

## VanE, a New Type of Acquired Glycopeptide Resistance in *Enterococcus faecalis* BM4405

MARGUERITE FINES,<sup>1†</sup> BRUNO PERICHON,<sup>1\*</sup> PETER REYNOLDS,<sup>2</sup>  
DANIEL F. SAHM,<sup>3</sup> AND PATRICE COURVALIN<sup>1</sup>

Unité des Agents Antibactériens, Institut Pasteur, 75724 Paris Cedex 15, France<sup>1</sup>; Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, United Kingdom<sup>2</sup>; and MRL Pharmaceutical Services, Herndon, Virginia 20171<sup>3</sup>

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***Enterococcus faecalis* BM4405 was resistant to low levels of vancomycin (MIC, 16 µg/ml) and was susceptible to teicoplanin (MIC, 0.5 µg/ml). No PCR product was obtained when the total DNA of this clinical isolate was used as a template with primers specific for glycopeptide resistance genes *vanA*, *vanB*, *vanC*, and *vanD*. However, a 604-bp PCR fragment was obtained when V1 and V2 degenerate primers were used and total DNA was digested with *Hind*III as a template. The product was cloned and sequenced. The deduced amino acid sequence had greater identity (55%) with VanC than with VanA (45%), VanB (43%), or VanD (44%). This was consistent with the fact that BM4405 synthesized peptidoglycan precursors that terminated in D-serine residues. After induction with vancomycin, weak D,D-dipeptidase and penicillin-insensitive D,D-carboxypeptidase activities were detected in cytoplasmic extracts of BM4405, whereas a serine racemase activity was found in the membrane preparation. This new type of acquired glycopeptide resistance was named VanE.**

Glycopeptide antibiotics are used for the treatment of infections caused by gram-positive bacteria. They form a complex with the C-terminal D-alanyl-D-alanine (D-Ala-D-Ala) of peptidoglycan precursors and block their incorporation into the bacterial cell wall (15).

Glycopeptide-resistant enterococci have a broad geographical distribution and are phenotypically and genotypically heterogeneous. Three types of acquired resistance to glycopeptides have been described in enterococci (3). VanA-type strains display high-level inducible resistance to both vancomycin and teicoplanin following acquisition of transposon Tn1546 or closely related elements (2). VanB-type strains have variable levels of inducible resistance to vancomycin only (3). VanD-type strains are resistant to various levels of vancomycin and teicoplanin (14). In strains of all three phenotypes, glycopeptide resistance is due to synthesis of peptidoglycan precursors that terminate in D-lactate (D-Lac) instead of D-Ala (5).

The VanA, VanB, and VanD ligases synthesize the depsipeptide D-Ala-D-Lac. In VanA- and VanB-type strains three other enzymes are required for resistance: the VanH dehydrogenase reduces pyruvate to D-Lac (6), whereas the VanX D,D-dipeptidase and the penicillin G-insensitive VanY D,D-carboxypeptidase (3) prevent synthesis of UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala (pentapeptide[Ala]). The VanA and VanB types of resistance, but not the VanD type, are generally transferable to other enterococci by conjugation.

VanC-type resistance, characterized by low-level resistance to vancomycin, is specific to *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens* (11, 13). The *vanC* gene product synthesizes D-Ala-D-serine (D-Ala-D-Ser), which is substituted for D-Ala-D-Ala in peptidoglycan precursors (18). Insertional inactivation of *vanC* in *E. gallinarum*

BM4174 led to vancomycin susceptibility and exclusive synthesis of precursors that end in acyl-D-Ala-D-Ala, indicating that intrinsically resistant enterococci also produce a D-Ala:D-Ala ligase and that *vanC* is necessary for expression of vancomycin resistance (7, 18). The level of synthesis of pentapeptide[Ala] in BM4174 is low due to the presence of weak D,D-dipeptidase and D,D-carboxypeptidase activities (18). It has been shown recently (i) that production in *E. gallinarum* BM4174 of VanXY<sub>c</sub>, a protein that has both D,D-dipeptidase and D,D-carboxypeptidase activities, allows hydrolysis of the dipeptide D-Ala-D-Ala and removal of the ultimate D-Ala from pentapeptide[Ala] and (ii) that the presence of *vanC1* and *vanXY<sub>c</sub>* is sufficient for resistance when D-Ser is added to the culture medium of *Enterococcus faecalis* JH2-2 into which the two genes had been introduced (17). A membrane-bound serine racemase, VanT, which produces D-Ser peptidoglycan synthesis in BM4174 has also been characterized (1).

