**vanE** Gene Cluster of Vancomycin-Resistant *Enterococcus faecalis* BM4405

Lorena Abadía Patiño, Patrice Courvalin, and Bruno Perichon*

*Unité des Agents Antibactériens, Institut Pasteur, 75724 Paris Cedex 15, France*

Received 7 May 2002/Accepted 28 August 2002

Acquired VanE-type resistance to low levels of vancomycin (MIC = 16 μg/ml) in *Enterococcus faecalis* BM4405 is due to the inducible synthesis of peptidoglycan precursors terminating in d-alanine–d-serine (Fines, M., B. Périchon, P. Reynolds, D. Sahm, and P. Courvalin, Antimicrob. Agents Chemother. 43:2161-2164, 1999). A chromosomal location was assigned to the vanE operon by pulsed-field gel electrophoresis and hybridization, and its sequence was determined. Three genes, encoding the VanE ligase, the VanXYE dipeptidase, and the VanT, VanR, VanSE, enzyme with DD-dipeptidase activity, responsible for hydrolysis of precursors, D-alanyl–D-lactate (D-Ala–D-Lac) and D-alanyl–D-serine (D-Ala–D-Ser), have been identified (4, 9, 33). Three genes, with the same orientation, were shown to be cotranscribed by Northern analysis and reverse transcription-PCR. The vanE, vanXYE, and vanT, VanR, VanSE operons confer inducible low-level resistance to vancomycin after cloning in *E. faecalis* JH2-2, probably following cross talk with a two-component regulatory system of the host.

Glycopeptide antibiotics bind with high specificity to the d-alanyl–d-alanine (D-Ala–D-Ala) termini of peptidoglycan late precursors and block the transglycosylation and transpeptidation steps in cell wall synthesis by gram-positive bacteria (32). In enterococci, glycopeptide resistance is due to production of modified peptidoglycan precursors which exhibit a lower binding affinity for vancomycin (32). Two substituted precursors, d-alanyl–d-lactate (D-Ala–D-Lac) and d-alanyl–d-serine (D-Ala–D-Ser), have been identified (4, 9, 33). Three genes of glycopeptide resistance, VanA, VanB, and VanD, result from the production of D-Ala–D-Lac-terminating peptidoglycan precursors, whereas the VanC, VanE, and VanG types are characterized by the synthesis of precursors ending in D-Ala–D-Ser. Production of D-Ala–D-Ser-containing precursors in *Enterococcus gallinarum* and *Enterococcus casseliflavus flavescens*, which display intrinsic resistance to vancomycin, is due to the presence of the chromosomal vanC operon. The cluster is composed of five genes: vanC, vanXYC, vanT, vanRC, and vanSC (1). The vanC gene encodes a ligase that synthesizes the dipeptide D-Ala–D-Ser; vanXYC specifies a bifunctional enzyme with DD-dipeptidase activity, responsible for hydrolysis of the dipeptide D-Ala–D-Ala, and DD-carboxypeptidase activity that removes D-Ala from UDP-MurNAc-pentapeptide-[D-Ala] (33); and the vanT gene directs the synthesis of a membrane-bound serine racemase that provides D-Ser for the resistance pathway (1). Expression of the resistance genes of the van operons is controlled by two-component regulatory systems (6). These systems comprise response regulators (VanR type) and histidine kinases which act as sensor proteins (VanS type) (6). Recently, VanE- and VanG-type vancomycin resistance was detected in strains of *Enterococcus faecalis*, a species which is not intrinsically resistant to vancomycin (15, 25). Clinical isolate BM4405, the first VanE-type strain, is resistant to a low level of vancomycin (MIC = 16 μg/ml) and susceptible to teicoplanin (MIC = 0.5 μg/ml). Attempts to transfer vancomycin resistance from BM4405 to a susceptible *E. faecalis* strain by filter mating were unsuccessful (15). We describe the genetic organization of the vanE operon in *E. faecalis* BM4405. The cluster includes three genes which are sufficient to confer resistance to vancomycin and two genes for a two-component system in which the sensor is most likely nonfunctional. We also demonstrated that the five genes are cotranscribed from a single promoter.

