ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1994, p. 407–408
0066-4804/94/$04.00+0

Vol.. 38, No. 2

Letter to the Editor

Analysis of the *penA* Gene of *Pseudomonas cepacia* 249

In the April issue of *Antimicrobial Agents and Chemotherapy* (6), Proenca et al. described the cloning and sequencing of a 1.1-kb DNA fragment reportedly containing the *penA* gene encoding the chromosomal β-lactamase of *Pseudomonas cepacia* 249 and deduced the primary structure of an unprocessed protein of 313 residues which was declared to belong to the AmpC family of β-lactamases, i.e., to structural class C (2). However, two important sequence elements which characterize these enzymes [Y150(A)SN and H314KTG] surprisingly were not found, and the putative active site serine at position 190 was much closer to the C terminus than it was in the other class C β-lactamases. If the proposed sequence is correct, the penicillinase of *P. cepacia* must be considered a representative of a new original class of β-lactamases, quite distinct from all other active site serine β-lactamases identified so far and constructed on a pattern dramatically different from that of all the presently known enzymes of this superfamily.

A careful analysis of the published sequence yielded the following findings.

(i) A search of the complete DNA sequence failed to identify the *Xmu*I and *Pst*I sites reported to be present in the sequenced *Eco*RI-*Eco*RI fragment, and the *Hin*cII site was found between 400 and 600 rather than between 200 and 400 bp.

(ii) DNA sequence comparisons (EMBL data bank) revealed a very high similarity of nucleotides 426 to 827 with phage M13mp18 nucleotides 5811 to 6224 (9), corresponding to a portion of the *lacI* gene. Interestingly, two stretches of extremely high similarity were interrupted by a short, 60-nucleotide sequence (nucleotides 638 to 697) coding for a 20-residue peptide around the putative active serine, i.e., Ile-174–Lys-193. This is the only peptide in the "PenA" protein which exhibits a clear analogy with the active serine containing peptides of class C enzymes. If this DNA stretch is not included in the comparison, a 93.4% identity is found between nucleotides 426 to 637 and 698 to 827 of the *penA* gene and nucleotides 5811 to 6033 and 6093 to 6224 of the phage vector. Strikingly, most of the differences are insertions or deletions near or in multiplets.

(iii) A simple search of the protein sequences in the SWISSPROT data bank revealed a much stronger similarity of PenA with *Escherichia coli* isoleucyl-tRNA synthetase (IleS, SWISSPROT : Syi_E coli [8]) than with any of the known class C β-lactamases. Indeed, for these β-lactamases, similarities were strictly restricted to the 20-residue peptide described above. The high similarity with the *E. coli* enzyme extends from residue 1 to 43 of the "PenA" protein with only three different aminio acids. A closer examination involving the six reading frames of the "*penA*" gene indicated that the similarity could be extended to a much longer portion of the gene product if four frameshifts were allowed. Under these conditions, 108 out of 112 residues could be identified to those of the Ile-tRNA synthetase. Furthermore, the sequence of nucleotides 7 to 69 of the putative *penA* gene was nearly identical to the corresponding part of the Ile-tRNA synthetase gene, and again, most of the differences as well as all of the frameshifts were due to insertions or deletions near multiplets.

(iv) Similarly, nucleotides 828 to 1133 were translated in the six reading frames and the peptide sequences thus obtained were compared with those in the SWISSPROT data base. Opening reading frame 6 for nucleotides 1096 to 1004 encodes a 31-residue peptide nearly identical (30 of 31 residues) to residues 1 to 31 of alkyl hydroperoxide reductase (F52A protein) of *Salmonella typhimurium* (SWISSPROT : Aphf_Salty [7]). Again, allowing two frameshifts, the similarity could be extended up to residue 81 of the alkyl reductase with 72 of 81 strict identities.

(v) A Kyte and Doolittle (3) hydrophobic profile failed to reveal a hydrophobic stretch at the N terminus of "PenA," which would be the signature of a signal peptide sequence.

(vi) Although the size of the putative PenA protein is credible for a β-lactamase, the protein is certainly significantly shorter than all of the presently known class C enzymes (1, 4, 5). The cystein content (nine residues) is also surprisingly high for a β-lactamase, this residue being completely absent from the mature forms of all class C enzymes whose sequences have been published.

In conclusion, only a short, 20-residue fragment containing the S*XXK tetrad, in which S* is the active site serine, can be satisfactorily aligned with the corresponding part of class C β-lactamases. The 60-base stretch of DNA encoding this part of the protein replaces a vector portion of identical length, and it is difficult to understand how this sequence can be found in such a position not as an insertion, but as a substitution of exactly the same number of nucleotides. It seems unlikely that the similarities with the *E. coli* and *S. typhimurium* proteins and with the vector found in our analysis represent simple coincidences. It is quite probable that the published sequence is not that of a genuine β-lactamase.

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Author's Reply

Dr. Joris et al. provide a detailed computer analysis of the sequence of the *penA* β-lactamase that we had previously described (1). As we reported, the active site serine was much closer to the C terminus than in any other reported β-lactamases, and Dr. Joris' homology searches for the predicted protein revealed strong similarity with an *E. coli* isoleucyl-tRNA synthetase, particularly when several frameshifts are allowed. Obviously, the DNA sequence alone does not support the contention that this is a β-lactamase. Moreover, as demonstrated in the initial description of this enzyme, the *penA* β-lactamase differs significantly from the chromosomal β-lactamases of other gram-negative organisms in its substrate profile and kinetics, although as demonstrated by Southern hybridization, it clearly is derived from *P. cepacia* 249 (2). As *P. cepacia* has a well-described facility for acquiring foreign genes from other organisms, we hypothesized that this β-lactamase was originally derived from members of the family *Enterobacteriaceae*.

Support for this hypothesis was provided by the series of genetic studies demonstrating that *penA* expression could be regulated by the *E. coli ampD* locus and that a putative *P. cepacia* AmpR homolog could positively regulate *Enterobacter cloacae ampC*. Moreover, the putative *P. cepacia ampR* was found to have homology to the *E. cloacae* ampR by Southern hybridization, and gel mobility shift assays further confirmed the ability of both the putative *P. cepacia* AmpR and the *E. cloacae* AmpR to bind to the DNA immediately upstream of the *penA* coding sequence.

Thus, the combination of these genetic studies, evidence of the appropriate protein-DNA interactions, and key sequence elements, including the presence of an active site serine and five separate regions of >69% DNA identity with *E. coli* and/or *E. cloacae ampC* DNA, supports our contention that *P. cepacia* acquired an *ampC*-type β-lactamase or at least a portion of the operon from the *Enterobacteriaceae*.

Furthermore, there is a precedent for the association of β-lactamase activity with genes found to have other biological functions. The amino acid sequence deduced from the nucleotide sequence of the region immediately upstream of the cytochrome *c* oxidase subunit II from *Paracoccus denitrificans* was found to have a very high degree of homology to gram-negative β-lactamases and codon usage more typical of *E. coli* (3), although *P. denitrificans* is not known to contain a β-lactamase. With *Streptomyces cacaoi*, nucleotide sequence analysis of the β-lactamase coding region revealed several open reading frames which contain sequences typical of DNA-binding activator proteins, and a minimal active site serine region but little homology to other well-characterized enzymes with β-lactamase activity (4). Thus, it is not unprecedented to find portions of genetic elements associated with β-lactamase activity which do not conform to the genuine enzymes which have been so well characterized by Dr. Joris and his collaborators. The ability of *P. cepacia* to acquire and express genes from diverse sources undoubtedly contributes to its wide metabolic capabilities and success as an opportunistic pathogen.

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