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Identification of Chromosomal Mobile Element Conferring High-Level Vancomycin Resistance in *Enterococcus faecium*

SANDRA HANDWERGER* AND JUSTIN SKOBLE

Laboratory of Microbiology, The Rockefeller University, New York, New York 10021

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A clinical isolate of *Enterococcus faecium* that contains a chromosomally encoded *vanA* gene cluster, Tn*1546*::IS*1251*, transferred vancomycin resistance to the plasmid-free strain *Enterococcus faecalis* JH2-2 during filter matings. Hybridization of a *vanHAXY* probe to *Sma*I restriction-digested genomic DNA separated by pulsed-field gel electrophoresis showed that the *vanA* gene cluster was located on a 40-kb fragment in the original donor strain and on fragments of different sizes (150 to 450 kb) in the transconjugants. No hybridization to *vanA* gene cluster probes was obtained with plasmid DNA preparations from the donor or transconjugants. These results suggested that in each case, the *van* genes had integrated into the recipient chromosome. The transconjugants in turn could act as donors of vancomycin resistance, and resistance was transferable to a Rec$^-$ recipient. The results of restriction analyses and DNA hybridizations of genomic DNA from the donor and transconjugants were consistent with the transfer of a mobile element that includes the 12.3-kb Tn*1546*::IS*1251* gene cluster and at least 13 kb of additional DNA. This element has been tentatively designated Tn*5482*. DNA sequence analysis of a fragment predicted to contain the left end of Tn*5482* revealed two insertion sequence-like elements: IS*1216V* and an apparently truncated IS*3*-like element. Restriction mapping and DNA hybridization patterns of the *van* gene clusters of three additional clinical isolates from New York City showed an element similar to Tn*5482*. Transfer of Tn*5482* and related elements may be involved in dissemination of vancomycin resistance.

Enterococci possess an impressive array of mechanisms for the transfer of antibiotic resistance determinants. Conjugative transfer of resistance genes may occur either via plasmids or via transposons. The enterococcal conjugative transposons, of which Tn*916* has been most intensively studied, differ from classic transposons in that they are capable of independent intercellular transfer (10, 41). These elements range in size from 16 kb to greater than 50 kb and usually confer tetracycline resistance (10, 37). Conjugative transposons are considered among the most promiscuous of mobile elements, since they may be transferred into a wide variety of gram-positive and gram-negative species (41).

Transposons that are not self-transferable between strains also play a role in dissemination of resistance determinants, via intracellular transposition onto conjugative plasmids. Two such classes of transposons have been identified in enterococci: the composite transposons and the Tn*3*-like transposons (31). Composite transposons, which consist of two (or more) flanking insertion sequences and a resistance determinant, have recently been identified in enterococci. For example, Tn*5281*, which contains IS*256* flanking the bifunctional aminoglycoside resistance gene *aphD-aacA*, has been located on conjugative plasmids of *Enterococcus faecalis* (23). A 26-kb element containing three copies of IS*256* and determinants encoding erythromycin and aminoglycoside resistance has recently been identified in the chromosome of *E. faecalis* (35).

Members of the Tn*3* family of transposons are characterized by the presence of transposase (*tnpA*) and resolvase (*tnpR*) genes and a resistance determinant (31). This family is exemplified by Tn*917*, a 5.3-kb element encoding erythromycin resistance which was identified from the *E. faecalis* plasmid pAD2 (11). Tn*3* family elements most often reside on plasmids and have a predilection for insertion into plasmid DNA (31, 44). In several strains of *Enterococcus faecium*, the genes conferring high-level vancomycin resistance (VanA phenotype) have been shown to reside on a transposon, Tn*1546*, that is a member of the Tn*3* family (3). This 10,851-bp transposon includes nine open reading frames (ORFs): a putative transposase and resolvase (ORF1 and ORF2, respectively) and seven ORFs that mediate resistance (*vanR*, *vanS*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ*) (2, 3). Although Tn*1546* was first identified on a nonconjugative plasmid (26), a number of conjugative plasmids that carry Tn*1546* or related elements have now been isolated (3, 14, 21, 22). Furthermore, numerous epidemiologic investigations have identified the *vanA* gene on plasmids in enterococcal strains of different genetic backgrounds by pulsed-field gel electrophoresis (PFGE) and ribotyping (4, 8, 46). Together, these observations suggest that transposition of Tn*1546* or related elements onto conjugative plasmids is an important mechanism for the dissemination of resistance.