**MATERIALS AND METHODS**

Strains, plasmids, and growth conditions. The bacterial strains and plasmids are described in Table 1. *E. faecalis* clinical isolate BM4405 is resistant to low levels of vancomycin (MIC = 16 μg/ml) (15). VanC-type *E. gallinarum* BM4174 (12) and VanA-type *Enterococcus faecium* BM4147 (20) were used as controls in pulsed-field gel electrophoresis experiments. *E. faecalis* JH2-2, used in electrophoresis experiments, is susceptible to glycopeptides and resistant to fusidic acid and rifampin (19). *Escherichia coli* BM83 (42) and Top10 (Invitrogen, Groningen, The Netherlands) were used as the hosts in cloning experiments. Strains were cultured in brain heart infusion broth or agar (Difco Laboratories, Detroit, Mich.) at 37°C. Susceptibility to glycopeptides was determined by agar dilution with 10⁵ CFU per spot on Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France) after 24 h of incubation.

Recombinant DNA techniques. Cleavage of DNA with restriction endonucleases (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England, and Gibco BRL-Life Technologies Inc.), purification of restriction fragments from agarose gel, and ligation with T4 DNA ligase (Amersham Pharmacia Biotech) were performed by standard methods (35).

Plasmid construction. The plasmids were constructed as follows (Fig. 1).

(i) Plasmid pAT664. Total DNA from BM4405 was partially digested with Sau3AI and ligated with pUC8 DNA cleaved by BamHI. Clones harboring recombinant plasmids were screened by colony hybridization (35) with the 513-bp fragment internal to vanD purified from pAT663 (15) as a probe.

(ii) Plasmids pAT667 and pAT668. A fragment encompassing the vanE, vanXYE, and vanT, VanR, VanSE genes, with or without 600 bp upstream from vanE, was amplified by using primer pairs E35-TE5 and E43-TE5, respectively, and *E. faecalis* BM4405 DNA as a template. Oligodeoxynucleotides E35 and E43 con-
tained a SacI site, and TE5 contained Sp6I site. The PCR products were digested with SacI and SphI and cloned in pAT29.

Plasmid pAT667 (600 bp upstream from vanE, vanEXY, vanTE) and pAT668 (vanEXY, vanTE) were introduced into E. faecalis JH2-2 by electrotransformation, and transformants were selected with spectinomycin (60 μg/ml).

Probes and hybridization. DNA was transferred onto Hybond N+ membranes (Amersham Pharmacia Biotech) and fixed under UV illumination. Plasmid pAT663 DNA labeled with [α-32P]dCTP (Amersham Pharmacia Biotech) by nick translation was used as a probe for colony hybridization, and Southern experiments were carried out under stringent conditions (35).

![Diagram](http://jb.asm.org/Downloaded)
TABLE 2. Oligonucleotides used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>E9</td>
<td>5' ACT GTG TTT CGG GTA GC</td>
<td>1722–1738</td>
</tr>
<tr>
<td>E12</td>
<td>5' TAT GGG AGT TGT GAA</td>
<td>2711–2725</td>
</tr>
<tr>
<td>E15</td>
<td>5' CAG AAG CTG AGC TAG T</td>
<td>2576–2581</td>
</tr>
<tr>
<td>E35</td>
<td>5' GGC ACG TCA CAG ATG AAA TCG GA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>E37</td>
<td>5' GGA TCA CGG AAG AAG GT</td>
<td>119–135</td>
</tr>
<tr>
<td>E38</td>
<td>5' CCA GGC ATT GTA TTG ATC T</td>
<td>932–941</td>
</tr>
<tr>
<td>E41</td>
<td>5' GCA ATT GTT AAT CCT AGA CC</td>
<td>5464–5445</td>
</tr>
<tr>
<td>E43</td>
<td>5' GGC ACG TCA ACA AAT ACT GGA GGT A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>XYE1</td>
<td>5' GTG CAT GGT CTT TTT TGT</td>
<td>1130–1141</td>
</tr>
<tr>
<td>XYE2</td>
<td>5' GCA GTT CTT CTT ATT GAC TC</td>
<td>1590–1580</td>
</tr>
<tr>
<td>TE4</td>
<td>5' GCA GGG TGT CAG GTG TT</td>
<td>2159–2174</td>
</tr>
<tr>
<td>TE5</td>
<td>5' GCC CAT GCA GCC ATT AAA CAT CCT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3783–3797</td>
</tr>
<tr>
<td>RE1</td>
<td>5' CCG AGA CAG CCA AAT</td>
<td>4410–4424</td>
</tr>
<tr>
<td>RE2</td>
<td>5' TCG ACT GTG GAC AAA T</td>
<td>4214–4199</td>
</tr>
<tr>
<td>RE6</td>
<td>5' AGA TCG ATT TAC TC</td>
<td>3913–3930</td>
</tr>
<tr>
<td>SE1</td>
<td>5' AGC TAA CAG ATT AGA ACA T</td>
<td>5027–5045</td>
</tr>
<tr>
<td>SE2</td>
<td>5' GGA GTT CTT AAG TCA TGT</td>
<td>4921–4904</td>
</tr>
<tr>
<td>VDV</td>
<td>5' GAT CTT TGG TTT TTA GAT</td>
<td>178–195</td>
</tr>
<tr>
<td>RDe2g</td>
<td>5' CCN ACH CCS CRB ACV GTT&lt;sup&gt;e&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>PE1</td>
<td>5' CCA ATG ACC TTC TTC GTG GAT CC</td>
<td>120–96</td>
</tr>
<tr>
<td>PE3</td>
<td>5' AAG CTT TCT TCT CCT GAC ATA GCC TC</td>
<td>3877–3851</td>
</tr>
<tr>
<td>SDe1g</td>
<td>5' ATS GSM ARH CCM ARW CC&lt;sup&gt;e&lt;/sup&gt;</td>
<td>NA</td>
</tr>
</tbody>
</table>

