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Letter to the Editor

Analysis of the *bla*$_{toho}$ Gene Coding for Toho-2-β-Lactamase

Toho-2 is a non-TEM, non-SHV-type extended-spectrum β-lactamase, produced by *Escherichia coli* TUM 1083 isolated in 1995 in Japan, a cefotaxime-resistant clinical isolate (8). Toho-2, like Toho-1 (7), belongs to a β-lactamase cluster which includes plasmid-mediated β-lactamases such as MEN-1 (3, 6), also referred as CTX-M-1 (4, 5), and chromosomally mediated β-lactamases such as those produced by *Proteus vulgaris* (9), *Klebsiella oxytoca* (11), and *Serratia fonticola* (10).

The cefotaxime resistance gene for Toho-2 was sequenced by Ma et al. (8). The deduced protein sequence consisted of a precursor of 327 residues. As reported by the authors (8), the Toho-2 β-lactamase protein sequence is close to that of Toho-1, but the sequence located at Ala-185 to Ala-219 had "almost no homology with the other class A β-lactamases. The sequence may constitute a loop structure at the substrate-binding site and has a deletion of 2 amino acid residues. . ."

In order to better understand the possible evolution of Toho-2 from an unknown precursor, I tried to introduce a few putative bases in the *bla*$_{toho}$ gene, as six were possibly lost (Fig. 1). I started at base 676, using the numbering of Ma et al. This corresponds to amino acid residue 186, following the numbering scheme of Ambler et al. (2). The last introduced base corresponds to amino acid residue 217. Thus, for the 32 residues of the novel deduced protein sequence, and in comparison with the Toho-1 β-lactamase sequence, 25 residues are conserved, 3 cannot be determined because of lack of information, and 4 are substituted. This shows that the corrected sequence is very close to that of Toho-1. Next, a Blast search of the National Center for Biotechnology Information database (8a) was performed by using a 90-base sequence starting at base 676 of the *bla*$_{toho2}$ gene (1). High alignment scores were obtained only for β-lactamases of the previously defined cluster. Two 17-base identities were obtained, respectively, for the *Mus cookii* DNA sequence, GenBank accession number M97512 (3′-tggtgacgtggctcaaa-5′, starting at base 735 of the *bla*$_{toho2}$ gene following the numbering of Ma et al. [8]), and *Streptomyces coelicolor* cosmid 2E1, GenBank accession number AL023797 (3′-gggtcatgcgctgggcg-5′, starting at base 701). Nevertheless, these two 17-base identities are meaningless.

These results suggest that the *bla*$_{toho2}$ gene sequence should be revised.

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DNA alignment
Toho-1  accacgccgctcgcgatggcgcagaccctgaaaaatctgacgctgggtaaagcgctggcg
        |||||| ||| ||  |||||||||  ||       | |||||||||| | ||||||||
Toho-2  accacggcgcgggct▼▼ggcgcagacgttg▼▼cgtcattacgctgggtcatgcgctgggc

Toho-1  gaaactcagcgggcacagttggtgacgtggcttaagggcaatactaccggtagcgcgagc
        ||||| ||| ||| |||||||||||||||||| || |||||||| ||||    ||  ||
Toho-2  gaaacccag▼cggcgcagttggtgacgtggctcaaaggcaatacgaccgc▼gcagccggc


Protein alignment
Toho-1:  T  T  P  L  A  M  A  Q  T  L  K  N  L  T  L  G  K  A  L  A
         |  |        |     |  |  |  |           |  |  |     |  |
Toho-2:  T  T  A  R  A  #  A  Q  T  L  #  V  I  T  L  G  H  A  L  G
ABL                    186
Toho-1:  E  T  Q  R  A  Q  L  V  T  W  L  K  G  N  T  T  G  S  A  S
         |  |  |     |  |  |  |  |  |  |  |  |  |  |  |  |     |
Toho-2:  E  T  Q  #  A  Q  L  V  T  W  L  K  G  N  T  T  G  A  A  G
ABL                                                    217
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FIG. 1. Local alignment of the *bla*$_{toho1}$ and *bla*$_{toho2}$ genes. Toho-1 starts at base 640 and Toho-2 starts at base 661, following the numbering system of Ma et al. (8). Six presumed bases, starting at position 676 of *bla*$_{toho2}$, were introduced. The protein alignment is deduced from the *bla*$_{toho1}$ gene and the *bla*$_{toho2}$ gene after introduction of the six bases. "ABL" represents Ambler's numbering scheme for amino acids (2). ▼, putative bases. Note that the four codons "gc▼" code for glycine (Gly-217).

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Author's Reply

We have known that the DNA sequences of Toho-1 (2) and Toho-2 (3) are more similar than the amino acid sequences. So, we can understand the comment of Dr. Labia. However, we confirmed the sequence, carrying out the DNA sequencing of the Toho-2-encoding gene from both sides; the sequence was also reconfirmed by another laboratory.

We have discussed the meaning of the deletion of Toho-2 in our report published last year (3), in which we state that the deletion of 2 amino acid residues is well consistent with the high affinity of this enzyme with tazobactam, which has the large moiety at the C-2 position, since the deletion affords an extra space to accommodate the large moiety based on the crystal structure of the mutant of Toho-1 (1).

Analyses of the mutant and the structure of Toho-2 are now under way. We believe that these studies will in the near future afford more details of the function of the deletion in Toho-2.

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