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Nonconjugative Transposition of the *vanB*-Containing Tn*5382*-Like Element in *Enterococcus faecium*

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The *vanB2* operon encoding glycopeptide resistance is an integral part of the putative conjugative transposon Tn*5382*. Characterization of clinical glycopeptide resistant derivatives from an epidemic ampicillin-resistant *Enterococcus faecium* strain showed precise chromosomal or plasmid insertions of a *vanB2*-containing Tn*5382*-like element. Conjugative transposition of the Tn*5382*-like element was not demonstrated in retransfer studies.

The 34-kb *vanB*-containing transposon Tn*1549*, which is similar to the Tn*916*-Tn*1545* family of conjugative transposons, was recently described (11). Tn*1549* appears to be identical to the previously described and partially sequenced transposon Tn*5382* (4). It is therefore designated a Tn*5382*-like element. The *vanB2* operon seems to be universally linked to the Tn*5382*-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 Tn*916*-Tn*1545* 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 Tn*5382* transposition has been described previously (4, 11). We present additional data for precise transposition of Tn*5382*-like elements under natural conditions describing the arrival of the Tn*5383*-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, IS*1216V* from *E. faecium* BM4147 (14), Tn*5382* 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-*Ceu*I (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 Tn*5382*-flanking sequences. The Tn*5382* left and right ends were amplified with internal divergent Tn*5382* primers after ligation of *Dra*I-, *Hin*fI-, or *Hph*I/*Bfa*I- and *Alu*I-, *Dde*I-, or *Sau*3AI-digested total DNA (1 to 3 μg), 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 Tn*5382* (Fig. 1C, lanes 3 and 4) probes. Cohybridization of *vanB* and rDNA probes to I-*Ceu*I PFGE fragments confirmed the chromosomal *vanB* location (data not shown). Inverse PCR and sequencing showed insertion of the Tn*5382*-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-*Ceu*I PFGE fragments (data not shown) revealed a plasmid-located *vanB*-containing Tn*5382*-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)
E. faecium		
TUH2-21	Epidemic human clinical strain; Ap^r Vm^s	12
TUH2-18	Clonally related to TUH2-21; chromosomal *vanB2;* Ap^r Vm^r	6, 12
TUH2-19	Clonally related to TUH2-21; plasmid *vanB2;* Ap^r Vm^r	12
TUH2-20	Clonally related to TUH2-21; chromosomal *vanB2;* Ap^r Vm^r	12
TUH2-55	Clonally related to TUH2-21; plasmid *vanB2;* Ap^r Vm^r	12
BM4105-RF	Recipient strain in transfer studies; Vm^s Rif^r Fus^r	20
E. faecalis		
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).

Tn*5382* 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 Tn*5382*-like element in an IS*1216V* element (GenBank accession no. AF289475 and AF289476). Hybridization of plasmid DNA (data not shown) provided evidence of the presence of a *vanB*- and IS*1216V*-containing plasmid in TUH2-19 and −55. Comigrating IS*1216V* probe-positive plasmid DNA fragments were observed in TUH2-18, −20, and −21, consistent with an IS*1216V*-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
vanB	CAAAGCTCCGCAGCTTGCATG TGCATCCAAGCACCCGATATAC	484	6
vanB long	GTTTGATGCAGAGGCAGACGACT ACAAGTTCCCCTGTATCCAAGTGG	5,959	6
Tn*5382*	GTTCTTATTCCGCAGGTGGTGATT ACGCCATGCTATTTACTTCCGGC	311	4
Tn*5382* left inverse	GCTATGGCAGTTTTCCGTGTG TCGCCTCCTTTCTCTATTTGG	Variable	This study
Tn*5382* right inverse	GAGGGGGAAATGGTGAGAGGT AACGCTTCTTCATGGCTCTTG	Variable	This study
TUH2-18 Tn*5382* left	TACTGCCAATGATGTCAACCC GTTCTTATTCCGCAGGTGGTGATT	721	This study
TUH2-18 Tn*5382* right	GAGGGGGAAATGGTGAGAGGT ATCCTTTGACGATCATCTTGG	413	This study
TUH2-18 Tn*5382* target	TACTGCCAATGATGTCAACCC ATCCTTTGACGATCATCTTGG	439	This study
TUH2-19 Tn*5382* left	CCGCAAGGGGATTTTAGTA GTTCTTATTCCGCAGGTGGTGATT	677	This study
TUH2-19 Tn*5382* right	GAGGGGGAAATGGTGAGAGGT CCACGGCTACAATAATCACA	248	This study
TUH2-19 Tn*5382* target	CCGCAAGGGGATTTTAGTA CCACGGCTACAATAATCACA	224	This study
Tn*5382*-like junction	GAGGGGGAAATGGTGAGAGGT GTTCTTATTCCGCAGGTGGTGATT	701[a]	This study
IS*1216V*	AAAGCAATTTCAGCAGGATG GTACGATGTTCTGTCCCTTG	456	This study
16S rDNA	TGCATTAGCTAGTTGGTGAGG TCGAATTAAACCACATGCTCC	726	7
23S rDNA	CGCATGTACAGGATAGGTAGG AGGTGGGCTTCACACTTAGAT	669	This study