<sup>a</sup> Nucleotide numbering begins at the first base of the vanE gene.
<sup>b</sup> The SnrI site is underlined.
<sup>c</sup> NA, not applicable.
<sup>d</sup> The SpI site is underlined.
<sup>e</sup> B = C, G, or T; H = A, C, or T; N = A, C, G, or T; R = A or G; S = G or T; V = A, C, or G.

<sup>f</sup> H = A, C, or T; M = A or C; R = A or G; S = G or T; W = A or T.
ucts were transferred from agarose gel to a Hybond N+/H11001 membrane (Amersham Pharmacia Biotech) and hybridized with specific probes (Fig. 1C).

(iv) Primer extension analysis. The synthetic oligodeoxynucleotide PE1 (Fig. 1D; Table 2) was 5’-end labeled with [γ-32P]ATP (4,500 Ci/mmol; Amersham Pharmacia Biotech) and T4 polynucleotide kinase (Amersham Pharmacia Biotech). After phenol-chloroform extraction, the labeled primer was precipitated with ethanol and redissolved in sterile water to a final concentration of 1 pmol/l.

Labeled primer (1 pmol) was annealed to 50 mg of total RNA at 65°C for 3 min, and extension was performed in a 20-l final volume with 40 U of Moloney murine leukemia virus modified reverse transcriptase (Superscript II; Gibco) for 45 min at 50°C. After addition of 5 l of stop solution (Amersham Pharmacia Biotech) and heat denaturation, the sample was immediately loaded onto 6% polyacrylamide–urea sequencing gels for electrophoresis. Sequencing reactions using the same primer and appropriate plasmid DNA templates were run in parallel to allow determination of the endpoints of extension products.

RESULTS AND DISCUSSION

Cloning of the vanE gene cluster. Fragments obtained after partial digestion of E. faecalis BM4405 total DNA with Sau3AI were cloned in pUC18 DNA cleaved with BamHI into E. coli, and transformants were screened by hybridization with a vanE internal probe (Fig. 1A). Plasmid pAT664 (vanE/XYE) carried an insert of 8 kb that was sequenced. Three open reading frames (ORFs), designated vanE, vanXYE, and vanTE, were found, but the two distal ones were truncated (Fig. 1). The 5’ portion of vanE and 1.5 kb upstream were obtained by successive inverted PCRs. We assumed that the vanE cluster had the same gene organization as the vanC operon, i.e., that vanTE should be followed by the vanRE and vanSE genes. We thus amplified BM4405 DNA by using oligodeoxynucleotide E12, specific for vanTE, and degenerate oligodeoxynucleotide RDeg2, complementary to the sequence encoding a conserved motif in the C-terminal part of VanS-type proteins, to amplify total DNA from BM4405 (Table 2). Determination of the sequence of the PCR product indicated the presence of the vanSE gene. A 1.5-kb fragment downstream from vanSE was obtained by inverted PCR and sequenced, but no ORF was found. The gene organization of the vanE cluster is shown in Fig. 1.

