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Sequences of MGH-1, YOU-1, and YOU-2 Extended-Spectrum β-Lactamase Genes

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Genes for MGH-1, YOU-1, and YOU-2 extended-spectrum β-lactamases have been cloned and sequenced. The gene for MGH-1 has the sequence of bla_{TEM-10}, YOU-2 has that of bla_{TEM-12}, and YOU-1 has that of bla_{TEM-26}. All have evolved from bla_{TEM-1b} but have the strong dual promoter sequence of bla_{TEM-2}.

MGH-1, YOU-1, and YOU-2 are extended-spectrum β-lactamases from strains of *Klebsiella pneumoniae* isolated in the Boston area in 1988 (8, 15). Each has a distinctive isoelectric point (pI), but all three belong to the TEM family and are encoded by high-molecular-weight IncHI2 plasmids. In enzymatic properties, MGH-1 resembled TEM-10, which was isolated at about the same time in Chicago (13); YOU-2 resembled TEM-12, which was isolated earlier in Cincinnati (1, 19); and YOU-1 appeared unlike other enzymes tested (15). We have determined the nucleotide sequence of the genes for MGH-1, YOU-1, and YOU-2 to extend the comparisons to a molecular level.

The gene for MGH-1 was separated from the gene for TEM-1, which is also carried on plasmid pMG223 (15), by cloning it on a 3.6-kb *Sal*I fragment into vector pMLC28 (6). The YOU-2 gene was cloned into vector pBC SK$^+$ (Stratagene, La Jolla, Calif.) from plasmid pMG224 (15) on ca. 2-kb *Bam*HI-*Sal*I and 2.2-kb *Eco*RV fragments and further subcloned on 1.2-kb *Xho*I-*Pst*I and 0.9-kb *Not*I-*Pst*I fragments from the former and a 0.6-kb *Xho*I-*Pst*I fragment of the latter recombinant plasmid. By using double-stranded plasmid DNA, the inserts were sequenced as described previously (5) by the dideoxy chain termination method (16) with α-^{35}S-dATP and the Sequenase Version 2.0 Kit (United States Biochemical Corp., Cleveland, Ohio) using synthetic primers T3 and T7 (Stratagene), pBR322 primers 1206 and 1207 (New England Biolabs, Beverly, Mass.), and custom primers derived from the sequence of TEM-1 at nucleotides 177 to 193 (primer T177), 255 to 239 (primer T255-), and 1100 to 1084 (primer T1100-) in the numbering scheme of Sutcliffe (18). The sequence of YOU-1 was determined by using cloned polymerase chain reaction (PCR) products. The early part of the gene was amplified by using primers A (11) and T255- or primers T177 and T560-, derived from the sequence of the bla_{TEM-1} gene at nucleotides 560 to 544. The sequence was confirmed and extended with the PCR product derived with primer T177 and a primer from nucleotides 1082 to 1065 of the TEM-1 gene (T1082-). Amplification products were cloned into vector pCRII (Invitrogen, San Diego, Calif.) and sequenced by using T7 and Sp6 primers, the AutoRead Sequencing Kit, and the Automated Laser Fluorescent DNA Sequencer (Pharmacia Biotech Inc., Piscataway, N.J.).

Table 1 shows the coding and consequent amino acid differences between TEM-1 or TEM-2 and MGH-1, YOU-1, and YOU-2. The latter three enzymes all had substitutions known to extend the substrate range of β-lactamases. Each had an Arg-to-Ser alteration at amino acid 162, a change identified previously in extended-spectrum β-lactamases TEM-5, TEM-7, TEM-8, TEM-9, TEM-10, TEM-12, TEM-24, and TEM-26 (2, 4, 10, 12, 14, 17). In addition, MGH-1

TABLE 1. Nucleotide and amino acid substitutions in genes for TEM-1, TEM-2, MGH-1, YOU-1, and YOU-2

Nucleotide no.	Amino acid no.	Nucleotide[a] (amino acid) in:					
		TEM-1 Tn*3*	TEM-1b Tn*2*	TEM-2 Tn*1*	MGH-1	YOU-1	YOU-2
32		C	C	T	T	T	T
175		A	G	A	G	G	G
226		C	T	C	T	T	T
317	37	C (Gln)	C (Gln)	A (Lys)	C (Gln)	C (Gln)	C (Gln)
436		C	T	T	T	T	T
512	102	G (Glu)	G (Glu)	G (Glu)	G (Glu)	A (Lys)	G (Glu)
604		G	T	G	T	T	T
692	162	C (Arg)	C (Arg)	C (Arg)	A (Ser)	A (Ser)	A (Ser)
917	237	G (Glu)	G (Glu)	G (Glu)	A (Lys)	G (Glu)	G (Glu)

[a] Numbering is according to Sutcliffe (18). Data for TEM-1b and TEM-2 are from Mabilat et al. (11). Position 32 lies within the Pa and Pb dual promoter region of Tn*1* (3).

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had a Glu-to-Lys change at position 237, while YOU-1 had a Glu-to-Lys change at position 102. The gene for MGH-1 has the nucleotide sequence recently reported for bla_{TEM-10} (14). The gene for YOU-2 has the alterations predicted by oligotyping of bla_{TEM-12} (10), and the gene for YOU-1 has the nucleotide sequence of bla_{TEM-26}, which encodes an extended-spectrum β-lactamase first found in Stanford, Calif. (12). It is noteworthy that a single nucleotide substitution in the gene for YOU-2 can thus give rise to YOU-1, a more active extended-spectrum β-lactamase that was found in the same hospital outbreak (15).

In addition to these amino acid changes, there are distinctive nucleotides at positions 175, 226, 436, and 604 that indicate derivation of the genes for MGH-1, YOU-1, and YOU-2 from bla_{TEM-1b} of Tn*2* rather than bla_{TEM-1a} of Tn*3*, although it should be noted that transposition of these genes has never been demonstrated (9). Furthermore, each of the genes for these extended-spectrum enzymes has a C-to-T change at nucleotide 32 which creates the dual overlapping promoters responsible for the 6- to 10-fold-higher expression of bla_{TEM-2} compared to bla_{TEM-1a} or bla_{TEM-1b} (3). The same C-to-T change has been found upstream from genes for TEM-4, TEM-5, and TEM-9 (11). The higher levels of β-lactamase gene expression thus produced presumably compensate for the loss in enzymatic efficiency which often accompanies mutations expanding the substrate spectrum (7).

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