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Sequences of the Genes for the TEM-20, TEM-21, TEM-22, and TEM-29 Extended-Spectrum β-Lactamases

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The sequences of the bla_{TEM} genes encoding TEM-20, TEM-21, TEM-22, and TEM-29 extended-spectrum β-lactamases were determined. Analysis of the deduced amino acid sequences indicated that TEM-20 and TEM-29 were derived from TEM-1 and that TEM-21 and TEM-22 were derived from TEM-2. The substitutions involved were Ser-238 and Thr-182 for TEM-20; His-164 for TEM-29; Lys-104, Arg-153, and Ser-238 for TEM-21; and Lys-104, Gly-237, and Ser-238 for TEM-22. The promoter region of the bla_{TEM-22} gene was identical to that of bla_{TEM-3}. High-level production of TEM-20 could result from a 135-bp deletion which combined the −35 region of the *Pa* promoter with the −10 region of the *P3* promoter and a G→T transition in the latter motif.

Extended-spectrum β-lactamases TEM-20 and TEM-21 were detected in two nosocomial *Klebsiella pneumoniae* strains isolated in Tunis, Tunisia, in 1986 and 1988, respectively (5). A high level of resistance to cefotaxime, ceftriaxone, ceftazidime, and aztreonam was observed for both strains. TEM-20 and TEM-21 showed hydrolysis rates for cefotaxime and ceftriaxone similar to those of TEM-3 (22) and TEM-4 (18). In contrast to TEM-20, TEM-21 showed a significant rate of ceftazidime hydrolysis that was similar to those of TEM-3 (22) and TEM-4 (18). Isoelectric point determination (5.4 for TEM-20 and 6.4 for TEM-21) and hybridization studies led to the designation of the enzymes (5). Point mutations in the corresponding structural genes detected by PCR-restriction fragment length polymorphism (RFLP) (3) indicated the following substitutions in the deduced amino acid sequences. TEM-20 had a Glu at position 104 as in TEM-1 and the same amino acid substitution, Ser-238 for Gly, as in TEM-19 (6, 12, 15), but the corresponding gene differed from the bla_{TEM-19} gene by a G→A transversion at position 925. TEM-21 was derived from TEM-2 and had the same substitutions enhancing the substrate spectrum as TEM-3 and TEM-4 but differed from TEM-3 by an additional substitution (His-153 for Arg), resulting in a pI difference (6.4 versus 6.3). TEM-22 (pI 6.3) from a *K. pneumoniae* clinical isolate was previously characterized by biochemical analysis only (4). Donor, transconjugant, and transformant strains producing this enzyme have a higher level of resistance to aztreonam than to ceftazidime and cefotaxime (4). This very unusual resistance phenotype led to the designation TEM-22. TEM-29 (pI 5.4) from three *Escherichia coli* clinical strains isolated in 1987 was reported in 1995 (3).

We report the sequence of the structural genes for TEM-20, TEM-21, TEM-22, and TEM-29 and of the promoters of the genes encoding TEM-20, and TEM-22. The strains used were *E. coli* J53-2 (pUD30, TEM-20) (5), *E. coli* J53-2 (pUD31, TEM-21) (5), *E. coli* HB101 (TEM-22) (4) and the clinical isolate *E. coli* DEL (TEM-29) (3). PCR amplification of the bla_{TEM} genes was performed using primers OT3 and OT4 (3) corresponding to positions 209 to 228 and 1047 to 1066, respectively, of the bla_{TEM-1} gene (26), which allow the amplification of the entire coding region. Both strands of the PCR products were sequenced (21) using the PCR primers, fluorescent dye-labeled dideoxynucleotides, thermal cycling with *Taq* polymerase, and an ABI 373A DNA sequencer (Applied Biosystems, Foster City, Calif.). In case of differences in the sequences obtained for the two strands, a PCR product obtained independently was subsequently sequenced. PCR amplification and DNA sequencing of the promoter and coding regions of the bla_{TEM-20} and bla_{TEM-22} genes were performed as described (16).

