The ORF1 Gene Located on the Class-1-Integron-Associated Gene Cassette Actually Represents a Novel Fosfomycin Resistance Determinant

The class 1 integrons are genetic elements capable of integrating gene cassettes by a site-specific recombination mechanism (1). Gene cassettes are mobile units composed of a gene, most often an antibiotic resistance gene, and a recombination site, the 59-base element (1). We have previously characterized a class 1 integron containing a VIM-2-type metallo-β-lactamase gene, blvM-2, an aacA4 gene, and an unknown function gene, designated as ORF1, which was identified in a Pseudomonas aeruginosa clinical isolate strain Mβ-7 (6). Partridge and Hall (5) recently searched using the predicted sequences of these search results, they proposed that both ORF1 and orf “i” genes are likely to confer fosfomycin resistance. To prove this hypothesis, we cloned the ORF1 gene and constructed an Escherichia coli transformant to examine its antibiotic resistance phenotype.

The ORF1 gene was amplified from P. aeruginosa strain Mβ-7 by PCR using primers ORF1 Exp5 EcoRI (5’-TCG GAA TTT GAT TAT CGG CAT CAA CCA C-3’) and ORF1 Exp3 HindIII (5’-TAA AGT CTG CGT CAT GGC ACC ACC GGC TT-3’). These primers were designed to clone the ORF1 gene to be in frame with the lacZ gene on the cloning vector pBCKS+ to construct transformant able to express the ORF1 fusion protein. Since these primers prime at the beginning and the end of the coding region, regulatory signals from the original P. aeruginosa gene were eliminated. The amplified fragment containing the ORF1 gene was digested with EcoRI and HindIII and cloned into pBCKS+ to form the recombinant plasmid, ORF1: pBCKS+. E. coli DH5 was transformed with the ORF1: pBCKS+ plasmid to form E. coli DH5 ORF1:pBCKS+. The transformants were selected on L agar plates containing 30 μg/ml chloramphenicol and 20 mM IPTG (isopropyl-β-d-thiogalactopyranoside). Antibiotic resistance phenotypes of the E. coli DH5 ORF1:pBCKS+, P. aeruginosa strain Mβ-7, and E. coli DH5 containing pBCKS+ (E. coli DH5 pBCKS+) were determined by the broth microdilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (3, 4) using commercially available plates, Dry-plate “Eiken” DP-21 and DP-25 (Eiken Kagaku Co., Tokyo, Japan).

P. aeruginosa strain Mβ-7 and E. coli DH5 ORF1:pBCKS+ showed a >32 μg/ml MIC of fosfomycin, while the control strain E. coli DH5 pBCKS+ was sensitive to this antibiotic. These results prove the hypothesis proposed by Partridge and Hall (5) that the ORF1 gene is the novel fosfomycin resistance determinant. It is important to clarify prevalence of this novel fosfomycin resistance determinant.

REFERENCES

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