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Nucleotide Sequence of SHV-2 β-Lactamase Gene

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The nucleotide sequence of plasmid-mediated β-lactamase SHV-2 from *Salmonella typhimurium* (SHV-2_{pHT1}) was determined. The gene was very similar to chromosomally encoded β-lactamase LEN-1 of *Klebsiella pneumoniae*. Compared with the sequence of the *Escherichia coli* SHV-2 enzyme (SHV-$2_{E.coli}$) obtained by protein sequencing, the deduced amino acid sequence of SHV-2_{pHT1} differed by three amino acid substitutions.

Transferable resistance to broad-spectrum cephalosporins, such as cefotaxime, is a developing phenomenon among members of the family *Enterobacteriaceae* and involves new β-lactamases which belong to class A in the scheme of Ambler (1) and are genetically derived from TEM or SHV enzymes (see reference 11 for a review). Since the discovery in 1983 of a plasmid-mediated β-lactamase related to SHV-1 (10), new SHV-type enzymes have been found, mainly in *Klebsiella* isolates (5, 7–9). Although the amino acid sequences of SHV-1 from *Klebsiella* spp. and SHV-2 from *Escherichia coli* A2302 (SHV-$2_{E.coli}$) have been determined (3, 4), little is known concerning the nucleotide sequence, the signal peptide, or the promoter of SHV-type enzymes. For this reason, we chose to sequence the *bla* gene coding for SHV-2 ($bla_{SHV-2(pHT1)}$), which was isolated from *Salmonella typhimurium* 122 (14). In a previous study, we showed that this gene was carried by a 12.5-kilobase plasmid designated pHT1 and encoded an enzyme (SHV-2_{pHT1}) which was indistinguishable from SHV-$2_{E.coli}$ β-lactamase by isoelectric point, substrate profile, and kinetic constants (6). The $bla_{SHV-2(pHT1)}$ gene was mapped to adjacent *Pst*I fragments of 0.86 and 0.79 kilobases and cloned into plasmid pBR322. For DNA sequencing, the *Pst*I fragments were subcloned into bacteriophages M13mp18 and M13mp19. In addition, deleted *Pst*I fragments were prepared by *Bal* 31 exonuclease digestion (Boehringer Mannheim, Meylan, France) to produce inserts with overlapping sequences. The deleted fragments were further subcloned into the same vectors. The DNA sequence was determined on both strands by the dideoxy-chain termination method described by Sanger et al. (12). We used the Sequenase sequencing kit from the United States Biochemical Corp., Cleveland, Ohio, as indicated by the supplier, and [α-^{35}S]dATP (600 Ci/mM) was purchased from Amersham France, Les Ulis, France. Labeled DNA was analyzed by electrophoresis at 50 W in buffer gradient gels (8 M urea, 6% polyacrylamide).

Figure 1 shows the nucleotide sequence of the $bla_{SHV-2(pHT1)}$ gene. An ATG codon at position 223 initiates a long open reading frame of 858 nucleotides which ends at position 1081 with a TAA codon. The initiation codon is preceded by a Shine-Dalgarno ribosome-binding sequence, AAGG, and a possible −10 region, TATTCT, and a −35 region, TTTGCA, of a promoter. The deduced sequence of 286 amino acids is shown in Fig. 2. The mature enzyme is 265 amino acids long and begins with a signal peptide of 21 residues. The sequence of SHV-2_{pHT1} shows a great similarity to that of chromosomally encoded β-lactamase LEN-1 of *Klebsiella pneumoniae* (2). These two proteins show 89% similarity in nucleotide sequence, and 234 amino acids are identical in the mature enzymes. LEN-1 β-lactamase is seven residues shorter than SHV-2_{pHT1} at the carboxy-terminal extremity. This is due to the deletion of a G residue at position 1054, which changes the reading frame and leads to a termination codon 7 nucleotides downstream in the LEN-1 sequence. If this deletion were disregarded, the deduced sequences of the last nine amino acids would be identical in the two enzymes. The similarity between LEN-1 and SHV-2_{pHT1} also includes the signal peptide in which 17 of 21 residues occupy identical positions. In addition to nucleotide similarity within the $bla_{SHV-2(pHT1)}$ and LEN-1 genes, the sequence is highly conserved in the noncoding region which stretches to 73 base pairs upstream from the initiation codon and includes the possible −10 locus of a promoter. In contrast, similarity is lacking between the LEN-1 and SHV-2_{pHT1} nucleotide sequences surrounding the −35 promoter region. This could indicate that, although very closely related, these two enzymes do not share the same promoter.

