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Letters to the Editor

First VanD-Type Vancomycin-Resistant *Enterococcus raffinosus* Isolate[▽]

The vancomycin-resistant *Enterococcus raffinosus* strain GV5 was resistant to vancomycin (MIC, 1,024 μg/ml) and teicoplanin (MIC, 256 μg/ml). The species of strain GV5 was determined by sequencing of a specific PCR product for the 16S rRNA gene of *E. raffinosus*. GV5 was isolated from a stool specimen and from a bedsore on the necrotic inferior limb of a diabetic 73-year-old man in Japan.

DNA sequence analysis of the *vanD* operon was performed by sequencing the PCR products with primers specific for each gene in the *vanD4* operon of *E. faecium* 10/96A (8); it showed that GV5 encodes a 5,654-bp *vanD* gene cluster consisting of $vanR_DS_DY_DH_DDX_D$, which is homologous to the corresponding genes in the reported VanD-type strains and is located on the chromosome (accession no. AB242319) (3, 6, 7, 9). The *vanD* gene cluster was compared with that of the corresponding genes of the *vanD4* gene cluster of *E. faecium* 10/96A (Fig. 1) (8). $vanR_D$ and $vanX_D$ were completely identical to the equivalent genes in 10/96A. There was one amino acid substitution in both $VanH_D$ and VanD, where Ile_{169} was converted to Phe and Gly_{121} was converted to Val, respectively. The reported $VanS_D$ contains five blocks of the conserved sequences H, N, G1, F, and G2 (2, 4, 8), which are contained in phosphate transmitters of two-component regulator systems (1, 11). Block H sequences consist of the residues L_{164}AHDL KTPLS$_{173}$, including a putative autophosphorylation site, His_{166} (14). The Thr_{170} residue in the block H sequence has been replaced by Ile in $VanS_D$ of GV5, suggesting that this mutation might result in the constitutive expression of resistance due to impaired $VanS_D$ function to dephosphorylate phosphorylated $VanR_D$. $vanY_D$ of GV5, which has a molecular size of 1,068 bp, is completely identical to that of 10/96A with the exception of an additional adenosine insertion in $vanY_D$ of 10/96A (8). The nucleotide sequence from position 346 to position 354 of GV5 $vanY_D$ is C_{346}AAAAAAAC$_{354}$, and the

