The First VanD-type Vancomycin-Resistant

*Enterococcus raffinosus*

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Vancomycin resistant *Enterococcus raffinosus* GV5 was resistant to vancomycin (MIC, 1024 µg/ml) and teicoplanin (MIC, 256 µg/ml). The species of GV5 strain was determined by sequencing of specific PCR product for 16S rRNA gene of *E. raffinosus*. The 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 specific primers for each gene in the VanD4 operon of *E. faecium* 10/96A (8), and showed that GV5 encoded a 5,654 bp VanD gene cluster consisting of *vanR<sub>D</sub>* / *S<sub>D</sub>* / *Y<sub>D</sub>* / *H<sub>D</sub>* / *D* / *X<sub>D</sub>* which are 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). The *vanR<sub>D</sub>* and *vanX<sub>D</sub>* were completely identical to the equivalent genes in 10/96A. There was one amino acid substitution in both VanH<sub>D</sub> and VanD, where Ile<sub>169</sub> was converted to Phe and Gly<sub>121</sub> was converted to Val, respectively. The reported VanS<sub>D</sub> contain 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 system (1, 11). Block H sequences consist of $^{164th}$L AHDLKTPL $^{172th}$s residues including putative autophosphorylation site His at position 166 (4). Thr$^{170}$ residue in the “H” block sequences had been substituted by Ile in VanSp of GV5, suggesting that this mutation might result in the constitutive expression of the resistance due to impaired VanSp function to dephosphorylate phosphorylated VanRd. The $vanY_d$ of GV5, which had a molecular size of 1,068 bp, was completely identical to that of 10/96A, with the exception of an additional adenosin (A) insertion in the $vanY_d$ of 10/96A (8). The nucleotide sequence from position 346 to 354 of the GV5 $vanY_d$ was $^ {346th} C$ AAAAAAAA $^ {354th} C$ and the sequence from position 346 to 355 of 10/96A $vanY_d$ was $^ {346th} C$ AAAAAAAA $^ {355th} C$. If an adenosine residue was inserted within the seven adenosines located between nucleotide 346 and 354 of GV5 $vanY_d$, the codon sequence at position 415 to 417 of the resulting gene would become the TGA translation stop codon as a result of the frameshift mutation and the translation would be terminated prematurely after amino acid 138, as in the $vanY_d$ protein of 10/96A (8).

In Northern hybridization with $vanY_d$ and $vanD$ probes, identical
hybridization bands with about 3.7 kb in size, which corresponded to transcript of \textit{vanY_DH_DX_D} (5), were observed both in the absence and the presence of vancomycin (Fig.1). The \textit{vanS_D} probe detected about 1.9 kb band, which is corresponding to the size of transcript of \textit{vanR_DS}, in the absence and presence of vancomycin (Fig.1). These results indicate that the \textit{vanD4} cluster in GV5 is expressed constitutively (2, 3, 6, 9, 12).

Analysis of the D-Ala-D-Ala ligase gene (\textit{ddl}) on the chromosome of the GV5 strain (accession no. AB242318) revealed that there were two amino acid substitutions, Asn$^{271}$ was converted to Asp, and Gly$^{319}$ was converted to Asp compared to the wild type DDL of \textit{E. raffinosus} JCM8733 (accession no. AB242317), which implied that the amino acid substitutions might result in an impaired function of the GV5 DDL (10).

Several VanD type VREs have been identified in \textit{E. faecium} and \textit{E. faecalis} (3, 6, 7, 9). We have described the first VanD type \textit{E. raffinosus} and showed evidence that there is species divergence in enterococcus that encodes VanD resistance as well as nucleotide divergence between the VanD determinants (3, 6, 7, 9).
References


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Figure legends

Fig. 1 Schematic representation of the vanD gene cluster from *E. raffinosus* strain GV5 and Northern blot analysis of vanD cluster. (A), Open arrows represent coding sequences and indicate the direction of transcription. The PCR fragments internal to the vanSD, vanYD, and vanD genes used in the hybridization experiments are indicated below the corresponding regions. Amino acids with arrows within parentheses indicate amino acid substitutions compared with reported VanD4 of *E. faecium* 10/96A (8). (B), Northern hybridization was performed according to the protocol described previously (13). RNAs were prepared from the strains cultured with 6 µg/ml of vancomycin (+VCM) or without vancomycin (-VCM) for 2 hours. Thirty µg RNA was used in each lane. The sizes of RNA was determined by using sizes of RNA molecular weight markers (Invitrogen, Inc.), and the arrows and the numbers on the left indicate the position and size of the largest bands in each experiment.
A

\( \text{vanR}_D \rightarrow \text{vanS}_D \rightarrow \text{vanY}_D \rightarrow \text{vanH}_D \rightarrow \text{vanD} \rightarrow \text{vanX}_D \)

- \( \text{vanS}_D \) probe: 232 aa
- \( \text{vanY}_D \) probe: 381 aa
- \( \text{vanH}_D \) probe: 355 aa
- \( \text{vanD} \) probe: 323 aa
- \( \text{vanX}_D \) probe: 202 aa

\( \text{vanRS-mRNA} \) 1.9 kb
\( \text{vanYHDX-mRNA} \) 3.7 kb

(170 Thr \( \rightarrow \) Ile) (169 Ile \( \rightarrow \) Phe) (121 Gly \( \rightarrow \) Val) 5,654 bp

B

- \( \text{vanS}_D \) probe
- \( \text{vanY}_D \) probe
- \( \text{vanD} \) probe

- VCM
- + VCM

23S rRNA degradation products
16S rRNA degradation products