Conjugal transfer of the vancomycin resistance determinant \textit{vanB} between enterococci involves the movement of large genetic elements from chromosome to chromosome

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Abstract: Resistance to various levels of vancomycin and susceptibility to teicoplanin in enterococci (VanB phenotype) is mediated by the \textit{vanB} gene cluster. VanB-type resistance was transferred by intra- and inter-specific conjugation between different strains of \textit{Enterococcus}. Analysis of \textit{SfiI}-digested genomic DNA by zero integrated-field electrophoresis followed by Southern hybridization revealed \textit{vanB}-containing chromosomal insertions of approximately 90–250 kb in the transconjugants. Thus, transfer of VanB-type resistance is associated with the movement of large genetic elements from chromosome to chromosome.

Key words: Enterococcus; Glycopeptide; Conjugal transfer; \textit{vanB}; Vancomycin resistance

Introduction

Vancomycin and teicoplanin are glycopeptide antibiotics that inhibit cell wall synthesis by forming complexes with the peptidyl-d-alanyl-d-alanine termini of peptidoglycan precursors at the cell surface, thereby blocking the transpeptidation and transglycosylation steps of peptidoglycan assembly. Acquired resistance to glycopeptides in clinical isolates of enterococci can be classified by the level of resistance to vancomycin and susceptibility or resistance to teicoplanin [1,2]. Resistance to high levels of vancomycin and teicoplanin defines the VanA phenotype mediated by the \textit{vanRSHAX} gene cluster which is carried by the 11-kb transposon \textit{Tn1546} and related elements [2]. The \textit{vanA} gene encodes a d-Ala:d-Ala ligase of modified substrate specificity that synthesizes d-alanyl-d-lactate which is incorporated into peptidoglycan precursors that have reduced affinity for glycopeptides [2]. Dissemination of this type of resistance is due to conjugal transfer of self-transferable plasmids that have acquired the mobile elements by transposition [2]. The VanB phenotype is characterized by various lev-
els of resistance to vancomycin (minimal inhibitory concentrations (MICs) 4 to > 1000 μg ml⁻¹) and susceptibility to teicoplanin [1]. Despite the wide range of vancomycin MICs of enterococci with VanB-type resistance, a portion of the vancomycin resistance gene \( \text{vanB} \) hybridizes to total DNA of all these strains, indicating that a single class of resistance determinant accounts for the VanB phenotype [1]. The \( \text{vanB} \) gene, like \( \text{vanA} \), appears to encode a ligase of altered specificity that synthesizes D-alanyl-D-lactate [3]. In this work we investigated the genetic location of the \( \text{vanB} \) determinant.

**Materials and Methods**

**Bacterial strains**

The strains studied are listed in Table 1. Clinical isolate *Enterococcus faecalis* UMH [4] was resistant by disc susceptibility testing to vancomycin, tetracycline, penicillin, erythromycin, gentamicin, kanamycin, tobramycin, and streptomycin. Clinical isolate BM4120 was resistant to vancomycin, penicillin, erythromycin, gentamicin, kanamycin, and tobramycin. Strains JH2-2 [5] and BM4113 [6] are derivatives of plasmid-free *E.

faecalis* JH2 [5]. *E.

faecalis* JH2-2 carries chromosomal mutations conferring resistance to fusidic acid and rifampicin. *E.

faecalis* BM4113 is a streptomycin-resistant mutant of *E.

faecalis* JH2 which contains pAT112 stably integrated into the chromosome. Integrative vector pAT112 is 4.9 kb in size and harbors the erythromycin (ermB) and kanamycin (aphA-3) resistance genes [6].

**Transfer of antibiotic resistance traits and growth conditions**

Mating on filters was performed as described [7]. The antibiotic concentrations used for selection of transconjugants were: rifampicin, 20 μg ml⁻¹; fusidic acid, 10 μg ml⁻¹; and vancomycin, 8 μg ml⁻¹; or streptomycin, 1000 μg ml⁻¹; erythromycin, 8 μg ml⁻¹; and vancomycin, 8 μg ml⁻¹. Frequency of transfer was expressed as the number of transconjugants per donor colony-forming unit after the mating period. Brain-heart infusion broth and agar were used. All incubations were at 37°C.