Recently, *E. faecium* clinical isolates from the northeastern United States were identified in which the *van* genes appeared to reside in the chromosome. One of these strains (strain GUC) contained a Tn*1546*-like element that was interrupted in the *vanS-vanH* intergenic region by the novel 1,496-bp insertion sequence (IS) element IS*1251* (21). Restriction analysis and DNA hybridization of 10 *E. faecium* strains from four hospitals in New York City showed a similar pattern (21), raising the possibility that Tn*1546*::IS*1251* resided on a mobile element with a predilection for integration into the chromosome. The aim of the study described here was to characterize this putative mobile element. We present evidence that in some *E. faecium* strains, Tn*1546*::IS*1251* resides within a trans-

* Corresponding author. Mailing address: Laboratory of Microbiology, Rockefeller University, 1230 York Ave., New York, NY 10021. Phone: (212) 327-8290. Fax: (212) 327-8688. Electronic mail address: handwes@rockvax.rockefeller.edu.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)[a]	Source or reference
Bacterial strains		
E. faecium		
GUC	Vm^r clinical isolate	21
Bat	Vm^r clinical isolate	21
Fair	Vm^r clinical isolate	21
COP	Vm^r clinical isolate	21
E. faecalis		
JH2-2	Plasmid free; Rif^r Fus^r	25
OG1X	Plasmid free; Str^r	24
UV202	Plasmid free; Rif^r Fus^r Rec^-	47
JG-7	Vm^r Rif^r Fus^r	GUC × JH2-2
JG-8	Vm^r Rif^r Fus^r	GUC × JH2-2
OJG-72	Vm^r Tet^r Str^r	JG-7(pCF10) × OG1X
OJG-72C1	Vm^r Str^r	Derivative of OJG-72[b]
Plasmids		
pHKK100	Vm^r; pheromone response	19
pCF10	Tet^r; pheromone response	13
pHKK702	Vm^r	22

[a] Abbreviations: Vm, vancomycin; Rif, rifampin; Fus, fusidic acid; Str, streptomycin; Tet, tetracycline.

[b] OJG-72C1 is a derivative of OJG-72 from which pCF10 has been cured by growth at 45°C.

poson with a size of at least 26 kb, tentatively designated Tn*5482*, that integrates into the chromosome. We propose that elements similar to Tn*5482* may be involved in dissemination of high-level vancomycin resistance.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains and plasmids used in this study are shown in Table 1. *E. faecium* blood culture isolates were provided by Timothy Kiehn, Memorial Hospital, New York, N.Y. (strain GUC); Judy Berger, St. Barnabus Hospital, Bronx, N.Y. (strains COP and Bat); and Amy Kresel, Bronx Municipal Hospital, Bronx, N.Y. (strain Fair). All four contain IS*1251* in the *vanS-vanH* intergenic region (21). *E. faecalis* JH2-2 (25) and UV202 (47) were gifts of Don B. Clewell. *E. faecalis* OG1X (24) and plasmid pCF10 (13) were gifts of Keith Weaver. Enterococcal strains were grown in brain heart infusion broth (Difco) at 37°C. *Escherichia coli* XL1-Blue (Stratagene) was grown in Luria-Bertani broth at 37°C with aeration.

Filter matings. Filter matings were performed by a modification of the procedure of Clewell et al. (9). Briefly, aliquots of late-log-phase cultures were mixed in a donor/recipient ratio of 1:10 and applied to one or more 45-μm-pore-diameter filters on the surface of a tryptic soy agar plate (Difco) supplemented with 4% sheep blood (Micropure Medical, White Bear Lake, Minn.). After overnight incubation, cells were suspended in 1 ml of brain heart infusion broth, and aliquots were plated on selective media. The concentrations of antibiotics used for selection of transconjugants were as follows: vancomycin, 10 μg/ml; rifampin, 20 μg/ml; fusidic acid, 20 μg/ml; tetracycline, 10 μg/ml; and streptomycin, 1,000 μg/ml.