We present in this report evidence that *E. faecalis* BM4405, which is resistant to low levels of vancomycin and which is susceptible to teicoplanin, is of a new glycopeptide resistance type which has similarities with the intrinsic VanC type of resistance.

### MATERIALS AND METHODS

**Strains.** *E. faecalis* BM4405 was isolated from the peritoneal dialysis fluid of a patient in a Chicago, Ill., hospital who was diagnosed with peritonitis and who had received vancomycin. *E. faecalis* JH2-2 is a derivative of strain JH-2, which is resistant to fusidic acid and rifampin (10).

**Antibiotic susceptibility testing.** MICs were determined by the agar dilution method on Mueller-Hinton agar (Diagnostics Pasteur, Marnes-la-Coquette, France) with an inoculum of 10<sup>8</sup> CFU per spot. Plates were incubated overnight at 37°C.

**Filter mating.** Transfer of vancomycin resistance from BM4405 to the recipient strain *E. faecalis* JH2-2 was attempted by filter mating (6) with selection on brain heart infusion (BHI) containing rifampin (20 µg/ml), fusidic acid (10 µg/ml), and spectinomycin (60 µg/ml) or vancomycin (4 µg/ml).

**DNA amplification, cloning, and sequencing.** Identification of strain BM4405 to the species level was performed by PCR (8). A PCR assay with primers specific for resistance genes *vanA*, *vanB*, *vanC1*, *vanC2*, and *vanD* (8, 14) was used to determine the glycopeptide resistance genotype of *E. faecalis* BM4405. Amplifications were carried out in a final volume of 100 µl containing 40 pmol of each oligonucleotide primer, 50 nmol of each 2'-5'-triphosphate deoxynucleoside, 100

\* Corresponding author. Mailing address: Unité des Agents Antibactériens, Institut Pasteur, 25, rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: (33) (1) 45 68 83 18. Fax: (33) (1) 45 68 83 19. E-mail: brunoper@pasteur.fr.

† Present address: Laboratoire de Microbiologie, CHU de la Côte de Nacre, 14033 Caen Cedex, France.

cag gga gta ttt gag tta tta gat att cca tat gta ggt tgt ggt atc gga gct gca gca  
 Q G V F F E L L L D I P Y V G G C G I G A A A  
 att ttc atg aat aaa ata atg ctc cat caa ttt gct gaa ata gtt ggt gta aaa agc atc  
 I S M N K I M L H Q F A E I V G V K S T  
 cct agt atg att ata gaa aag gga cag cta caa aaa gtc gat gaa ttt ggc aaa ata  
 P S M I I E K G Q D L Q K V D E F A K I  
 cat gga ttt cct tta tat atc aaa ccg aag gag gca ggc tca tca aaa gga att agc aag  
 H G F P L Y I K P N E A G S S K G I S K  
 gta gaa caa aaa agt gat tta tat aaa gca ata gac gaa gct tca aaa tat gat agc cgt  
 V E Q K S D L Y K A I D E A S K Y D S R  
 att tta att. caa aag gaa gtg aaa ggg gta gaa att. ggc tgt ggg att. tta ggg aat gaa  
 I L I Q K E V K G V E I G C G I L G N E  
 caa ttg gtc gtt gga gaa tgt gat caa att agt ctt gtg gat ggc ttt ttc gac tat gaa  
 Q L V V G E C D Q I S L V D G F F D Y E  
 gag aaa tac aat tta gta aca gca gaa att ttg tta cca gct aaa cta tca ata gac aaa  
 E K Y N L V T A E I L L P A K L S I D K  
 aaa gaa gac atc cag ata aaa gca aaa aaa cta tac aga cta tta ggg tgc aaa gga tta  
 K E D I Q I K A K K L Y R L L G C K G L  
 gcg aga atc gac ttt ttc tta acg gat gac gga gaa att tta tta aat gag atc aac acc  
 A R I D F F L T D D G E I L L N E I N T  
 ctc c  
 L

FIG. 1. Sequence of the 604-bp PCR product internal to the *vanE* gene of *E. faecalis* BM4405 and the corresponding amino acid sequence.