The BLASTN (1) program at the National Center for Biotechnology Information was used for database searches, and the Clustal V program (11) was used to align multiple protein sequences. The EMBL accession numbers for the nucleotide sequences are Y17581 for bla_{TEM-20}, Y17582 for bla_{TEM-21}, Y17583 for bla_{TEM-22}, and Y17584 for bla_{TEM-29}. The changes in the genes are shown in Table 1, and those in the deduced amino acid sequences are shown in Table 2.

The sequence of bla_{TEM-20} revealed that this gene was derived from the bla_{TEM-2}-like group (silent mutations of bla_{TEM-2} and C at position 317) (7) whereas we proposed, following PCR-RFLP, that TEM-20 was derived from TEM-1 (3). Two mutations, T_{747}→C and G_{914}→A, resulting in amino acid substitutions Met-182→Thr and Gly-238→Ser, were found. Substitution Ser for Gly at position 238 has been observed in TEM-19 (12) and TEM-25 (8) and extends the substrate profile of the enzyme to cefotaxime (14), as for TEM-25 (19). Similar levels of resistance to expanded-spectrum cephalosporins were observed in the clinical strains producing these enzymes. The second substitution (Thr for Met at position 182) in TEM-20, which was not detected by PCR-RFLP (3), distinguished the enzyme from TEM-19 and TEM-25. The same substitution occurs in TEM-32 (IRT-3), TEM-43 (6), and TEM-52 (20). Alone, this substitution does not seem to extend the substrate profile; however, when combined with Lys-104 and Ser-238, Thr-182 confers moxalactam resistance, as observed for TEM-52 (20).

Analysis of the sequence of bla_{TEM-21} showed that this gene

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TABLE 1. Nucleotide substitutions among the bla_{TEM} genes from Tn*1* (bla_{TEM-2}), Tn*2* (bla_{TEM-1B}), and Tn*3* (bla_{TEM-1A}) and those encoding TEM-3, TEM-20, TEM-21, TEM-22, and TEM-29

Position[a]	Gene							
	bla_{TEM-1A}	bla_{TEM-1B}	bla_{TEM-2}	bla_{TEM-3}	bla_{TEM-20}	bla_{TEM-21}	bla_{TEM-22}	bla_{TEM-29}
Promoter region								
32	C	C	T	T	Deleted	ND[b]	T	ND
147	T	T	T	A	Deleted	ND	A	ND
162	G	G	G	G	T	ND	G	ND
175	A	G	A	A	A	ND	A	ND
Coding region								
226[c]	C	T	C	C	C	C	C	C
317	C	C	A	A	C	A	A	C
346[c]	A	A	G	G	G	G	G	A
436[c]	C	T	T	T	T	T	T	T
512	G	G	G	A	G	A	A	G
604[c]	G	T	G	G	G	G	G	T
660	A	A	A	A	A	G	A	A
682[c]	T	T	C	C	C	C	C	T
693	G	G	G	G	G	G	G	A
747	T	T	T	T	C	T	T	T
912	C	C	C	C	C	C	G	C
914	G	G	G	A	A	A	A	G
925[c]	G	G	A	A	A	A	A	G

[a] Numbering according to Sutcliffe (26).
[b] ND, not determined.
[c] Position at which only silent mutations occur.

derived from bla_{TEM-2} (A at position 317). The deduced TEM-21 protein had the same substitutions responsible for resistance to broad-spectrum cephalosporins and aztreonam as TEM-3 (24) and TEM-4 (derived from TEM-1) (25). The enzyme had a His-153→Arg substitution recently found in a TEM-21 produced by a clinical isolate of *Morganella morganii* (27). The consequence of this substitution on the catalytic activity of the β-lactamase is unknown and possibly insignificant since residue 153 is far from the β-lactam binding site (13).

bla_{TEM-22} differs from bla_{TEM-3} by a C→G mutation at position 912 leading to a Gly for Ala substitution at position 237, probably involved in the high-level aztreonam resistance of the host. Replacement of Ala-237 by threonine, as in TEM-5 and TEM-24 (6), increases the affinity of the enzyme for extended-spectrum β-lactams (14). Replacement by saturated mutagenesis of Ala-237 by Asp or Thr but not by Gly increases the catalytic activity of TEM-1 against cephems (10). The effect of these substitutions on the catalytic efficiency against aztreonam has not been studied.

