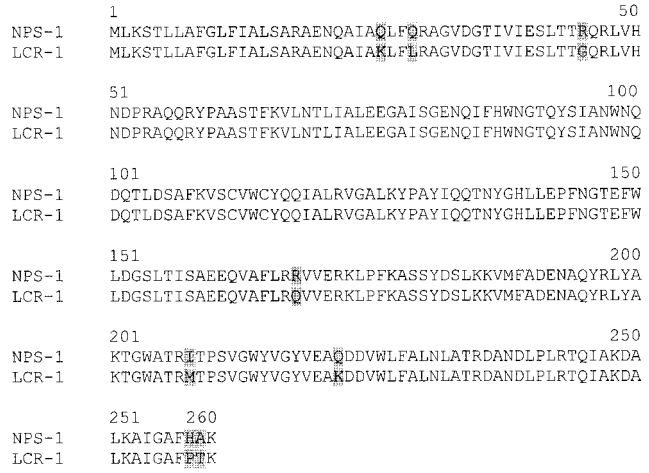

FIG. 1. Comparison of the amino acid sequences of the NPS-1 and LCR-1 β-lactamases. Amino acids that differ between the two enzymes are shaded.

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