

Nonconjugative Transposition of the *vanB*-Containing Tn5382-Like Element in *Enterococcus faecium*

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The *vanB2* operon encoding glycopeptide resistance is an integral part of the putative conjugative transposon Tn5382. Characterization of clinical glycopeptide resistant derivatives from an epidemic ampicillin-resistant *Enterococcus faecium* strain showed precise chromosomal or plasmid insertions of a *vanB2*-containing Tn5382-like element. Conjugative transposition of the Tn5382-like element was not demonstrated in retransfer studies.

The 34-kb *vanB*-containing transposon Tn1549, which is similar to the Tn916-Tn1545 family of conjugative transposons, was recently described (11). Tn1549 appears to be identical to the previously described and partially sequenced transposon Tn5382 (4). It is therefore designated a Tn5382-like element. The *vanB2* operon seems to be universally linked to the Tn5382-like element and is the most widespread *vanB* subtype (7, 8, 15, 16, 17, 19). Conjugative transposons encode the functions for excision to a circular intermediate and intercellular transfer (5). The staggered cleavage of Tn916-Tn1545 during transposition results in the transfer of a coupling sequence from the previous target. The circular intermediates are joined at the ends by mismatched strands that are repaired in enterococci (18), representing sequences from flanking regions on either side of the previous insertion. Transposon-flanking regions thus represent the target sequence on one side and one of the mismatched strands on the other (3, 21). Evidence of Tn5382 transposition has been described previously (4, 11). We present additional data for precise transposition of Tn5382-like elements under natural conditions describing the arrival of the Tn5382-like element in a Norwegian ampicillin-resistant *Enterococcus faecium* outbreak strain (TUH2-21).

Relevant characteristics of the strains used are listed in Table 1. Isolation of DNA (2, 22), pulsed-field gel electrophoresis (PFGE), Southern hybridization, matings, PCRs, and DNA sequencing (6, 7) were performed as described previously. Amplicons and primer sequences are given in Table 2. Various bacteria were used as templates for PCR probe synthesis: 16S rRNA gene (rDNA) probe from *Enterococcus faecalis* DS16C2 (10), 23S rDNA from *E. faecium* ATCC 19434, IS1216V from

E. faecium BM4147 (14), Tn5382 from *E. faecium* C68 (4), and *vanB* from *E. faecalis* V583 (9). A chromosomal *vanB* location was assessed by sequential *vanB*, 16S rDNA, and 23S rDNA hybridizations of PFGE-separated, I-CeuI (New England Biolabs)-digested DNA. Plasmid *vanB* localization was examined by sequential *vanB* and 16S rDNA hybridizations of plasmid DNA. Inverse PCR was performed to characterize Tn5382-flanking sequences. The Tn5382 left and right ends were amplified with internal divergent Tn5382 primers after ligation of *Dra*I-, *Hinf*I-, or *Hph*I/*Bfa*I- and *Alu*I-, *Dde*I-, or *Sau*3AI-digested total DNA (1 to 3 μ g), respectively. The Sequence Navigator Software Package (Perkin-Elmer) and the Blastn and Blastx local alignment search tools (1) were used for sequence analyses.

The outbreak due to the ampicillin-resistant strain (TUH2-21) and the four VanB-type strains (TUH2-18, TUH2-19, TUH2-20, and TUH2-55) has been reported previously (12). The *vanB2* genotype was confirmed as previously described (6). *Sma*I PFGE patterns of TUH2-18 and -20 (Fig. 1A, lanes 3 and 4) were closely related to that of TUH2-21 (Fig. 1A, lane 2). The only difference was the replacement of a 270-kb *Sma*I fragment in TUH2-21 with an approximately 300-kb fragment in TUH2-18 and -20 that hybridized with the *vanB* (Fig. 1B, lanes 3 and 4) and Tn5382 (Fig. 1C, lanes 3 and 4) probes. Cohybridization of *vanB* and rDNA probes to I-CeuI PFGE fragments confirmed the chromosomal *vanB* location (data not shown). Inverse PCR and sequencing showed insertion of the Tn5382-like element in a chromosomal region of TUH2-18 between the putative genes for ribonucleotide reductase and membrane proteins (GenBank accession no. AF289471 and AF289472). TUH2-18 flanking sequences were identical to TUH2-21 and TUH2-19 chromosomal sequences except for six additional base pairs (ATAATT) at the right extremity of the transposon (GenBank accession no. AF289469 to AF289472; Fig. 2A).

