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linG, a new integron-associated gene cassette encoding a lincosamide nucleotidyltransferase

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Abstract

Lincosamide antibiotics include lincomycin, a compound produced by several actinomycetes, and its semisynthetic chlorinated derivative clindamycin. These antibiotics block the peptidyltransferase activity of the 50S subunit of the bacterial ribosome, inhibiting protein synthesis, and are active against most gram-positive cocci and anaerobes. However, they are not generally effective against gram-negative bacilli due to intrinsic resistance.

Keywords

lincosamide, encoding, cassette, gene, associated, integron, nucleotidyltransferase, ling

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Letters to the Editor

linG, a New Integron-Associated Gene Cassette Encoding a Lincosamide Nucleotidyltransferase

Lincosamide antibiotics include lincomycin, a compound produced by several actinomycetes, and its semisynthetic chlorinated derivative clindamycin. These antibiotics block the peptidyltransferase activity of the 50S subunit of the bacterial ribosome, inhibiting protein synthesis, and are active against most gram-positive cocci and anaerobes. However, they are not generally effective against gram-negative bacilli due to intrinsic resistance (3, 5).

Resistance to lincosamides is most commonly due to N^6 dimethylation of an adenine residue in the 23S rRNA, which usually confers broad-spectrum cross-resistance to macrolides, lincosamides, and streptogramin B antibiotics or to efflux (5). However, antibiotic inactivation by nucleotidylation (1, 2) has also been described as a mechanism of resistance. Despite the fact that lincosamides are not used to treat enterobacterial infections, a gene, *linF*, that confers low levels of resistance to both lincomycin (fourfold) and clindamycin (twofold) was recently found in a gene cassette in a class 1 integron recovered from an *Escherichia coli* blood isolate (4). The *linF* gene encodes a 273-amino-acid lincosamide nucleotidyltransferase. The LinF protein shares approximately 35% identity with the nucleotidyltransferases encoded by the *linB* gene from *Enterococcus faecium* (1) (GenBank accession no. AF110130) and *linB′* from *Enterococcus faecalis* (GenBank accession no. AF408195).

We have identified a second *lin* gene in a gene cassette. This cassette was recovered from a multiply antibiotic-resistant *Salmonella enterica* serovar Stanley strain (SRC54) isolated in

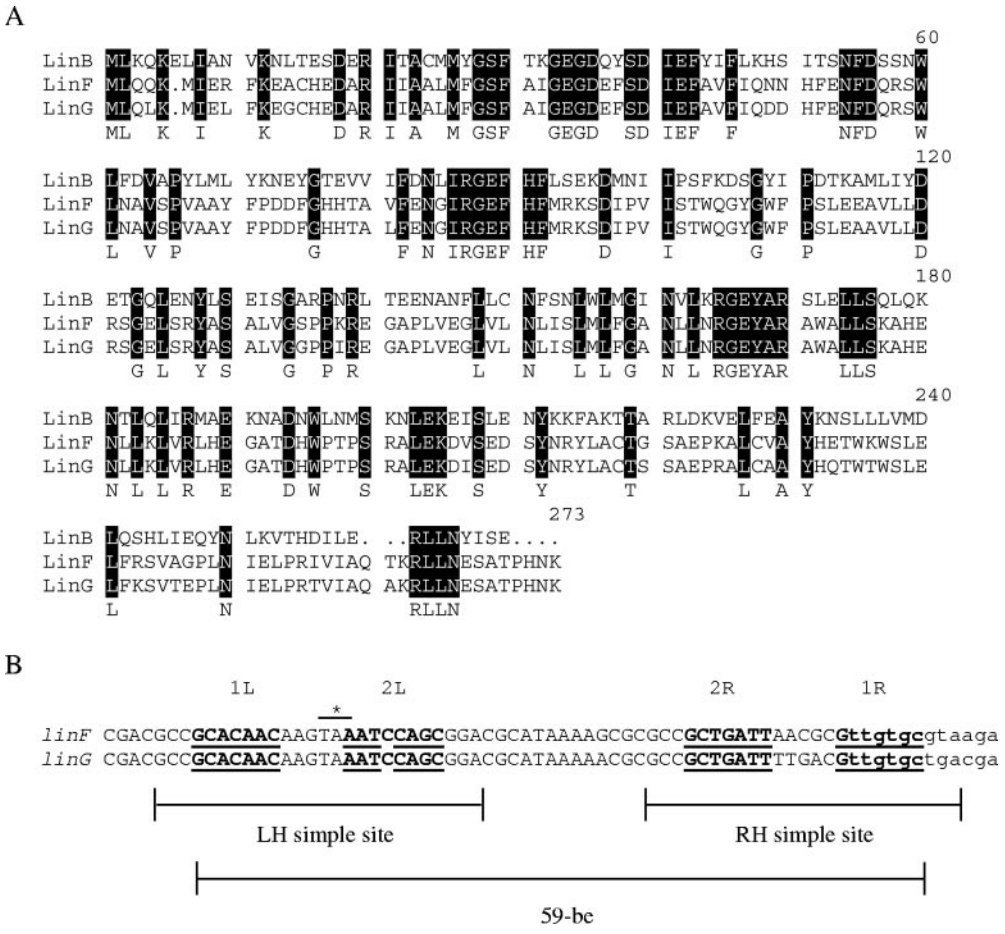

FIG. 1. Analysis of LinG sequences. (A) Alignment of Lin proteins. Amino acids that are completely conserved across all sequences are shown as white letters on a black background. The protein sequences of LinB and LinF were from GenBank accession numbers AF110130 and AJ561197, respectively. The LinG protein sequence is from this study. (B) Alignment of *linF* and *linG* 59-be. The core sites are in bold and are labeled 1L, 1R, 2L, and 2R (7). The boundaries of the LH (left-hand) and RH (right-hand) simple sites and the 59-be are indicated by bars. The bases in lowercase are those derived from the beginning of the cassette. The stop codons of the LinG and LinF proteins are indicated by the asterisk. The sequence of each 59-be came from the sources mentioned for panel A.

2001 from a traveler who had recently returned from Thailand. This strain was resistant to chloramphenicol, gentamicin, kanamycin, spectinomycin, streptomycin, sulfathiazole, and tetracycline but susceptible to ampicillin and nalidixic acid at levels described previously (6). It displayed intermediate resistance to ciprofloxacin. The gene cassette array was amplified by using standard primers (L2 and R1) in the 5′ conserved sequence and 3′ conserved sequence of class 1 integrons, and the 2.25-kb amplicon was cloned into pPCRscript and sequenced as previously described (see reference 6 for primer details). *E. coli* strain DH5α containing pPCR-Script with the cassette array was at least 10-fold more resistant to lincomycin (MIC, ≥2,000 μg/ml) than DH5α containing only pPCR-Script (MIC, 180 μg/ml).

The first cassette in the array was identical to the *aadA2* cassette in GenBank accession no. L06822. The second cassette was 937 bp long and 93.4% identical to the *linF* gene cassette. It encoded a 273-amino-acid protein that is 93.1% identical to LinF (17 amino acid differences). An alignment of these proteins with LinB is shown in Fig. 1A. The sequence of the *aadA2-linG* cassette array was identical to a region found in GenBank accession no. AY522431. The 59-base elements (59-be; *attC* sites) of the *linG* and *linF* cassettes are 58 bp long (Fig. 1B) and not closely related to any other known 59-be. They retain the critical features of 59-be, namely, complementary sites 1L-1R and 2L-2R (7).

Nucleotide sequence accession numbers. The sequence of the *aadA2-linG* cassette array has been deposited in GenBank under accession no. DQ836009.

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