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

HYUNJOO PAI1,2,3 AND GEORGE A. JACOBY2,3*

Division of Infectious Disease, College of Medicine, Dankook University, Chonan, Korea1; Edith Nourse Rogers Memorial Veterans Hospital, Bedford, Massachusetts 017302; and Lahey Clinic, Burlington, Massachusetts 018053

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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 EcoRI as a 7-kb insert into vector plasmid pBC SK (Stratagene, La Jolla, Calif.) encoding chloramphenicol resistance to produce plasmid pMG264. For sequencing, a Tn7-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 D101B (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 been 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

* Corresponding author. Mailing address: Lahey Clinic, 41 Mall Rd., Burlington, MA 01805. Phone: (781) 744-8608. Fax: (781) 744-1264. E-mail: george.a.jacoby@lahey.org.

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 Tn412 (6), which has been sequenced (GenBank accession no. L36547). The homology between sequence downstream from bla

Nucleotide sequence accession number. The nucleotide sequence of bla

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REFERENCES


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.