ng of template DNA, and 2 U of *Taq* DNA polymerase. The reactions were performed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) as described previously (8, 14). Amplification of fragments internal to genes encoding related ligases with degenerate V1 and V2 primers was performed as described previously (7). Prior to amplification, total DNA from BM4405 was digested for 2 h at 37°C with *Hind*III (Pharmacia LKB, Saint-Quentin-en-Yvelines, France). PCR fragments were purified on Microspin S-400 HR Columns (Pharmacia LKB), cloned into pCR2.1 (Invitrogen, Leek, The Netherlands), and sequenced by the dideoxynucleotide chain termination method (19) with T7 DNA polymerase (Pharmacia LKB) and  $\alpha$ -<sup>35</sup>S-dATP (Amersham Radiochemical Center). Specific amplification of a 513-bp fragment internal to *vanE* was obtained with primers VANE1 (5'-TGTGGTATCGGAGCTGCAG-3') and VANE2 (5'-GTTCGATTCTCGCTAATCC-3') under the following conditions: 30 s at 94°C, 30 s at 52°C, and 30 s at 72°C (30 cycles).

**Sequence analysis.** The programs included in the GCG package (Genetics Computer Group, Madison, Wis.) were used for sequence editing, translation, and alignment.

**Analysis of peptidoglycan precursors.** Analysis of the peptidoglycan precursors of BM4405 was performed as described previously (16). The strain was grown in BHI with or without vancomycin (8  $\mu$ g/ml) to the midexponential phase ( $A_{600} = 1$ ). Ramoplanin was added, and incubation was continued for 20 min. The bacteria were harvested by centrifugation (12,000  $\times$  g, 2 min, 4°C), and the cytoplasmic precursors which had accumulated were extracted with 8% trichloroacetic acid (15 min, 4°C), desalted, and analyzed by high-performance liquid chromatography.

|      |            |            |             |            |             |            |    |     |
|------|------------|------------|-------------|------------|-------------|------------|----|-----|
| VanE | QGVP       | ELLDIPYVGC | GIGANAISMN  | KIMLHQFAEI | VGKSTPSMI   | IEKGG...   | D  | 50  |
| VanC | QCLL       | ELMNLPIVGC | HVAASALCMN  | KWLHLQIADT | MGTASAPTLI  | LSRYE...   | ND | 51  |
| VanA | QCLP       | ELSGIPVGC  | DIQSSAICMD  | KSLEYIVAKN | AGIATPAFKY  | INKDD...   | RP | 51  |
| VanB | QCLP       | ELSGIPVGC  | DIQSSAACMD  | KSLEYIVAKN | AGIATPAFKY  | INKDD...   | RP | 51  |
| VanD | QCLL       | ELSGIPVGC  | DIQSSVICMD  | KALAYTVVKN | AGITVGFRI   | LQESD...   | RL | 48  |
| Efa  | QGFH       | ETINMPYVGA | GIVLASVNAM  | KIMPKYLLQT | VGIPQVFPV   | VLRSDWKGF  |    | 54  |
|      |            |            |             |            |             |            |    |     |
| VanE | LQKVDFAKI  | HGFFLYIKPN | BAGSSKGIK   | VEQKSDLYKA | IDEASKYDSR  | LIQKEVKGV  |    | 110 |
| VanC | PATIDRFIQ  | HGFFLIFIKN | BAGSSKGIK   | VVDKTLQSA  | LTTAFAYGST  | VLIQKAIAGI |    | 111 |
| VanA | V.....AAT  | FTYFVFKPA  | RGSSFGVKK   | VNSADELDYA | IESARQYDSK  | LIIEQAVSGC |    | 105 |
| VanB | E.....ART  | LYYPVFKPA  | RGSSFGVTK   | VNSTEELNAA | IEAAGQYDYGK | LIIEQAISGC |    | 105 |
| VanD | E.....TED  | FVYPVFKPA  | RGSSFGVKN   | VCKAELQAA  | IEEARKYDSK  | LIIEEAVTGS |    | 102 |
| Efa  | KEVEKCEGS  | LYYPVFKPA  | NGSSVGIK    | VENREELQEA | LEEAFRYDAR  | AIIVEQGIAR |    | 114 |
|      |            |            |             |            |             |            |    |     |
| VanE | EIGCGILGNE | QLVVGECDD  | ISLVDGFF... | DFEKKYQL   | VTAEILLPAK  | LSIDKKEDIQ |    | 165 |
| VanC | EIGCGILGNE | QLTIGACDA  | ISLVDGFF... | DFEKKYQL   | ISATITVPAP  | LPIALESQIK |    | 166 |
| VanA | EVGCAVLGNS | AALVVGVDQ  | IRLQYGFRI   | HOEVEPEKGS | ENAVITVPAD  | LSAEBGRIO  |    | 165 |
| VanB | EVGCAVVGNE | DDLIVGVDQ  | IRLSHGIFRI  | HOENEPEKGS | ENAMIIVPAD  | IPVEERNRVO |    | 165 |
| VanD | EVGCAILGNS | NDIMAVGVDQ | IELRHGFKEI  | HQEAQPEKGS | ENAVIRVPA   | LPDEVRRQIQ |    | 162 |
| Efa  | EIEVALIGNE | DVRRITLIGE | VVKDVAFYDY  | DAKYIN.NTI | E...MQIPAH  | VPEEVARQAQ |    | 169 |
|      |            |            |             |            |             |            |    |     |
| VanE | IKAKILYRLL | CCKGLARIDF | FLTDDGEILL  | NEINTEI    |             |            |    | 201 |
| VanC | EQAQILYRNL | GLTGLARIDF | FVYNGSAYLV  | NEINRM     |             |            |    | 202 |
| VanA | ETAKILYKAL | GCRLARVDM  | FLQDNGRIVL  | NEVNTL     |             |            |    | 201 |
| VanB | ETAKIVYRVL | GCRLARVDM  | FLQEDGQIVL  | NEVNTL     |             |            |    | 201 |
| VanD | ETAKILYRIL | GCRLARIDF  | FLREDCGIVL  | NEVNTF     |             |            |    | 198 |
| Efa  | EYAKKAYIML | DGSLRSRDF  | FLTSKNELEF  | NEIINTM    |             |            |    | 205 |

