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satG, Conferring Resistance to Streptogramin A, Is Widely Distributed in *Enterococcus faecium* Strains but Not in Staphylococci

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A gene almost identical to *satG* was isolated from an *Enterococcus faecium* strain. This gene was transferred to a *Staphylococcus aureus* recipient strain where it conferred resistance to streptogramin A. *satG* was found to be widely distributed among *E. faecium* strains but not detected among staphylococci.

Streptogramin, virginiamycin, pristinamycin, and synergistin are produced by *Streptomyces* and consist of synergistic mixtures of two chemically different molecules: A and B compounds (10). In some European countries and in Algeria, these mixtures are used both orally and topically, mostly against staphylococcal infections. Virginiamycin is used as a growth promoter in animal feed in Europe and in the United States. Virginiamycin-resistant *Enterococcus faecium* is prevalent in fecal and intestinal samples from turkeys, pigs, broilers, and farmers in Europe and America (1, 14, 19, 20, 21). As bacteria can be transferred via food from animals to humans, this is alarming, in particular because quinupristin-dalfopristin (see supplement to J. Antimicrob. Chemother. volume 30 [1992]), an injectable mixture of semisynthetic streptogramins soon to be released for commercial use, is expected to be widely used, mainly to treat vancomycin-resistant *E. faecium* infections.

The *satA* gene (18) encoding an acetyltransferase that inactivates streptogramin A compounds was identified as part of a plasmid from an *E. faecium* isolate. It was found in only 29% of the 140 tested *E. faecium* strains isolated in Dutch and Danish farms and resistant to the above-mentioned mixtures (13, 14). Five of the *E. faecium* strains isolated in Denmark harbored a large plasmid which conferred resistance to the mixtures and which was transferable by filter mating experiments to an *E. faecium* recipient (13). None of the transconjugants harboring these plasmids carried *satA*, *vat*, *vatB*, *vga*, or *vgaB* (13). These results suggested that the *E. faecium* strains contained another, unidentified streptogramin A resistance gene(s). We investigated this possibility.

The 51 *E. faecium* strains included in this study (MICs of virginiamycin ranged from 8 to 32 mg · liter^{-1}) were those described by Jensen et al. (14). They were isolated from fecal samples from poultry ($n = 22$), pigs ($n = 5$), farmers ($n = 19$), and suburban residents ($n = 5$) in the Netherlands (Table 1). *satA* was previously found in 19 strains, and *vgb* was found in a single strain by PCR (14). The *E. faecium* strains were analyzed by Southern hybridization at high stringency (65°C) (2) with intragenic probes to screen for the following eight genes, previously found in staphylococcal and enterococcal plasmids conferring resistance to the mixtures: *satA* (18) (with primers *satA1* at nucleotide [nt] positions 189 to 210 and *satA2* at nt positions 760 to 782 [accession no. L12033]), *vat* (9), *vatB* (3), *vatC* (6), *vga* (7), and *vgaB* (4) conferring resistance to A compounds and *vgb* (8) and *vgbB* (6), encoding lactonases which hydrolyze B compounds. A total of 19 of the strains carried *satA*, and the combination of *vat* and *vgb* was detected in a single strain, KH6 (Table 1). *vat* and *vgb* were contiguous and in the same relative position in KH6 as in the staphylococcal plasmids in which the *vat-vgb* combination is carried by a DNA fragment originating from the *E. faecalis* plasmid pAMß1 (5).

A total of 31 of the tested *E. faecium* strains did not hybridize to any of the eight gene probes tested. PCR experiments were carried out at a low annealing temperature (40°C) with a pair of degenerate primers, M and N (3, 16), designed to amplify a DNA fragment from any sequence encoding a streptogramin A acetyltransferase containing two well-conserved motifs, III and IV (3, 6, 16). A DNA fragment of the expected size (147 nt) was amplified from the cellular DNA of all the strains. The amplicon obtained with strain K14 was sequenced with oligonucleotides M and N as the primers. Its sequence was only 60.4 to 68.6% similar to those of the SgA acetyltransferase genes (*vat*, *vatB*, *vatC*, and *satA*), suggesting that the amplicon was from a different gene. A 5-kb *Hin*dIII fragment hybridizing with the sequenced amplicon was isolated from the cellular DNA of strain K14 and inserted into the *Hin*dIII site of pUC18. The resulting plasmid, pIP1798, was used to sequence 1,080 nt of the insert, including the sequences hybridizing with the 147-bp amplicon.