Analysis of the proteins encoded by the vanE gene cluster. The deduced amino acid sequence of vanE exhibited 53 and 41% identity with the VanC (2) and VanG (25) D-Ala:D-Ser ligases, respectively (Fig. 2). The EKYQ motif conserved in the VanC-type resistance ligases (13) was found in VanE (EKYN) at positions 198 to 201. The phylogenetic tree based on the alignment of the D-Ala:Lac and D-Ala:D-Ser ligases confirmed that VanE was related to VanC (Fig. 3).

FIG. 2. Comparison of the D-Ala–D-Ser gene clusters. Arrows represent coding sequences and indicate the direction of transcription. The asterisk indicates the stop codon in vanSE. The guanosine-plus-cytosine content (% G+C) is indicated in the arrows. The percentages of amino acid (aa) identity between the deduced proteins are indicated under the arrows.

FIG. 3. Phylogenetic tree derived from the alignment of D-Ala:Lac and D-Ala:D-Ser ligases. The tree was constructed by the neighbor-joining method, taking into account the results of maximum-parsimony and bootstrapping analysis.
with the VanXYC bifunctional enzyme (1) (Fig. 2). VanXYE
displayed higher identity with VanY and VanYB DD-carboxy-
peptidases (23 and 16%, respectively) than with VanX and
VanXb DD-dipeptidases (13 and 16%, respectively). The iden-
tity was lower with VanYG1 than with VanYG2 (19 and 32%,
respectively). The consensus sequences found in VanX DD-di-
peptidases (23), VanY DD-carboxypeptidases (5), and VanXY
DD-dipeptidases (34) were also present in VanXYE. An SnHxxGx
AxD motif, in which the histidine and aspartate are zinc li-
gands, was found in VanXYE (S$^\text{E}$PHEIGLAVD$_\text{E}$). Fur-
more, another histidine ligand to zinc, conserved in the zinc
binding domain of DD-peptidases (33), was found in VanXYE
(H157). A conserved glutamate/aspartate residue functioning
as a catalytic base (1) was present in VanXYE (E154). The
hydropophicity profile of VanXYE suggested a cytoplasmic
localization for the protein (data not shown). Previous study of
vancomycin-induced E. faecalis BM4405 indicated weak DD-
dipeptidase and DD-carboxypeptidase activities in cytoplasmic
extracts (15). Both activities are also found in the cytoplasm of
VanC-type E. gallinarum BM4174 (1).

The third ORF, vanTE, encoded a putative protein with 47
and 33% identity with VanTEC (2) and VanTEG (25) serine
racemases, respectively (Fig. 2). The N-terminal half of VanTE
contained 11 clusters of hydrophobic amino acids, suggesting
that, like VanTEC, it may be a membrane-associated protein.
The serine racemase activity present in the membrane fractions
of BM4405 is ca. 10-fold higher than that of E. gallinarum
BM4174 (15). The C-terminal domain of VanTEC had substan-
tial sequence identity (28%) with that of alanine racemase Alr1
from E. coli. The putative pyridoxal 5’-phosphate attachment
motif, which is highly conserved in alanine racemases and in
VanT (33), was found in VanTEC (V$_\text{E}$373VKANAYGCG$_\text{E}$). Fur-
thermore, the residues implicated in the hydrogen-bonding
interactions with the phosphate group of pyridoxal 5’-phosphate
in VanT (Y379, S540, and N688) (2) were present in VanTEC.
Finally, residues which putatively play a structural role and
maintain the geometry of the active site of alanine race-
mases and VanT (2) were identified in VanTEC: A377, A379,
Y380, R410, G619, D622, R626, and E688.

The two genes downstream from the three resistance deter-
minants are likely to encode a two-component regulatory sys-
tem (Fig. 1A). The putative VanR$_\text{E}$ protein exhibited 61 and
43% identity with VanR$_\text{C}$ (1) and VanR$_\text{G}$ (25), respectively
(Fig. 2). The conserved aspartate and lysine residues typical of
response regulators in two-component systems from gram-posi-
tive bacteria (28) were present in VanR$_\text{E}$ (D10, D53, and
K102). VanR$_\text{E}$ displayed 44% identity with the CheY-like re-
sponse regulator of Clostridium acetobutylicum (29).