Comparision of the amino acid sequence of SHV-2_{pHT1} β-lactamase from *S. typhimurium* with those of SHV-1 (4) and SHV-$2_{E.coli}$ (3) shows four and three amino acid substitutions, respectively. Compared to SHV-1, SHV-2_{pHT1} and SHV-$2_{E.coli}$ share the same substitution of serine for glycine at position 234. It has been reported that this mutation is the only change observed within the amino acid sequences of SHV-1 and SHV-$2_{E.coli}$ and is responsible for the cefotaxime-hydrolyzing activity of SHV-2 β-lactamase (3). In contrast, SHV-2_{pHT1} differs from SHV-1 and SHV-$2_{E.coli}$ by three additional substitutions: a glutamine (CAA) instead of a leucine (CTN, TTA, or TTG) at position 31, an alanine (GCC) instead of a threonine (ACN) at 136, and a threonine (ACC) instead of an alanine (GCN) at 137. Remarkably, these positions are occupied by the same three residues and are encoded by the same codons within the LEN-1 sequence. From the most likely codon that would be present for the SHV-$2_{E.coli}$ enzyme, a single base change is sufficient to account for each substitution. Since SHV-2_{pHT1} and SHV-$2_{E.coli}$ are indistinguishable by their enzymatic activities, these mutations are not likely to be involved in the catalytic activity, but they could cast light on the evolutionary relationships among the SHV-type enzymes. If all these enzymes are related to the progenitor LEN-1 β-lactamase, they could have evolved independently in different bacterial hosts. On the basis of sequence analysis, LEN-1 appears to

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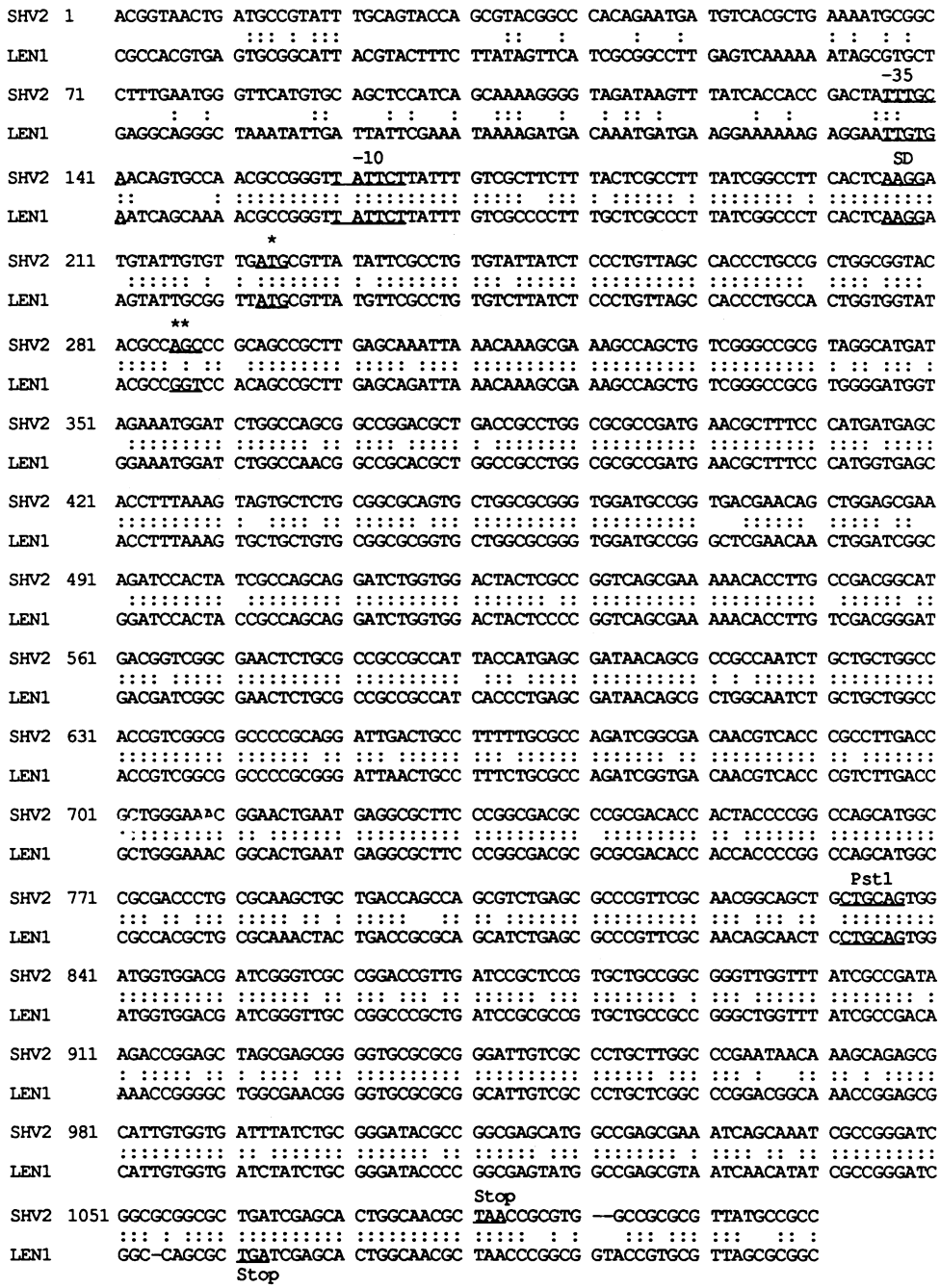

FIG. 1. Nucleotide sequence of $bla_{SHV\text{-}2(pHT1)}$ gene. Nucleotides are aligned by comparison with the sequence of the LEN-1 enzyme (2); a colon represents identity. The initiation (*) and the stop codons are indicated. ** represents the beginning of the mature enzyme. Upstream from the $bla_{SHV\text{-}2(pHT1)}$ gene, SD represents a putative Shine-Dalgarno consensus sequence, and the possible −10 and −35 regions are underlined. The *Pst*I site within the gene is indicated.

```
                1        10        20   22       30        40        50
SHV2            MRYIRLCIISLLATLPLAVHA ! SPQPLEQIKQSESQLSGRVGMIEMDLASGRTLTAWR
