**fexA**, a Novel *Staphylococcus lentus* Gene Encoding Resistance to Florfenicol and Chloramphenicol

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The *Staphylococcus lentus* plasmid pSCFS2 carries a novel florfenicol-chloramphenicol resistance gene, designated *fexA*, encoding a protein of 475 amino acids with 14 transmembrane domains. The FexA protein differs from all previously known proteins involved in the eflux of chloramphenicol and florfenicol. Induction of *fexA* expression by chloramphenicol and florfenicol occurs via translational attenuation.

Florfenicol is a fluorinated derivative of chloramphenicol which was licensed in Germany for the control of bacterial respiratory tract infections in 1995 for cattle and in 2000 for swine. In contrast to chloramphenicol, florfenicol is exclusively used in veterinary medicine (11), and the known chloramphenicol acetyltransferases are unable to inactivate florfenicol (6). While chloramphenicol acetyltransferase genes (cat genes) have been detected in a wide variety of gram-positive bacteria, including staphylococci of human and animal origin (5, 16), only a single gene, cfr, from bovine *Staphylococcus sciuri*, has been observed to mediate combined resistance to florfenicol and chloramphenicol by a yet-unknown mechanism (13). The cfr gene is located on the 17-kb plasmid pSCFS1, which also confers resistance to macrolide-lincosamide-streptogramin B antibiotics and spectinomycin (12).

In this study, the bovine *Staphylococcus lentus* isolate no. 8, obtained from the nasal swab of a calf suffering from a respiratory tract infection, was shown to be resistant to chloramphenicol, clindamycin, erythromycin, florfenicol, streptomycin, and tetracycline by agar disk diffusion (7) and was shown to carry six plasmids of ca. 2 to 34 kb. After transformation into protoplasts of *Staphylococcus aureus* RN4220, a 34-kb plasmid, designated pSCFS2, mediated only resistance to florfenicol and chloramphenicol. MICs for *S. aureus* RN4220:pSCFS2 were 32 μg of florfenicol/ml and 64 μg of chloramphenicol/ml (Table 1) as determined by the microdilution broth method (7). Preincubation in the presence of either 0.5 μg of chloramphenicol/ml or 0.5 μg of florfenicol/ml led to a fourfold increase of both MICs (Table 1). Since PCR assays and hybridization experiments did not reveal the presence of the cfr gene, cloning experiments using BglII-digested pSCFS2 DNA and BamHI-digested vector pBluescript II SK(+) (Stratagene, Amsterdam, The Netherlands) were performed. After transformation into *Escherichia coli* JM109, only transformants that carried a 7-kb BglII insert grew on Luria-Bertani agar plates supplemented with 10 μg of florfenicol/ml. They exhibited MICs of 16 μg of florfenicol/ml and 64 μg of chloramphenicol/ml, which could be increased to 64 μg of florfenicol/ml and 128 μg of chloramphenicol/ml, respectively, by preincubation in the presence of either 0.5 μg of chloramphenicol/ml or 0.5 μg of florfenicol/ml (Table 1). These observations suggested that the resistance gene in question is expressed inducibly in gram-positive and gram-negative hosts and that both antimicrobial agents, florfenicol and chloramphenicol, are effective as inducers.

Within the 7-kb BglII fragment, a single EcoRI site was detected. Subclones which carried EcoRI/BglII inserts of 5.2 and 1.8 kb proved to be susceptible to florfenicol and chloramphenicol, suggesting that this EcoRI site is located either within the gene in question or in its regulatory region. Sequence analysis was performed on both strands by primer walking, starting at the EcoRI sites of both subclones and using the M13 universal and reverse primers. The nucleotide sequence of a 1,674-bp fragment of plasmid pSCFS2 was determined. Analysis of this region confirmed the presence of two open reading frames (ORFs), one of them coding for a protein of 475 amino acids (aa) (position 177 to 1604), the other coding for a small peptide of 9 aa (position 118 to 147) preceding the aforementioned ORF.

The reading frame for the 475-aa protein was designated *fexA* (for florfenicol exporter), and the EcoRI site was located within this reading frame at position 1334. Analysis of the *fexA* upstream region revealed striking homologies to the translational attenuators upstream of chloramphenicol-inducible staphylococcal *cat* genes (4) and also to that of the chloramphenicol-inducible gene *cmlA* from Tn1696 (14). In addition to the reading frame for the 9-aa peptide, a pair of inverted repeated sequences (IR1 and IR2) of 11 bp was detected. These inverted repeats might be able to form a stable mRNA stem-loop structure (ΔG = −74.4 kJ/mol) (15) with the *fexA*-associated ribosome binding site (5′-AGGAGG-3′; position 164 to 169) located within IR2 (Fig. 1a). The sequence of the codons 2 to 5 of the 9-aa peptide (5′-GTGAAAGCAGTG-3′;
aureus 16S rRNA are displayed in bold capital letters, whereas mismatch. Matching bases in the stall sequences with regard to the stability of the binding (15), such pairing was not considered a mismatch. Stalling of a ribosome in the reading frame of the 9-aa peptide might prevent the formation of an mRNA secondary structure between IR1 and IR2 and thus also allow translation of the fexA transcript by a second ribosome.

Comparisons of the fexA nucleotide sequence using the National Center for Biotechnology Information standard nucleotide BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) revealed no significant homology to known sequences. The TMpred program (http://www.ch.embnet.org/software/TMPRED_form.html) predicts that the FexA protein has 14 transmembrane helices. This feature is seen in a wide variety of efflux proteins of the major facilitator superfamily (8), including a cluster of efflux proteins from gram-positive bacteria which confer resistance to antimicrobial agents such as tetracyclines and lincomycin. The deduced FexA amino acid sequence reveals only minor homologies to other proteins deposited in the databases. Closest similarities of 28 or 29% amino acid sequence identity were observed between FexA and a multidrug efflux protein from Lactobacillus planatarum (accession no. NP_84696.1) as well as a metal-tetracycline/H+ antiporter from Bacillus halodurans (NP_242832.1). Comparisons of the amino acid sequences revealed no more than 19% amino acid identity between FexA and known chloramphenicol or florfenicol/chloramphenicol exporter proteins (Fig. 2). Thus, FexA represents a novel type of florfenicol/chloramphenicol efflux system which is distinctly different from those of the FloR superfamily and the CmlA subgroup, both found in clinically important gram-negative bacteria, as well as from the chloramphenicol exporters so far detected in soil and environmental bacteria of the genera Streptomyces, Corynebacterium, and Rhodococcus (Fig. 2).

The detection of the florfenicol-chloramphenicol resistance gene fexA in staphylococci is to the best of our knowledge the first report of a gene coding for a florfenicol efflux protein in staphylococci. The location of this gene on a plasmid and the observation that it is functionally active even in E. coli suggest a potential transfer of fexA-mediated resistance between members of different bacterial species and genera.

**Nucleotide sequence accession number.** The sequence of the fexA gene has been deposited with the EMBL database under accession number AJ549214.
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