The deduced amino acid sequence of vanSE showed 41 and
31% identity with VanS$_\text{C}$ (1) and VanS$_\text{G}$ (25), respectively
(Fig. 2). The N-terminal part of VanS$_\text{E}$ contained transmem-
brane segments characteristic of the sensor proteins of two-
component systems (6). The carboxyl-terminal part of VanS$_\text{E}$
had four of the five conserved amino acid motifs (H, N, F, and
G2) characteristic of transmitter modules of histidine protein
kinases (30, 38). However, a stop codon at position 78 of vanSE
will result in the production of a truncated protein, suggesting
that VanS$_\text{E}$ is nonfunctional. The level of phosphorylation
of VanR-type proteins is controlled by the kinase and phospha-
tase activities of VanS-type sensors (3, 7, 42). However, kinases
encoded by the host chromosome are able to activate the
VanR response regulator (3, 8, 37), and it has been demon-
strated that both PhoR and acetylphosphate are capable of
activating VanR (18). In the absence of a functional VanS$_\text{E}$,
inducibility of vancomycin resistance expression in BM4405
(15) could be due to cross talk either with another two-com-
ponent system or of VanRE with an heterologous histidine
kinase.

Location of the vanE gene cluster. Fragments of E. faecalis
BM4405 total DNA digested with I-CeuI, an intron-encoded
endonuclease specific for rRNA genes (21), were separated by
pulsed-field gel electrophoresis and transferred onto a nylon
membrane which was hybridized successively to 16S rDNA
and vanE-specific probes. The probes cohybridized with a
c. 350-kb fragment from BM4405, indicating a chromosomal
location for the vanE cluster (data not shown).

Genes necessary for vancomycin resistance in E. faecalis
BM4405. To test if the vanE, vanXYE, and vanTEE genes were
sufficient to confer vancomycin resistance to the host, a 3.8-kb
fragment encompassing the three structural genes but devoid
of any 5’ upstream sequence was cloned in pAT29, leading to
plasmid pAT668 (vanEXE) ($\text{E}$). The plasmid was introduced
into E. faecalis JH2-2 by electrotransformation, and, irrespec-
tive of the absence or presence of a low concentration of van-
comycin in the culture medium (1 or 2 g/ml), the transfor-
mants remained susceptible to vancomycin (MIC = 2 g/ml)
(Table 3). A 4.4-kb fragment containing the structural genes
together with 600 bp upstream from vanE was then cloned in
pAT29, generating plasmid pAT667 (600 bp, vanEXE) ($\text{E}$) (Fig.
1B). In the absence of induction, strain JH2-2 harboring
pAT667 was susceptible to vancomycin. However, a reproduc-
able threefold increase in the vancomycin MIC was observed
after growth in the presence of vancomycin (Table 3). Taken
altogether, these results indicate that the vanE, vanXYE, and
vanTEE genes are sufficient to confer vancomycin resistance
and that the region upstream from the vanE cluster may act as a
promoter for activation of transcription of the resistance
genes, as in the vanC operon (1). When JH2-2/pAT667 was
grown in the absence of vancomycin, a very high proportion
(89%) of precursors ending in D-Ala was found in the
cells. In contrast, precursors ending in D-Ala–Ser were represen-
ted 53% of total peptidoglycan precursors in cells grown in
the presence of vancomycin (Table 3), confirming that the
resistance genes were inducibly expressed. In vancomycin-in-
TABLE 3. Glycopeptide MICs and nature of peptidoglycan
precursors in E. faecalis strains

<table>
<thead>
<tr>
<th>E. faecalis</th>
<th>MIC (µg/ml) of vancomycin</th>
<th>Precursors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetra</td>
<td>Penta-D-Ser</td>
<td>Penta-D-Ala</td>
</tr>
<tr>
<td>BM4405</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>BM4405 (Vma4)$^a$</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>JH2-2</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>JH2-2/pAT667</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>JH2-2/pAT667 (Vma4)</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>JH2-2/pAT668</td>
<td>2</td>
<td>ND</td>
</tr>
</tbody>
</table>


$^a$ ND, not determined.
duced JH2-2/pAT667 cells, nearly half of the peptidoglycan precursors were of the susceptible type. This might indicate that, possibly due to inefficient cross talk, expression of the \textit{vanE} operon was lower in induced JH2-2/pAT667 than in BM4405. This finding could account for the fact that the transformant was inhibited by a vancomycin concentration lower than that for strain BM4405 (Table 3).