                :::*:::*:::::::::*:*:   *:::::::::::::::::::::*:::::::::*:::
LEN1            MRYVRLCVISLLATLPLVVYA ! GPQPLEQIKQSESQLSGRVGMVEMDLASGRTLAAWR
SHV2EC/SHV1                                     L

                  60        70        80        90       100       110
SHV2            ADERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHYRQQDLVDYSPVSEKHLADGMTVGE
                :::::::*:::::*:::::::::::::*:::::*:::::::::::::::::::*::::*::
LEN1            ADERFPMVSTFKVLLCGAVLARVDAGLEQLERRIHYRQQDLVDYSPVSEKHLVDGMTIGE

                  120       130       140       150       160       170
SHV2            LCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALPGDARDTT
                :::::::*:::::*:::::::::::::::::::::::::::::::::*::::::::::::
LEN1            LCAAAITLSDNSAGNLLLATVGGPAGLTAFLRQIGDNVTRLDRWETALNEALPGDARDTT
SHV2EC/SHV1                     TA

                  180       190       200       210       220       230
SHV2            TPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAGPLIRSVLPAGWFIADKTGASERG
                :::::::::::::::*:*::::::*:::::::::::::::::*:::*:::::::::::*:::
LEN1            TPASMAATLRKLLTAQHLSARSQQQLLQWMVDDRVAGPLIRAVLPPGWFIADKTGAGERG
SHV1                                                                      G
SHV2EC                                                                    S

                  240       250       260       270       280   286
SHV2            ARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHWQR
                ::::::::::**:*:::::::::::::::::::::*:::::**
LEN1            ARGIVALLGPDGKPERIVVIYLRDTPASMAERNQHIAGIGQR
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FIG. 2. Amino acid sequence of SHV-2_{pHT1} β-lactamase. According to general usage for SHV enzymes (11), amino acid residues are numbered two less than for TEM-1 (13). Therefore, the first residue of the mature enzyme is numbered 22. The cleavage site after the signal peptide is indicated with an exclamation mark. The SHV-2_{pHT1} sequence has been aligned with the LEN-1 sequence (2); a colon represents identity, and an asterisk indicates a substitution. Only the residues which are not retained in SHV-1 (4) and SHV-$2_{E.coli}$ (SHV2EC) (3) are indicated.