DNA analysis. Small-scale preparations of plasmid DNA were prepared as previously described (21). Large-scale preparations of *E. coli* plasmid DNA were performed by the alkaline lysis method as described by Maniatis et al. (29). Genomic DNA was prepared by the method of Wilson (45), except that washed cells were incubated with 10 mg of lysozyme per ml for 30 min prior to the addition of sodium dodecyl sulfate. Restriction digestions were performed as recommended by the manufacturer (Gibco BRL).

DNA was prepared for PFGE analysis by the method of de Lencastre et al. (12) with minor modifications, including suspension of the harvested cells 1:1 in 1.5% agarose and lysis of the cell suspensions in 100 μg of lysozyme per ml with 50 mg of RNAse A (Sigma) per ml. DNA was incubated with *Sma*I overnight at 25°C. PFGE was performed as described by de Lencastre et al. (12), except that gels were prepared with 0.8% agarose. Gels were electrophoresed in a contour-clamped homogeneous electric field apparatus (CHEF-DRII; Bio-Rad) at 200 V for 26 h at 7°C, with the pulse time linearly ramped from 1 s to 30 s.

DNA hybridization. The ORF1, *vanR*, and *vanHAXY* probes were prepared from pHKK100 DNA (19). The ORF1 (0.75 kb) and *vanR* (0.50 kb) probes were obtained by PCR amplification as previously described (21), with primers from the sequences of pIP816 (3) and pHKK100 (18), respectively. The *vanHAXY* probe was prepared from the *Sal*I-*Eco*RI fragment as previously described (21). The *vanYZ* probe was prepared from pHKK702 DNA (22a) and was obtained by elution of the 1.09-kb *Ava*I-*Kpn*I fragment from an agarose gel. The IS*1251* probe (0.39 kb) was prepared from the *Eco*RI-*Hin*dIII fragment of a pBluescript clone containing the *vanS-vanH* intergenic region of *E. faecalis* JG-7 (previously described as GUCJ7 [21]). The 0.9-kb probe near the right end of Tn*5482* was prepared from a *Sal*I-*Kpn*I digest of a pBluescript clone containing a 4.5-kb *Eco*RV fragment of OJG-72 by using the *Sal*I site present in the *Eco*RI adapter.

Two probes (IS*1216* and IS*3*-like) were synthesized by PCR amplification of plasmid DNA from pBluescript containing a 7-kb *Eco*RV fragment of *E. faecalis* JG-7 on the basis of the DNA sequence obtained (see below). The primers used are shown in Figure 3. PCR was performed with a DNA Thermal Cycler 480 (Perkin-Elmer Cetus) by the protocol recommended by the manufacturer. Oligonucleotides were obtained from Genosys (Woodlands, Tex.).

DNA probes were labelled with digoxigenin-11-dUTP by random-primed labelling (Genius; Boehringer Mannheim). Restriction-digested DNA was transferred to nylon membranes (Hybond-N; Amersham) by vacuum blotting and was cross-linked to the membranes by exposure to UV light. Hybridization and washings were performed as directed by the manufacturer (Boehringer Mannheim). Hybridizing fragments were identified with an anti-digoxigenin alkaline phosphatase conjugate with a chromogenic substrate.

DNA cloning and sequencing. Genomic libraries of *E. faecalis* JG-7 and OJG-72 were constructed in LambdaZapII (Stratagene). DNA was restriction digested by incubation overnight with *Eco*RV, *Eco*RI adapters (Gibco BRL) were added by ligation with T4 ligase (Gibco BRL), and the excess adapters were removed by purification with a Wizard column (Promega). The resulting library of fragments was ligated to *Eco*RI-digested, alkaline phosphatase-treated LambdaZapII arms, packaged, and used to infect *E. coli* XL1-Blue MRF′ as directed by the manufacturer (Stratagene). Plaques were transferred to nylon membranes (Hybond N; Amersham), and the lambda clone containing the desired insert was identified by hybridization with the ORF1 (for JG-7) or IS*1216* (for OGJ-72) probe. After plaque purification, pBluescript containing the insert was excised by rescue with helper phage.

Double-stranded DNA sequencing of the pBluescript insert from JG-7 was performed by the dideoxy chain termination method of Sanger et al. (39) with incorporation of [^{35}S]dATP, with the Sequenase 2.0 kit (U.S. Biochemical Corp.). Initial primers used were the T7 22-mer designed for use with pBluescript and a primer (5′-GGA GAA TAC CAT TTT AAC TCA G-3′) from within the published sequence of ORF1 (3). Sequencing was continued with internal sequence-specific primers, which were obtained from Genosys.