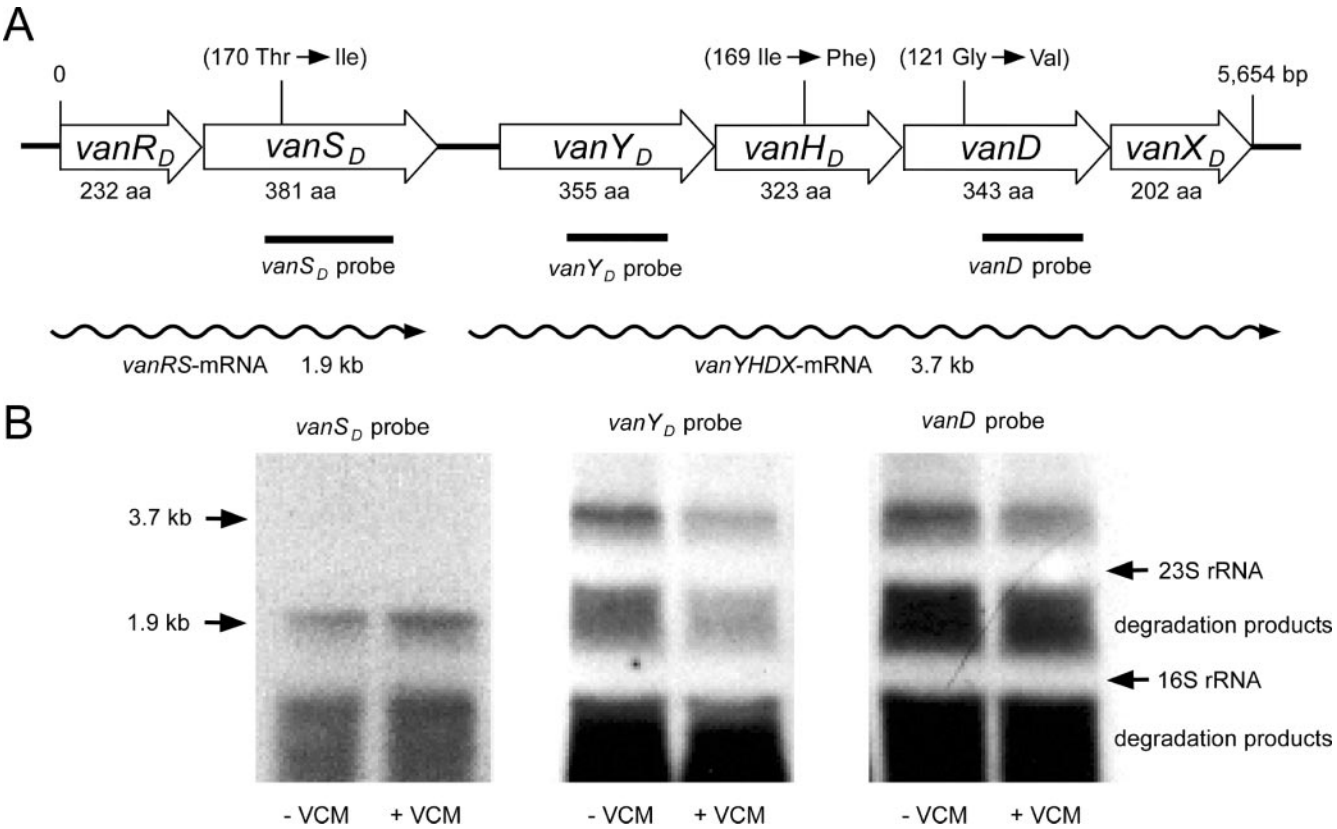

FIG. 1. Schematic representation of the *vanD* gene cluster from *E. raffinosus* strain GV5 and Northern blot analysis of the *vanD* cluster. (A) Open arrows represent coding sequences and indicate the direction of transcription. The PCR fragments internal to the $vanS_D$, $vanY_D$, and *vanD* genes used in the hybridization experiments are indicated below the corresponding regions. Amino acids with arrows within parentheses indicate substitutions compared with the reported sequence of the *vanD4* operon of *E. faecium* 10/96A (8). (B) Northern hybridization was performed according to a protocol described previously (13). RNAs were prepared from strains cultured with 6 μg/ml of vancomycin (+VCM) or without vancomycin (−VCM) for 2 h. Thirty micrograms of RNA was used in each lane. The sizes of the RNAs were determined by using the sizes of RNA molecular weight markers (Invitrogen, Inc.), and the arrows and the numbers on the left indicate the positions and sizes of the largest bands in each experiment.

sequence from position 346 to position 355 of 10/96A $vanY_D$ is $C_{346}AAAAAAAAC_{355}$. If an adenosine residue were inserted within the seven adenosines located between nucleotides 346 and 354 of GV5 $vanY_D$, the codon sequence at positions 415 to 417 of the resulting gene would become the TGA translation stop codon as a result of the frameshift mutation, and translation would be terminated prematurely after amino acid 138, as in the $VanY_D$ protein of 10/96A (8).

In Northern hybridizations with $vanY_D$ and *vanD* probes, identical bands of about 3.7 kb in size, which correspond to the transcript of $vanY_DH_DDX_D$ (5), were observed in both the absence and the presence of vancomycin (Fig. 1). The $vanS_D$ probe detected an approximately 1.9-kb band, which corresponds to the size of the transcript of $vanR_DS_D$, in the absence and presence of vancomycin (Fig. 1). These results indicate that the *vanD4* cluster in GV5 is expressed constitutively (2, 3, 6, 9, 12).

Analysis of the D-Ala:D-Ala ligase gene (*ddl*) on the chromosome of strain GV5 (accession no. AB242318) revealed that there are two amino acid substitutions—Asn_{271} is converted to Asp, and Gly_{319} is converted to Asp—compared to the wild-type DDL of *E. raffinosus* JCM8733 (accession no. AB242317), which implies that the amino acid substitutions might result in impaired function of GV5 DDL (10).

Several VanD-type vancomycin-resistant enterococci have been identified among *E. faecium* and *E. faecalis* (3, 6, 7, 9). We have described the first VanD-type *E. raffinosus* strain and showed evidence that there is species divergence in enterococci that encode VanD resistance as well as nucleotide divergence between the VanD determinants (3, 6, 7, 9).

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