**Zero integrated field gel electrophoresis (ZIFE)**

Agarose plugs were prepared [8] and digested overnight with 40–80 units of SfiI or NorI (Boehringer) according to the manufacturer’s recommendations. The software-assisted ZIFE electrophoresis apparatus (Auto Base, Q-Life System Inc.) was used with ROM cards that optimize DNA fragment separations in the 100–700 kb (card no. 4) or 100–1100 kb (card no. 5) size ranges [9].

**Southern hybridization**

Transfer of DNA, labelling of probes with [α-³²P]dCTP and Southern hybridization were

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### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Resistance characters a</th>
<th>MIC (μg ml⁻¹)</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vancomycin</td>
<td>Teicoplanin</td>
</tr>
<tr>
<td><em>E. faecalis</em> JH2-2</td>
<td>Fu Rif</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td><em>E. faecalis</em> BM4113</td>
<td>Em Km Sm</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td><em>E. faecalis</em> UMH</td>
<td>Em Km Sm Vm</td>
<td>512</td>
<td>1</td>
</tr>
<tr>
<td><em>E. faecium</em> BM4120</td>
<td>Km Vm</td>
<td>64</td>
<td>1</td>
</tr>
<tr>
<td><em>E. faecalis</em> BM4260 to BM4269</td>
<td>Fu Rif Vm</td>
<td>512–1024</td>
<td>0.5</td>
</tr>
<tr>
<td><em>E. faecalis</em> BM4270</td>
<td>Em Km Sm Vm</td>
<td>512</td>
<td>0.5</td>
</tr>
<tr>
<td><em>E. faecalis</em> BM4271</td>
<td>Em Km Sm Vm</td>
<td>512</td>
<td>0.5</td>
</tr>
<tr>
<td><em>E. faecalis</em> BM4272 to BM4284</td>
<td>Fu Rif Vm</td>
<td>48–128</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a Em, resistance to erythromycin; Km, resistance to kanamycin; Rif, resistance to rifampicin; Sm, resistance to streptomycin; and Vm, resistance to vancomycin.
performed as described [1]. The vanB probe consisted of the purified 550-bp fragment internal to the vanB gene [10] and the pAT112 probe consisted of the entire plasmid [6].

Results and Discussion

E. faecalis UMH, which harbors vanB-related sequences [1] and is highly resistant to vancomycin (MIC 512 μg ml⁻¹), was used as a donor in filter matings with E. faecalis JH2-2. Transfer of vancomycin resistance was obtained at a frequency of about 10⁻⁸, which is lower than that usually observed with enterococcal conjugative plasmids [11]. Screening of ten transconjugants (BM4260–BM4269) from ten independent experiments indicated that only resistance to vancomycin was transferred (Table 1). Total DNA of the transconjugants and of JH2-2 was digested with SfiI and resolved by zero integrated-field electrophoresis using two sets of electrophoresis conditions (Fig. 1A, C). Six SfiI fragments (1100, 610, 490, 400, 290, and 90 kb) were present in JH2-2. The SfiI restriction profiles of the transconjugants were similar to that of JH2-2 except for the replacement of the 610-kb fragment by a 700-kb fragment (Fig. 1A, Table 2). In Southern hybridizations, the vanB-specific probe hybridized to the 700-kb SfiI fragment of the transconjugants but not to JH2-2 DNA (Fig. 1A). These results indicate that a 90-kb fragment which does not contain a SfiI site and carries the vanB determinant was inserted into the JH2-2 chromosome. In agreement with these data, NorI-generated restriction profiles and Southern hybridizations also indicated acquisition of a 90-kb insertion by the transconjugants (data not shown).