FIG. 2. Alignment of the deduced partial amino acid sequence of VanE and of the corresponding regions of VanA, VanB, VanC, VanD, and Efa, the D-Ala:D-Ala ligase of *E. faecalis*. The amino acids conserved in all the sequences are printed in boldface. Dots indicate gaps introduced to optimize sequence similarity. The conserved motif EK(Y/Q)N present in VanC-type and VanE D-Ala:D-Ser ligases is underlined. GenBank accession numbers are as follows: X56895 for VanA, U35369 for VanB, M75132 for VanC, AF130997 for VanD, AF136925 for VanE, and U00457 for the D-Ala:D-Ala ligase for *E. faecalis*.

TABLE 1. Sequence identity between the deduced amino acid sequence of VanE and related ligases

| Sequence compared | % Sequence identity |      |      |      |      |
|-------------------|---------------------|------|------|------|------|
|                   | VanE                | VanC | VanA | VanB | VanD |
| VanC              | 55                  |      |      |      |      |
| VanA              | 45                  | 43   |      |      |      |
| VanB              | 43                  | 39   | 76   |      |      |
| VanD              | 44                  | 43   | 69   | 69   |      |
| Efa <sup>a</sup>  | 32                  | 35   | 34   | 36   | 38   |

<sup>a</sup> Efa, D-Ala:D-Ala ligase of *E. faecalis*.

**D,D-Dipeptidase and D,D-carboxypeptidase activities.** The enzymatic activities were assayed in BM4405 extracts as described previously (4). The D-Ala released from D-Ala-D-Ala by D,D-peptidase and from pentapeptide terminating in D-Ala (pentapeptide[Ala]) by D,D-carboxypeptidase was determined by using D-amino acid oxidase (12). Bacteria were lysed by treatment with lysozyme (2 mg/ml) at 37°C, followed by sonication, and the membrane fraction was pelleted (100,000  $\times$  g, 45 min). Activities were measured in the supernatant and in the resuspended pellet fraction.

**Serine racemase activity.** The assay of serine racemase was carried out as described previously (1). Cell fractions were prepared by osmotic lysis of bacteria treated with lysozyme (400  $\mu$ g ml<sup>-1</sup>) and M1 muramidase (70  $\mu$ g ml<sup>-1</sup>). The membrane fraction was separated from the cytoplasm by centrifugation at 100,000  $\times$  g for 45 min.

**Nucleotide sequence accession number.** The sequence was submitted to GenBank and was assigned accession no. AF136925.

RESULTS AND DISCUSSION

**Characterization of strain BM4405.** Clinical isolate *E. faecalis* BM4405 was resistant to low levels of vancomycin (MIC, 16  $\mu$ g/ml) but was susceptible to teicoplanin (MIC, 0.5  $\mu$ g/ml); it was also resistant to spectinomycin (MIC, 64  $\mu$ g/ml). To identify BM4405 to the species level, primers specific for genes encoding D-Ala:D-Ala ligases in enterococci were used (8), and an amplification product was obtained with the primer pair specific for the *E. faecalis* *ddl* gene. To determine the glycopeptide resistance genotype of BM4405 we used primers specific for resistance genes *vanA*, *vanB*, *vanC1*, *vanC2*, and *vanD* (8, 14), but no PCR product was obtained. Furthermore, in Southern blots with nonstringent washing conditions, none of the probes specific for these resistance genes hybridized with total DNA from BM4405. These results indicate that BM4405 is an *E. faecalis* strain with a newly acquired glycopeptide resistance genotype.