TABLE 2. Amino acid substitutions in TEM-type β-lactamases

β-lactamase	Amino acid located at position[a]:						
	39	104	153	164	182	237	238
TEM-1	Gln	Glu	His	Arg	Met	Ala	Gly
TEM-2	Lys						
TEM-3	Lys	**Lys**					**Ser**
TEM-11	Lys			**His**			
TEM-19							**Ser**
TEM-20					**Thr**		**Ser**
TEM-21	Lys	**Lys**	**Arg**				**Ser**
TEM-22	Lys	**Lys**				**Gly**	**Ser**
TEM-29				**His**			

[a] Numbering according to Ambler et al. (2). Amino acids indicated in bold extend the spectrum of β-lactamase activity.

The sequence of bla_{TEM-29} showed that this gene was derived from bla_{TEM-1B} and differed by a silent mutation, T_{226}→C, as in bla_{TEM-1A}. An additional mutation, G_{693}→A (also detected by PCR-RFLP) (3), leads to the amino acid substitution His for Arg at position 164, which increases ceftazidime resistance (14). However, Ser for Arg is the most common substitution at this position in TEM variants from clinical

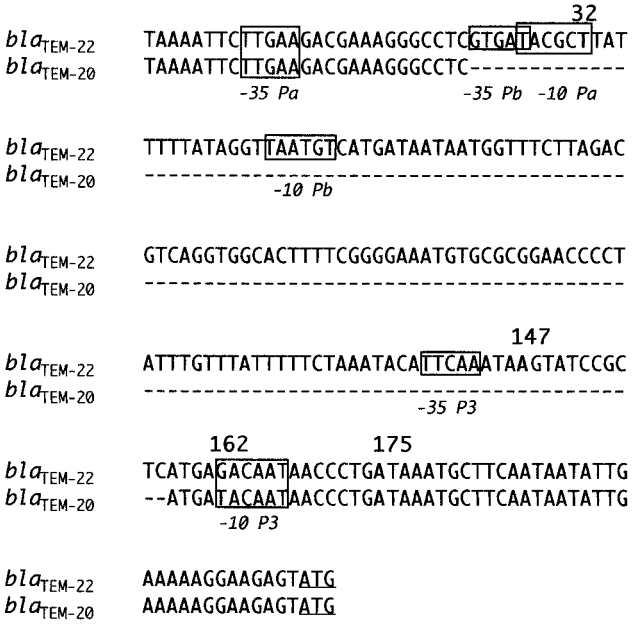

FIG. 1. Promoter sequence of the bla_{TEM-20} and bla_{TEM-22} genes. The differences in the promoter region of Tn*2* are shown in bold and numbered according to Sutcliffe (26). The −35 and −10 regions of *Pa*, *Pb*, and *P3* are boxed. The start codon is underlined. Deleted nucleotides are indicated by dashes.

isolates (6). The Gly-for-Arg substitution confers ceftazidime resistance in TEM-1 mutants obtained by random mutagenesis (17). In clinical isolates, this substitution is rarely observed alone, except for TEM-11 (CAZ-lo) derived from TEM-2 (28). The low-level resistance to ceftazidime resulting from this substitution may account for the difficulty in detecting such variants.

Our results confirm that sequencing is the only truly definitive method for DNA analysis, even if PCR-RFLP is a useful approach for epidemiological studies.

Analysis of the promoter regions of bla_{TEM-20} and bla_{TEM-22} indicated that bla_{TEM-22} had the same T-for-C change at position 32 as that observed in Tn*1*, bla_{TEM-3}, bla_{TEM-4}, and bla_{TEM-5} (Fig. 1) (24, 25). This change converts the weak *P3* promoter of bla_{TEM-1} (Tn*3*) into the two overlapping *Pa* + *Pb* promoters and results in a large increase in β-lactamase production (9). There was a 135-bp deletion in the promoter region of bla_{TEM-20}. This deletion was associated with a point mutation, T for G in the −10 region of *P3*, leading to greater similarity to the consensus promoter sequence, as previously observed for bla_{TEM-1} in *Shigella flexneri* (23). Combination of the deletion and the mutation, leading to the association of the −35 region of *Pa* with the more efficient −10 region of *P3*, may account for high-level resistance to oxyiminocephalosporins of the strain producing TEM-20 due to high-level enzyme production (5).

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