Transcription analysis of the \textit{vanE} gene cluster. The \textit{vanA}, \textit{vanB}, and \textit{vanD} operons are cotranscribed from their respective \textit{PH} (6), \textit{PYB} (37), and \textit{PYD} (10) promoters. The start codons of the \textit{vanXYE}, \textit{vanE}, \textit{vanXYE}, and \textit{vanT} \textit{E} genes overlap the termination codons of \textit{vanE} and \textit{vanXYE}, respectively, suggesting that the \textit{vanE}, \textit{vanXYE}, and \textit{vanT} \textit{E} genes are cotranscribed. The \textit{vanS} \textit{E} start codon also overlaps the \textit{vanR} \textit{E} stop codon, suggesting the existence of a second transcription unit. Total RNA from BM4405 was extracted and analyzed by Northern hybridization with probes internal to every gene in the \textit{vanE} operon (Fig. 1C). A single transcript of ca. 5800 nucleotides was observed, which hybridized with all of the probes, including those internal to \textit{vanR} \textit{E} and \textit{vanS} \textit{E} (Fig. 4). The size of the transcript and the absence of a smaller mRNA encompassing the last two \textit{van} genes are consistent with the production of a single mRNA corresponding to the five genes and originating from a promoter upstream from \textit{vanE}. Cotranscription of the entire \textit{vanE} gene cluster was tested by RT of total RNA from BM4405 with primer TE5, internal to \textit{vanT} \textit{E} (Fig. 1D; Table 2). The cDNA was amplified by PCR with primers VDV and E15, internal to \textit{vanE} and \textit{vanT} \textit{E}, respectively (Fig. 1D; Table 2). A PCR product of the expected size of 2.4 kb that cohybridized with probes specific for \textit{vanE}, \textit{vanXYE}, and \textit{vanT} \textit{E} (Fig. 1D and Fig. 5) was obtained. To confirm that a single transcript corresponded to the five genes, RT of total RNA from BM4405 with primer PE3, internal to \textit{vanR} \textit{E} (Fig. 1D; Table 2), was performed. The cDNA was then amplified using primers internal to \textit{vanT} \textit{E} (TE4) and \textit{vanR} \textit{E} (PE3) (Fig. 1D; Table 2). A PCR product of ca. 1.7 kb, which cohybridized with the \textit{vanT} \textit{E} and \textit{vanR} \textit{E} probes, was obtained (data not shown), indicating that the genes for the two-component system were cotranscribed with the resistance genes. Based on these observations, primer extension was performed to locate the transcriptional start site for \textit{vanE} by using primer PE1, complementary to the 5’ end of that gene (Fig. 1D; Table 2) (Fig. 6). The proposed initiation codon for \textit{vanE} was preceded by a putative ribosome binding site (5’-TATACTGGAGGN8ATG) that displayed high complementarity to the 3’ extremity of \textit{Bacillus subtilis} 16S rRNA (3’-OH-UCCUUCCUC) (27). The \textit{PE} promoter region contained two overlapping putative −10 regions, TTT CAA and TTCAAT, similar to the −10 σ70 recognition consensus. Both regions were at a correct distance, 10 and 11 bp, respectively, from the transcription start.
stream from the proposed −10 sequences lies a TTGAGG putative −35 sequence. However, due to spacing, it remains open whether this sequence plays a role in the recognition of the promoter region by the σ^70 RNA polymerase complex. Furthermore, expression of the vanE operon is likely to depend on the VanR regulon, transcriptional activator, which is known to render the −35 sequence dispensable for expression (11).

In conclusion, the vanE operon comprises five genes, with three of them being sufficient to confer vancomycin resistance whereas the last two encode a two-component system postulated to regulate expression of the operon. However, since VanS appears not to be functional, inducibility of resistance by vancomycin is likely to depend on cross talk reactions with another two-component regulatory system of the host. Comparative analysis of the vanE operon indicated that VanE-type resistance in E. faecalis BM4405 was due to the presence of a chromosomal operon related to vanC. It has been demonstrated that transfer of vancomycin resistance among enterococci can be associated with the movement of large genetic elements from chromosome to chromosome (31). Our results suggest acquisition by E. faecalis of a cluster of genes from an intrinsically resistant species such as E. gallinarum or E. casseliflavus-flavescens. To find a clue as to the mechanism of acquisition of the resistance operon, we are determining the sequence of the flanking regions.

ACKNOWLEDGMENTS

We thank T. Msadek and P. Reynolds for technical advice on RNA preparation and peptidoglycan precursor determination, respectively, and M. Chippaux, F. Delpadiou, and I. Marchand for helpful discussions.

This work was supported in part by a Bristol-Myers Squibb unrestricted Biomedical Research Grant in Infectious Diseases. L.A.P. was a recipient of a grant from the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) of the Venezuelan government.

REFERENCES