**Determination of the glycopeptide resistance genotype of BM4405.** The degenerate primers V1 and V2, which allow amplification of fragments internal to genes that encode related ligases (7), were used in a PCR with total DNA of BM4405 as the template. A ca. 600-bp fragment was obtained, cloned into *Escherichia coli*, and sequenced. The 10 clones analyzed exhibited a sequence identical to that of part of the *E. faecalis* *ddl* ligase gene. *E. faecalis* *ddl* has a *Hind*III restriction site which is absent from the *vanA*, *vanB*, *vanC*, and *vanD* ligase resistance genes. After digestion of total BM4405 DNA with *Hind*III, a PCR with V1 and V2 primers was performed and a ca. 600-bp fragment was obtained with low efficiency. This PCR product was digested with *Hind*III, to eliminate putative partial digests of total DNA, and a second PCR was performed under the same conditions. The reaction resulted, with high efficiency, in the production of a ca. 600-bp fragment which was cloned, and both strands were sequenced (Fig. 1). The deduced amino acid sequence was compared with those of the host D-Ala:D-Ala ligase, the D-Ala:D-Lac ligases of VanA, VanB, and VanD strains, and the VanC1 D-Ala:D-Ser ligase (Fig. 2); the percentages of identity were calculated from this

TABLE 2. Peptidoglycan precursors synthesized by *E. faecalis* BM4405<sup>a</sup>

| Culture                             | % Synthesis             |                              |                              |
|-------------------------------------|-------------------------|------------------------------|------------------------------|
|                                     | UDP-MurNAc-tetrapeptide | UDP-MurNAc-pentapeptide[Ala] | UDP-MurNAc-pentapeptide[Ser] |
| Uninduced                           | <1                      | 100                          | <1                           |
| Induced (Vm <sup>b</sup> , 4 µg/ml) | 10                      | <1                           | 90                           |

<sup>a</sup> Cultures were incubated with ramoplanin to inhibit peptidoglycan synthesis for 20 min. Cell extracts were prepared, and precursors were analyzed by high-performance liquid chromatography (4). The individual precursors were characterized by amino acid analysis and mass spectroscopy (19).

<sup>b</sup> Vm, vancomycin.

alignment (Table 1). The sequence obtained had a higher degree of identity with the corresponding portion of VanC1 (55%) than with that of VanA, VanB, or VanD (from 43 to 45%). The motifs conserved in the related ligases were present, and of the four amino acids that are invariably present in the VanC-type D-Ala:D-Ser ligases (EKYQ, at positions 142 to 145 [9]; Fig. 2 numbering), three (EKY) were conserved. The 604-bp product used as a probe in a Southern blot under stringent binding and washing conditions hybridized with total DNA from BM4405 but not with total DNA from *E. faecium* BM4147 (VanA), *E. faecalis* V583 (VanB), *E. gallinarum* BM4174 (VanC), or *E. faecium* BM4339 (VanD) (data not shown). These results confirm that the 604-bp fragment is internal to a new glycopeptide resistance gene that we have called *vanE*. Primers specific for the new gene were synthesized and used in an attempt to amplify fragments internal to genes encoding related ligases from *Enterococcus* strains BM4147 (VanA), V583 (VanB), BM4174 (VanC), BM4339 (VanD), and BM4405. A PCR fragment of the expected size of 513 bp was obtained only with BM4405 DNA, and direct sequencing of this product confirmed that the oligonucleotides were specific for *vanE*. The 513-bp fragment hybridized only with BM4405 DNA (data not shown).

**Transfer of vancomycin resistance.** Attempts to transfer vancomycin resistance from BM4405 to *E. faecalis* JH2-2 by filter mating were unsuccessful. Only transfer of spectinomycin resistance, which is not associated with vancomycin resistance, was obtained.