[a] Including 6-bp coupling sequence.

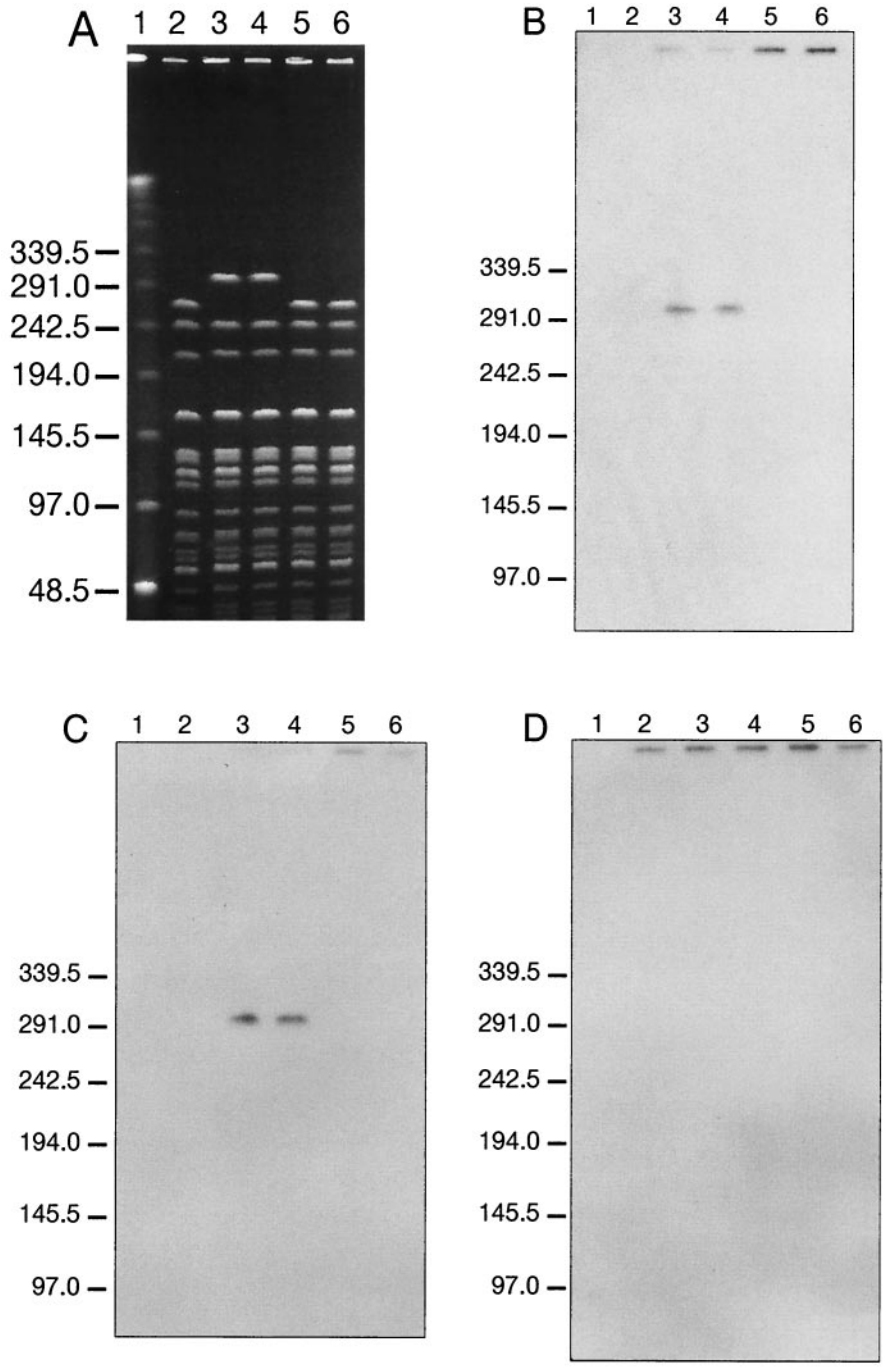

FIG. 1. PFGE of *Sma*I-digested total DNAs of four vancomycin-resistant strains and the prototype ampicillin-resistant *E. faecium* outbreak strain (TUH2-21) (A) and corresponding Southern hybridization with a *vanB* probe (B), a Tn*5382* probe (C), and an IS*1216V* probe (D). Lanes: 1, low-range PFGE marker (New England Biolabs); 2, TUH2-21; 3, TUH2-20; 4, TUH2-18; 5, TUH2-19; 6, TUH2-55.

the IS*1216V* signals corresponding to the locations of the wells of the four VanB strains and TUH2-21 (Fig. 1D). The Tn*5382*-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 Tn*5382*-like element (GenBank accession no. AF289473 to AF289476; Fig. 2B). Taken together, these observations indicate transposition of the Tn*5382*-like element into a preexisting IS*1216V*-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 Tn*5382*-like element in TUH2-18 and TUH2-19 are linked to the opposite ends of the transposon, indicating introduction of the Tn*5382*-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).

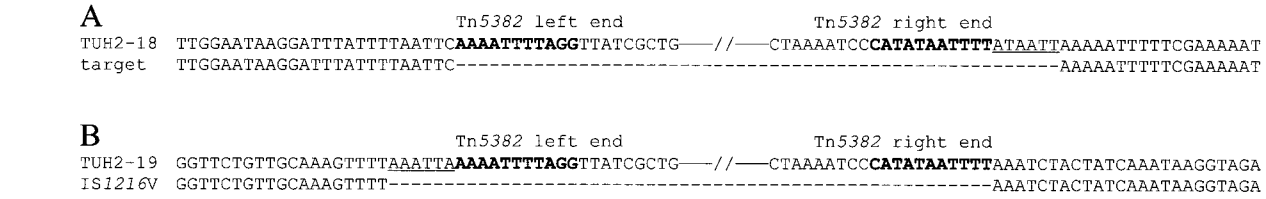

FIG. 2. Target sequence at the site of integration of the Tn*5382*-like transposon into the chromosome of TUH2-18 and in a plasmid in TUH2-19. The nucleotide sequences of the Tn*5382*-like elements chromosomally located in TUH2-18 (A) and plasmid borne in TUH2-19 (B) were aligned with the target sequence in TUH2-21. Tn*5382*-like element inverted repeats are in bold letters. The 6-bp coupling sequence is underlined.

Tn*5382* junction PCR analysis (Table 2) of TUH2-18 and TUH2-19 revealed the expected 701-bp product indicating the presence of an excised free Tn*5382* circular intermediate (results not shown). Unique insertions of the Tn*5382*-like element (4, 11; this study) and the presence of circular intermediates confirmed by PCR amplification (4, 11; this study) thus suggest that the Tn*5382*-like element is a functional mobile genetic element in enterococci. However, direct evidence of conjugative transposition of the Tn*5382*-like element in enterococci has not been demonstrated. Secondary transfer of the Tn*5382*-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 Tn*5382*-like element. Rather, the chromosomal Tn*5382*-like element in TUH2-18 is transferred as an integral part of variable-size DNA elements and the TUH2-19 Tn*5382*-like element is transferred as part of a mobilizable plasmid (data not shown). The lack of firm evidence that the Tn*5382*-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 Tn*5382*-like element in order to understand and control the spread of such determinants.

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