Comparative PFGE analysis of TUH2-21, -19, and -55 showed indistinguishable *Sma*I patterns (Fig. 1A, lanes 2, 5, and 6, respectively). Hybridization of plasmid DNA and I-CeuI PFGE fragments (data not shown) revealed a plasmid-located *vanB*-containing Tn5382-like element in TUH2-19 and -55, consistent with the observed colocalization of *vanB* and

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TABLE 1. Bacterial strains used in this study

Strain	Relevant characteristics ^a	Reference(s)
<i>E. faecium</i>		
TUH2-21	Epidemic human clinical strain; Ap ^r Vm ^s	12
TUH2-18	Clonally related to TUH2-21; chromosomal <i>vanB2</i> ; Ap ^r Vm ^r	6, 12
TUH2-19	Clonally related to TUH2-21; plasmid <i>vanB2</i> ; Ap ^r Vm ^r	12
TUH2-20	Clonally related to TUH2-21; chromosomal <i>vanB2</i> ; Ap ^r Vm ^r	12
TUH2-55	Clonally related to TUH2-21; plasmid <i>vanB2</i> ; Ap ^r Vm ^r	12
BM4105-RF	Recipient strain in transfer studies; Vm ^s Rif ^r Fus ^r	20
<i>E. faecalis</i>		
JH2-2	Recipient strain in transfer studies; Vm ^s Rif ^r Fus ^r	13
UV202	Recombination-deficient derivative of JH2-2	23

^a Ap^r, ampicillin resistant; Vm^s, vancomycin susceptible; Vm^r, vancomycin resistant; Rif^r, rifampicin resistant; Fus^r, fucidic acid resistant. The TUH strains were isolated at Haukeland University Hospital, Bergen, Norway (12).

Tn5382 hybridization signals corresponding to the PFGE wells (lanes 5 and 6, Fig. 1B and C, respectively). Sequencing of inverse PCR products from TUH2-19 revealed insertion of the Tn5382-like element in an *IS1216V* element (GenBank accession no. AF289475 and AF289476). Hybridization of plasmid

DNA (data not shown) provided evidence of the presence of a *vanB*- and *IS1216V*-containing plasmid in TUH2-19 and -55. Comigrating *IS1216V* probe-positive plasmid DNA fragments were observed in TUH2-18, -20, and -21, consistent with an *IS1216V*-containing plasmid. These data are compatible with

TABLE 2. PCR primers used in this study

Amplicon	Primer sequence (5' - 3')	Size of amplicon (bp)	Reference
<i>vanB</i>	CAAAGCTCCGCAGCTTGCATG TGCATCCAAGCACCCGATATAC	484	6
<i>vanB</i> long	GTTTGATGCAGAGGCAGACGACT ACAAGTTCCCTGTATCCAAGTGG	5,959	6
Tn5382	GTTCTTATTCCGCAGGTGGTGATT ACGCCATGCTATTTACTTCCGGC	311	4
Tn5382 left inverse	GCTATGGCAGTTTTCCGTGTG TCGCCTCCTTCTCTATTTGG	Variable	This study
Tn5382 right inverse	GAGGGGGAAATGGTGAGAGGT AACGCTTCTCATGGCTCTTG	Variable	This study
TUH2-18 Tn5382 left	TACTGCCAATGATGTCAACCC GTTCTTATTCCGCAGGTGGTGATT	721	This study
TUH2-18 Tn5382 right	GAGGGGGAAATGGTGAGAGGT ATCCTTTGACGATCATCTTGG	413	This study
TUH2-18 Tn5382 target	TACTGCCAATGATGTCAACCC ATCCTTTGACGATCATCTTGG	439	This study
TUH2-19 Tn5382 left	CCGCAAGGGGATTTTAGTA GTTCTTATTCCGCAGGTGGTGATT	677	This study
TUH2-19 Tn5382 right	GAGGGGGAAATGGTGAGAGGT CCACGGCTACAATAATCACA	248	This study
TUH2-19 Tn5382 target	CCGCAAGGGGATTTTAGTA CCACGGCTACAATAATCACA	224	This study
Tn5382-like junction	GAGGGGGAAATGGTGAGAGGT GTTCTTATTCCGCAGGTGGTGATT	701 ^a	This study
<i>IS1216V</i>	AAAGCAATTTTCAGCAGGATG GTACGATGTTCTGTCCCTTG	456	This study
16S rDNA	TGCATTAGCTAGTTGGTGAGG TCGAATTAACACATGCTCC	726	7
23S rDNA	CGCATGTACAGGATAGGTAGG AGGTGGGCTTCACTTAGAT	669	This study

^a Including 6-bp coupling sequence.