be more closely related to SHV-2_{pHT1} than to SHV-1 and SHV-$2_{E.coli}$, in which three additional mutations have occurred. Lastly, the determination of the SHV-2_{pHT1} nucleotide sequence will allow the development of specific oligonucleotide probes and the performance of site-directed mutagenesis to study the effect of point mutations on enzyme activity.

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LITERATURE CITED

1. **Ambler, R. P.** 1980. The structure of β-lactamases. Philos. Trans. R. Soc. London B Biol. Sci. **289**:321–331.
2. **Arakawa, Y., M. Ohta, N. Kido, Y. Fujii, T. Komatsu, and N. Kato.** 1986. Close evolutionary relationship between the chromosomally encoded β-lactamase gene of Klebsiella pneumoniae and the TEM β-lactamase gene mediated by R plasmids. FEBS Lett. **207**:69–74.
3. **Barthélémy, M., J. Peduzzi, H. Ben Yaghlane, and R. Labia.** 1988. Single amino acid substitution between SHV-1 β-lactamase and cefotaxime-hydrolyzing SHV-2 enzyme. FEBS Lett. **231**:217–220.
4. **Barthélémy, M., J. Peduzzi, and R. Labia.** 1988. Complete amino acid sequence of p453-plasmid-mediated PIT-2 β-lactamase (SHV-1). Biochem. J. **251**:73–79.
5. **Buré, A., P. Legrand, G. Arlet, V. Jarlier, G. Paul, and A. Philippon.** 1988. Dissemination in five French hospitals of *Klebsiella pneumoniae* serotype K25 harboring a new transferable enzymatic resistance to third-generation cephalosporins and aztreonam. Eur. J. Clin. Microbiol. **7**:780–782.
6. **Garbarg-Chenon, A., H. Vu Thien, R. Labia, H. Ben-Yaghlane, V. Godard, P. Deny, F. Bricout, and J. C. Nicolas.** 1989. Characterization of a plasmid coding for resistance to broad-spectrum cephalosporins in *Salmonella typhimurium*. Drugs Exp. Clin. Res. **15**:145–150.
7. **Gutmann, L., B. Ferré, F. W. Goldstein, N. Rizk, E. Pinto-Schuster, J. F. Acar, and E. Collatz.** 1989. SHV-5, a novel SHV-type β-lactamase that hydrolyzes broad-spectrum cephalosporins and monobactams. Antimicrob. Agents Chemother. **33**:951–956.
8. **Jarlier, V., M.-H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended-broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10**:867–878.
9. **Kliebe, C., B. A. Nies, J. F. Meyer, R. M. Tolxdorff-Neutzling, and B. Wiedemann.** 1985. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob. Agents Chemother. **28**:302–307.
10. **Knothe, H., P. Shah, V. Kreméry, M. Antal, and S. Mitsuhashi.** 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole, and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection **11**:315–317.
11. **Philippon, A., R. Labia, and G. Jacoby.** 1989. Extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **33**:1131–1136.
12. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **79**:5463–5467.
13. **Sutcliffe, J. G.** 1978. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc. Natl. Acad. Sci. USA **75**:3737–3741.
14. **Vu Thien, H., J. J. Baudon, and M. Rolland.** 1987. Salmonella typhimurium: résistance aux céphalosporines de troisième génération. Med. Mal. Infect. **6/7**:398–400.