Transconjugants BM4260 and BM4261 were used, in turn, as vanB donors in conjugation experiments with BM4113. Vancomycin-resistant transconjugants BM4270 and BM4271 were ob-

![Fig. 1. Analysis of SfiI digested genomic DNA by zero integrated-field electrophoresis (ZIFE) and by Southern hybridization using a vanB-specific probe. ZIFE conditions in (A) and (B) were optimized to separate DNA fragments with sizes ranging from 100 to 700 kb and in (C) to resolve fragments sized from 100 to 1100 kb.](image-url)
Two independent matings with transfer rates of approx. $10^{-8}$. Similar analysis of these strains (Fig. 1B, Table 2) revealed that transfer of vancomycin resistance was associated with the presence of 90-kb insertions in the 610-kb SfiI fragment. Since the SfiI restriction profiles of BM4261 were indistinguishable from BM4270 and BM4271, the recipient and transconjugants were probed with pAT112. Only BM4113, BM4270 and BM4271 had the probe (data not shown). The SfiI results also indicated that pAT112 was in the 1100-kb SfiI fragment, which suggested that the SfiI restriction profiles of H2-2 and BM4113 were similar. Thus, the vanB determinant from E. faecalis H2-2 and from JH2-2 derivatives was associated with the movement of a transposon from chromosome to chromosome. The isolate E. faecium BM4120 is resistant to vancomycin (MIC 32 μg ml$^{-1}$) and has vanB-related sequences [1]. Intercellular transfer of vancomycin resistance from BM4120 to E. faecalis JH2-2 was at a frequency of about $10^{-8}$, similar to the species transfer. Analysis of 13 interconjugants (BM4272–BM4284) revealed 12 chromosomal insertions of approx. 90 and 250 kb (Fig. 2, Table 2). Insertion sizes were detected in the 290- or 400-kb SfiI fragments of JH2-2 (Fig. 2, Table 2). Insertion sizes were consistent with those of vanB-containing chromosomal insertions.

<table>
<thead>
<tr>
<th>Transconjugant (no.)</th>
<th>Insertion size (kb)</th>
<th>SfiI integration fragment (kb)</th>
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</thead>
<tbody>
<tr>
<td>BM4260 to BM4269 (10)</td>
<td>90</td>
<td>610</td>
</tr>
<tr>
<td>BM4270 (1)</td>
<td>90</td>
<td>610</td>
</tr>
<tr>
<td>BM4271 (1)</td>
<td>90</td>
<td>610</td>
</tr>
<tr>
<td>BM4272 to BM4273 (2)</td>
<td>220</td>
<td>290</td>
</tr>
<tr>
<td>BM4274 to BM4279 (6)</td>
<td>250</td>
<td>290</td>
</tr>
<tr>
<td>BM4280 (1)</td>
<td>220</td>
<td>400</td>
</tr>
<tr>
<td>BM4281 to BM4284 (4)</td>
<td>250</td>
<td>400</td>
</tr>
</tbody>
</table>

Fig. 2. Analysis of SfiI-digested genomic DNA by zero integrated-field electrophoresis (ZIFE) and by Southern hybridization using a vanB-specific probe. BM4280 and BM4281 are representative of JH2-2 transconjugants which contain fragment insertions of 200 and 250 kb, respectively, in the 400-kb SfiI. The autoradiogram shows hybridization of the vanB probe with the DNA fragments containing the insertions. The ZIFE conditions used optimally separated DNA fragments with sizes ranging from 100 to 700 kb. The 90 and 1100 kb SfiI fragments are not included in this figure.
seen by analysis with NotI (data not shown). In the transconjugants the vanB probe hybridized with the novel bands containing the insertions (Fig. 2, and data not shown).

Our results indicate that conjugal transfer of VanB-type resistance among enterococci is associated with the movement of large genetic elements carrying vanB from chromosome to chromosome. Since there is only 20–30% homology between the genomes of E. faecalis and E. faecium [12], integration of these elements in the chromosome of these distantly related species most probably results from illegitimate recombination. In contrast to vanA, which is carried by non-conjugative transposons, the genes that mediate VanB-type resistance are apparently part of large (90–250 kb) conjugative elements. Large (> 50 kb) conjugative elements have already been detected in Streptococcus pyogenes [13], S. agalactiae [14] and in S. pneumoniae [15]. Related but smaller (15–25 kb) elements have also been described in enterococci [11].

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References