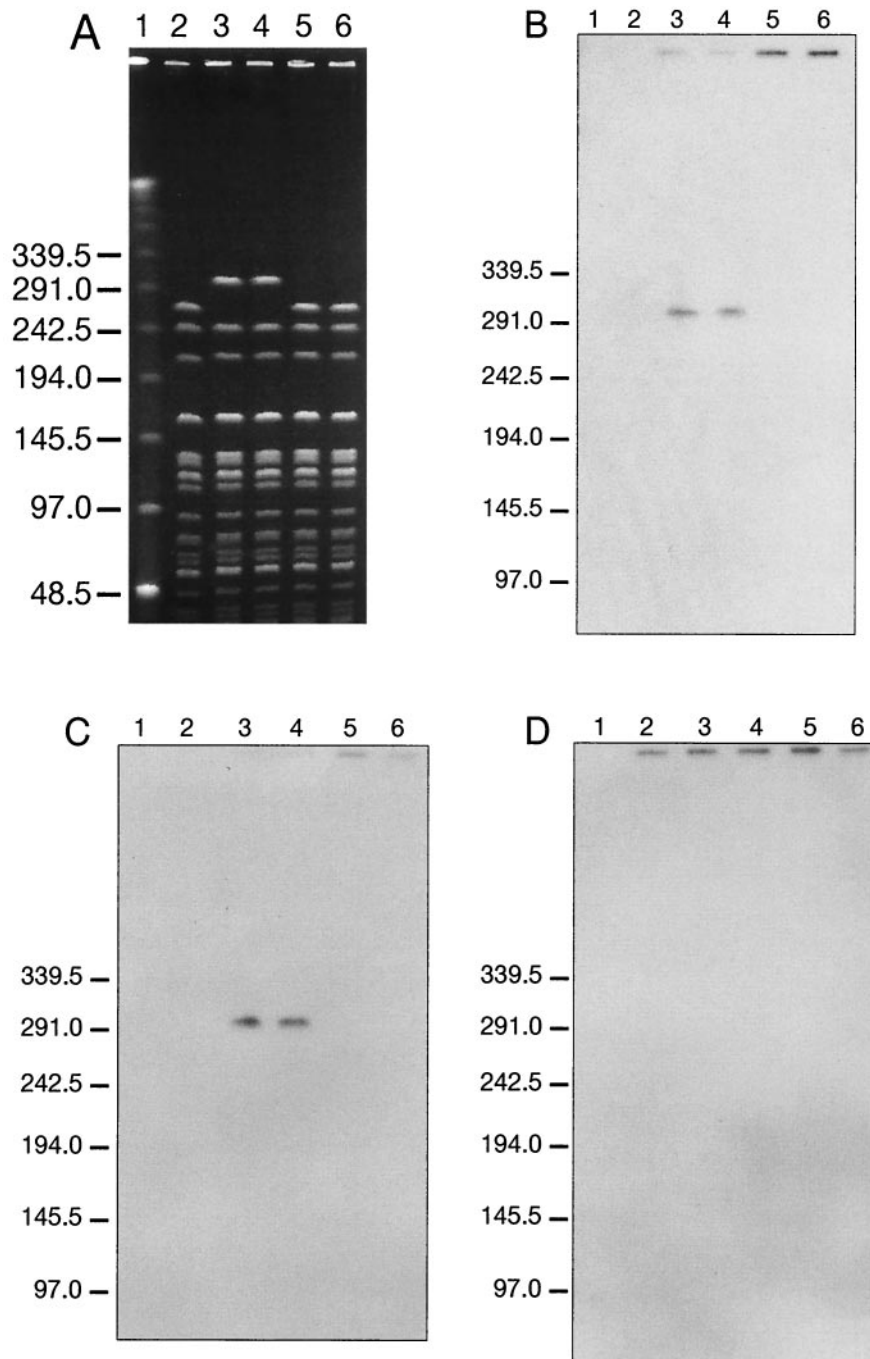


FIG. 1. PFGE of *Sma*I-digested total DNAs of four vancomycin-resistant strains and the prototype ampicillin-resistant *E. faecium* outbreak strain (TUH2-21) (A) and corresponding Southern hybridization with a *vanB* probe (B), a Tn5382 probe (C), and an IS1216V probe (D). Lanes: 1, low-range PFGE marker (New England Biolabs); 2, TUH2-21; 3, TUH2-20; 4, TUH2-18; 5, TUH2-19; 6, TUH2-55.