The sequence contains a 642-bp gene including an ATG start codon preceded, 6 nt upstream, by a putative ribosome-binding site. The calculated free energy of association of the most stable structure between this site and the 3′ terminus of the 16S rRNA is -61.5 kJ · mol^{-1}. This gene, not distinguishable from the recently described *satG* (22), is similar to those encoding SgA acetyltransferases, *satA*, *vat*, *vatB*, and *vatC* (54.3, 58.0, 60.0, and 60.1% similarity, respectively). *satG* encodes a putative 214-amino-acid protein of 23,775 Da similar to xenobiotic acetyltransferases (17). It is most similar to the SgA acetyltransferases, SatA, Vat, VatB, and VatC (48.5, 50.0, 59.9, and 50.9% identical amino acids, respectively).

Most *vat*-related genes in staphylococcal plasmids are contiguous to and downstream from another streptogramin resistance (Sg^{r}) gene. The pairs of genes are probably cotranscribed (12). However, analysis of the 270- and 170-nt sequences flanking *satG* did not suggest the presence of any contiguous Sg^{r} gene.

A DNA fragment of 885 nt containing *satG* (nt 98 to 982) was amplified from pIP1798 with a primer (nt 957 to 982) containing a single *Hae*III site and a primer (nt 98 to 120) whose sequence was modified to create an *Eco*RI site. This amplicon

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TABLE 1. Relevant characteristics of the 51 *E. faecium* strains isolated in The Netherlands

Sg^r gene(s)	Size (kb) of the hybridizing *Hin*dIII fragment	Strain origin(s) (no. of isolates and city)
satA	2.3[a]	Turkey farmer (1)
	3.8[a]	Pigs (2, Weert)
	3.9	Turkey farmer (1)
	3.9[a]	Turkey farmer (1), chicken farmers (2), suburban inhabitants (1, Weert; 2, Roermond)
	4.0[a]	Broiler (1), chicken farmer (1), suburban inhabitant (1, Weert)
	4.3[a]	Chicken farmer (1)
	4.5[a]	Chicken farmer (1), pig (1, Weert)
	5.6[a]	Chicken farmer (1)
	6.0[a]	Turkey (1)
	7.0[a]	Broiler (1)
satG	1.4	Turkeys (3)
	1.8	Pigs (2, Weert)
	1.9	Suburban inhabitant (1, Weert)
	2.0	Chicken farmer (1), turkey farmer (1)
	2.0[a]	Chicken farmer (1)
	2.3	Turkey farmer (1)
	2.5	Turkey farmers (2)
	2.5[a]	Turkey farmer (1)
	3.6[a]	Broilers (3), turkey (1)
	4.3[a]	Broilers (5)
	5.0[a]	Turkeys (4), turkey farmer (1), broilers (2)
	7.3[a]	Turkey farmer (1)
	>10.0[a]	Broiler (1)
satG plus *vat-vgb*	4.6[a] 8.9	Turkey farmer (1)[b]

[a] This fragment was among those detected in extrachromosomal DNA bands (≥40 kb) migrating above the chromosomal DNA fragments of the uncleaved total cellular DNA by agarose gel electrophoresis in Tris-acetate buffer. In the other strains, the hybridizing bands comigrated with the chromosomal fragments, but the hybridization signals were as strong as those of the extrachromosomal DNA, suggesting that they may be carried by plasmids.

[b] Strain KH6 was the only strain in which the *vat-vgb* combination was detected.

was cleaved with *Hae*III and *Eco*RI and inserted between the *Eco*RI and *Sma*I sites of the shuttle vector, pOX7 (11). The resulting plasmid, pIP1801, introduced by electroporation into the *Staphylococcus aureus* recipient, RN4220 (15), conferred resistance to pristinamycin IIA [MICs were as follows: 2 mg · liter^{-1} for RN4220(pOX7) and 8 mg · liter^{-1} for RN4220(pIP1801)].

The presence of *satG* in other strains was tested by Southern hybridization experiments using high stringency conditions (2) and a DNA fragment amplified from *satG* by PCR with the following pair of primers: *satG*-F (nt positions 354 to 378 in *satG*) and *satG*-R (nt positions 878 to 899 in *satG*) (accession no. AF153312). DNA hybridizing with *satG* probe was detected in the 32 strains which did not carry *satA*, including the strain containing *vat-vgb* (Table 1). Total cellular DNA of strain KH6 was subjected to agarose gel electrophoresis. The *satG* and *vat-vgb* sequences migrated to different positions, suggesting that they are not carried by the same plasmid. None of the 53 different *S. aureus* strains resistant to streptogramin A and described previously (2) contained DNA hybridizing with *satG*.

The distribution of the streptogramin resistance genes in the collection of *E. faecium* studied was clearly different from that found in staphylococci (2). None of the *satA* or *satG* genes found to be prevalent among *E. faecium* strains was found in staphylococci.

Nucleotide sequence accession number. Sequence data from this study has been registered in the GenBank EMBL Data Library under accession no. AF153312.

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