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Letter to the Editor

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, bla_{VIM-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 the proteins encoded by gene cassette-encoded open reading frames and revealed that two proteins encoded by *ORF1* and *orf "i"* (2) genes showed a similarity with the amino acid sequence, along with the presence of a number of conserved key amino acids, of known fosfomycin resistance determinants including FosA, FosB, PA1129, lmo1702, and mlr3345. Based on 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 TTC AAT GAT TAC CGG CAT CAA TCA C-3′) and ORF1 Exp3 HindIII (5′-TTA AAG CTT CGT CAG CTC CAC ACC AGC CCC TT-3′). These primers were designed to clone the *ORF1* gene to be in frame with the *lacZ* gene on the cloning vector pBCSK+ to construct transformant able to express the ORF1 fusion protein. Since these primers prime at the beginning and the end of the *ORF1* coding region, respectively, all 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 pBCSK+ to form the recombinant plasmid, ORF1:pBCSK+. *E. coli* DH5 was transformed with the ORF1:pBCSK+ plasmid to form *E. coli* DH5 ORF1:pBCSK+. 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:pBCSK+, *P. aeruginosa* strain Mβ-7, and *E. coli* DH5 containing pBCSK+ (*E. coli* DH5 pBCSK+) 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, Dryplate "Eiken" DP-21 and DP-25 (Eiken Kagaku Co., Tokyo, Japan).

P. aeruginosa strain Mβ-7 and *E. coli* DH5 ORF1:pBCSK+ showed a >32 μg/ml MIC of fosfomycin, while the control strain *E. coli* DH5 pBCSK+ 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.

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