the IS1216V signals corresponding to the locations of the wells of the four VanB strains and TUH2-21 (Fig. 1D). The Tn5382-like element-flanking sequences in TUH2-19 were identical to TUH2-21 and -18 plasmid sequences, except for six additional base pairs (AAATTA) at the left extremity of the Tn5382-like element (GenBank accession no. AF289473 to AF289476; Fig. 2B). Taken together, these observations indicate transposition of the Tn5382-like element into a preexisting IS1216V-containing plasmid or a 270-kb *Sma*I chromosomal fragment in

TUH2-21, generating the TUH2-19 and TUH2-18 strains, respectively. The six different additional base pairs of the Tn5382-like element in TUH2-18 and TUH2-19 are linked to the opposite ends of the transposon, indicating introduction of the Tn5382-like element into TUH2-21 by two separate events. Knowing the target sequence for integration in TUH2-21, the additional six base pairs most likely represent flanking sequences from the prior insertion rather than target duplication upon insertion, as earlier hypothesized (4).

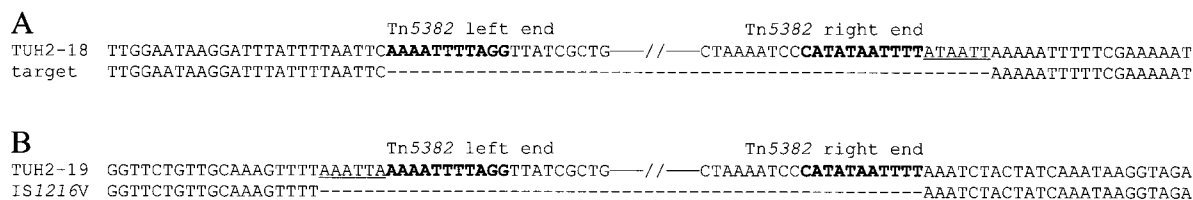


FIG. 2. Target sequence at the site of integration of the Tn5382-like transposon into the chromosome of TUH2-18 and in a plasmid in TUH2-19. The nucleotide sequences of the Tn5382-like elements chromosomally located in TUH2-18 (A) and plasmid borne in TUH2-19 (B) were aligned with the target sequence in TUH2-21. Tn5382-like element inverted repeats are in bold letters. The 6-bp coupling sequence is underlined.

Tn5382 junction PCR analysis (Table 2) of TUH2-18 and TUH2-19 revealed the expected 701-bp product indicating the presence of an excised free Tn5382 circular intermediate (results not shown). Unique insertions of the Tn5382-like element (4, 11; this study) and the presence of circular intermediates confirmed by PCR amplification (4, 11; this study) thus suggest that the Tn5382-like element is a functional mobile genetic element in enterococci. However, direct evidence of conjugative transposition of the Tn5382-like element in enterococci has not been demonstrated. Secondary transfer of the Tn5382-like element was therefore attempted with *vanB* donors (TUH2-18 and TUH2-19) and the recipient *E. faecium* BM4105-RF or *E. faecalis* JH2-2 or UV202. The plasmid-associated *vanB2* operon (TUH2-19) showed a transfer frequency of about 2×10^{-5} transconjugants per donor with *E. faecium* BM4105-RF. Low-frequency (6×10^{-9}) transfer of the chromosomal *vanB2* element (TUH2-18) was obtained only with *E. faecium* BM4105-RF. Molecular analyses of five transconjugants from each experiment did not reveal conjugative transposition of the Tn5382-like element. Rather, the chromosomal Tn5382-like element in TUH2-18 is transferred as an integral part of variable-size DNA elements and the TUH2-19 Tn5382-like element is transferred as part of a mobilizable plasmid (data not shown). The lack of firm evidence that the Tn5382-like element is a conjugative transposon in enterococci stands in contrast to the molecular data presented in references 4 and 11 and in this study, as well as the finding of several open reading frames with significant homology to proteins involved in conjugative transfer at the left end of the transposon (11). Further studies are needed to address the mechanisms of intracellular transposition and conjugative mobilization of the Tn5382-like element in order to understand and control the spread of such determinants.

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