DNA and deduced amino acid sequences were analyzed with the BESTFIT and PILEUP programs (University of Wisconsin Genetics Computer Group [GCG] package, version 7.0-UNIX). The BLAST algorithm (1) was used to identify GenBank sequences with similarity.

Nucleotide sequence accession number. The nucleotide sequence shown in Fig. 3 has been assigned GenBank accession number L40841.

RESULTS

Transfer of a chromosomal element conferring vancomycin resistance. Filter mating of *E. faecium* GUC with the plasmid-free recipient *E. faecalis* JH2-2 yielded vancomycin-resistant transconjugants at a frequency of 1.6×10^{-9} per donor. Plasmid DNA from the donor and two transconjugants from separate matings (JG-7 and JG-8) showed no hybridization with the *vanHAXY* probe (not shown). To determine if the *van* genes were integrated into the chromosome, *Sma*I-digested genomic DNA from the donor and transconjugants was separated by PFGE (Fig. 1). Hybridization with the *vanHAXY* probe revealed that the *van* gene cluster was present on *Sma*I fragments with a size of approximately 40 kb in the donor, *E. faecium* GUC, and also on those with sizes of approximately 370 and 160 kb in the transconjugants, JG-7 and JG-8, respectively.

Comparison of the PFGE restriction patterns revealed differences in the electrophoretic mobility of two or more fragments in each transconjugant compared with that of the corresponding recipient (Fig. 1). For example, the pattern of JG-7 lacks two fragments with sizes of approximately 350 and 120 kb that are present in JH2-2 and contains two new fragments with sizes of approximately 370 and 160 kb. However, only one new fragment in each transconjugant hybridized with the *vanHAXY* probe.

Although differential hybridization of plasmid and genomic DNA had suggested that the *van* genes were present in the

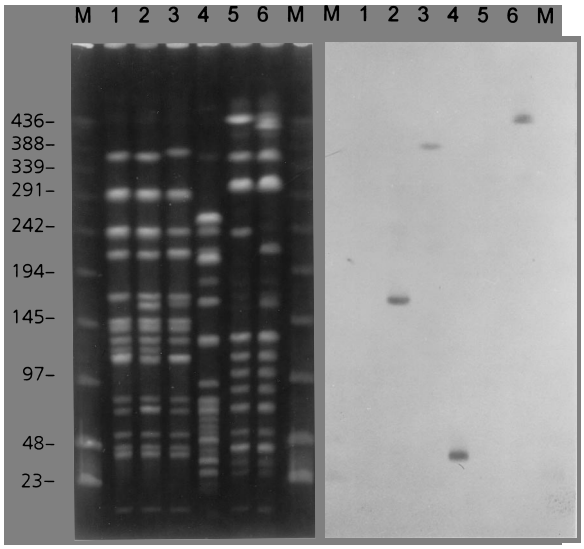

FIG. 1. PFGE analysis of *Sma*I restriction-digested genomic DNA prepared from *E. faecium* GUC; the transconjugants *E. faecalis* JG-7, JG-8, and OJG-72; and the recipients *E. faecalis* OG1X and JH2-2. Left panel, agarose gels stained with ethidium bromide; right panel, Southern hybridization with the *vanHAXY* probe. Lanes: 1, JH2-2; 2, JG-8; 3, JG-7; 4, GUC; 5, OG1X; 6, OJG-72. M, molecular size standards (lambda concatemers; New England Biolabs).