**Characterization of peptidoglycan precursors of BM4405.** To analyze the cytoplasmic peptidoglycan precursors, cultures of *E. faecalis* BM4405, grown with or without vancomycin (8 µg/ml), were incubated in the presence of ramoplanin to inhibit cell wall synthesis after formation of the precursors. The results obtained indicated that, in the absence of vancomycin, UDP-MurNAc-pentapeptide was the unique precursor synthesized, whereas after incubation with vancomycin, UDP-MurNAc-pentapeptide[Ser] and UDP-MurNAc-tetrapeptide were the main compounds produced (Table 2). These data indicate that resistance in BM4405 is inducible by vancomycin and are consistent with the finding that *vanE* is more closely related to *vanC* than to other ligase genes (*vanA*, *vanB*, and *vanD*). Incubation with vancomycin did not induce resistance to teicoplanin.

**D,D-Dipeptidase and D,D-carboxypeptidase activities of *E. faecalis* BM4405.** D,D-Dipeptidase (VanX) and D,D-carboxypeptidase (VanY) hydrolyze the dipeptide D-Ala-D-Ala and remove the terminal D-Ala residue of precursors ending in acyl-D-Ala-D-Ala, respectively (3). After centrifugation at 100,000 × *g* the D,D-dipeptidase activity in the supernatant of lysed BM4405 that had been grown in the absence or in the

TABLE 3. Enzymatic activities in extracts from *E. faecalis* BM4405

| Vancomycin concn (µg/ml) | Activity (nmol min <sup>-1</sup> mg of protein <sup>-1</sup> ) |                      |                      |                      |                    |                      |
|--------------------------|--|----------------------|----------------------|----------------------|--------------------|----------------------|
|                          | D,D-Dipeptidase  |                      | D,D-Carboxypeptidase |                      | Serine racemase    |                      |
|                          | Mem-brane fraction   | Cyto-plasmic extract | Mem-brane fraction   | Cyto-plasmic extract | Mem-brane fraction | Cyto-plasmic extract |
| 0                        | <1   | <1                   | <1                   | <1                   | 1                  | <1                   |
| 8                        | 1  | 16                   | 1                    | 9                    | 60                 | <1                   |

presence (8 µg/ml) of vancomycin was assayed (Table 3). Cytoplasmic extracts from vancomycin-induced *E. faecalis* BM4405 possessed weak D,D-dipeptidase activity, comparable to that found in *E. gallinarum* BM4174 (VanC) (16), and no activity was detected in extracts from uninduced bacteria. No D,D-carboxypeptidase activity was found in membrane preparations of induced or uninduced cells, even in the absence of penicillin G in the assay (Table 3). However, weak D,D-carboxypeptidase activity was detected in cytoplasmic extracts of induced BM4405 cells. This activity, which was insensitive to penicillin G, could account for the presence of tetrapeptide peptidoglycan precursors. Interestingly, the localization of D,D-carboxypeptidase activity in the cytoplasm is similar to that in the VanC-type strain *E. gallinarum* BM4174 (18) but different from that of VanA- and VanB-type strains, in which VanY is predominantly membrane bound. It is possible that the D,D-dipeptidase and D,D-carboxypeptidase activities of BM4405 are encoded by a single gene, as in BM4174 (17).

**Serine racemase activity.** VanC-type resistance requires three proteins: VanC and VanXY<sub>c</sub>, which catalyze synthesis of D-Ala-D-Ser (18) and which eliminate precursors ending in D-Ala-D-Ala (17), and VanT, a membrane-bound serine racemase for production of D-Ser (1). BM4405 (VanE) synthesizes peptidoglycan precursors that end in D-Ala-D-Ser and therefore also requires a source of D-Ser. Serine racemase activity was present in the membrane fraction of BM4405 (Table 3), and the enzyme was inducible by vancomycin. The serine racemase activity was ca. 10-fold greater than that of BM4174, which could account for the higher ratio of pentapeptide[Ser]:tetrapeptide (9:1) present in cytoplasmic extracts of ramoplanin-inhibited BM4405 than in BM4174, for which the ratio was between 0.5:1 and 1:1 (data not shown). All the serine racemase activity was membrane bound, whereas alanine racemase activity was present almost exclusively in the cytoplasm (data not shown). The distribution of the alanine and serine racemases between the cytoplasm and the membrane fractions was identical in BM4405 (VanE) and BM4174 (VanC).

In conclusion, VanE-type glycopeptide resistance in *E. faecalis* BM4405 is due to synthesis of late peptidoglycan precursors ending in D-Ala-D-Ser. The VanC and VanE types of resistance are biochemically and phenotypically similar. The *vanE* gene cluster in BM4405 is under study.

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