chromosome of the donor *E. faecium* GUC (21), the possibility could not be excluded that the 40-kb *Sma*I fragment observed by PFGE was derived from a large plasmid that was not easily purified rather than from the chromosome. Therefore, to determine whether the *van* genes could be transferred from the chromosome of the donor to that of a recipient, the JH2-2 transconjugants were used as donors in filter matings with *E. faecalis* OG1X. JG-8 transferred resistance at a frequency of 9×10^{-9} per donor, but no transconjugants were obtained with JG-7 as a donor (frequency of $<2 \times 10^{-10}$ per donor). In order to enhance the ability of JG-7 to transfer vancomycin resistance, the conjugative plasmid pCF10 was introduced into this strain by filter mating. The presence of pCF10 in transconjugants (selected for tetracycline resistance) was confirmed by plasmid DNA analysis. Mating of JG-7(pCF10) with OG1X yielded one vancomycin-resistant transconjugant (frequency of 4×10^{-9} per donor), which was also resistant to tetracycline. *Sma*I-digested genomic DNA from the transconjugant, OJG-72, showed hybridization of a 450-kb fragment with the *van HAXY* probe (Fig. 1). *Eco*RI-digested plasmid DNA preparations of JG-7(pCF10) and OJG-72 contained only restriction fragments corresponding to those of pCF10 (7) and did not hybridize with the *vanHAXY* probe.

Both OJG-72 and its cured derivative OJG-72C1, which contained no detectable plasmid DNA, were able to transfer resistance by filter mating to JH2-2 (frequencies of 8×10^{-7} and 1×10^{-7} per donor, respectively). OJG-72 also transferred resistance to the recombination-deficient recipient UV202 (frequency of 1.4×10^{-8} per donor). Hybridization of plasmid and genomic DNA prepared from four independent UV202 transconjugants was consistent with the presence of the *vanA* gene cluster in the chromosome. The transfer of the *van* genes from the donor chromosome to different locations within the recipient chromosomes, including that of a recombination-deficient host, suggested that the *van* genes resided on a transposon.

Restriction mapping of the transposon containing Tn*1546*:: IS*1251*. To determine the size of this putative transposon, restriction analysis of the *van* gene cluster region of strains GUC, JG-7, and OJG-72 was performed by hybridization of single and double restriction-digested genomic DNA with the ORF1, *vanR*, *vanHAXY*, *vanYZ*, and IS*1251* probes. The results are shown in Fig. 2. As expected, the *vanR*, *vanHAXY*, *vanYZ*, and IS*1251* probes hybridized with restriction fragments of identical size in the donor and the transconjugants when these fragments lay within Tn*1546*::IS*1251*. However, several restriction endonucleases that were predicted to generate fragments extending beyond the limits of Tn*1546*::IS*1251* also yielded hybridizing fragments that were of identical size in the donor and transconjugants, as shown in Fig. 2. This suggested that approximately 1.5 kb of DNA 3′ to ORF1 of Tn*1546*::IS*1251* (to the left in Fig. 2) was similar, if not identical, in the three strains. In contrast, restriction fragments that included more than 2.1 kb of the region 3′ to ORF1 were not identical in size in the two strains.

Hybridization with the *vanYZ* probe also yielded several restriction fragments that were identical in the donor and transconjugants (Fig. 2). Taken together, these results suggested that Tn*1546*::IS*1251* resides within a mobile element with a size of at least 26 kb, which extends approximately 1.5 to 2.1 kb 3′ to ORF1 and at least 13 kb 3′ to *vanZ*. This element has been tentatively designated Tn*5482*.

Identification of a Tn*5482*-like element in clinical isolates of *E. faecium*. A previous study identified 10 clinical isolates of *E. faecium* from hospitals in the New York area that appeared to contain Tn*1546*::IS*1251* (21). To determine whether such isolates contained Tn*5482* or a related element, three of the strains (*E. faecium* COP, Bat, and Fair) were selected for more detailed mapping and hybridization. Figure 2 shows the results for strain COP, which is clonally distinct from GUC by PFGE (not shown). Restriction mapping with the ORF1 probe indicated all three clinical isolates contained a 1.5-kb region 3′ to ORF1 that is similar or identical to that of Tn*5482*. When genomic DNA was restriction digested with enzymes that yielded fragments containing up to 7 kb 3′ to *vanZ*, the sizes of restriction fragments hybridizing with the *vanYZ* probe were identical in the clinical strains and transconjugants. In contrast, hybridizing restriction fragments obtained by digestion with *Xho*I and *Sal*I differed in size. On the basis of the locations of the *Sal*I and *Xho*I sites within Tn*1546*::IS*1251*, these fragments contain at least 12.5 kb of DNA lying 3′ to *vanZ*. Thus, between 7 and 12.5 kb of DNA lying 3′ to *vanZ* appears to be similar or identical among the isolates. These findings suggest that strains COP, Bat, and Fair contain an element similar but not identical to Tn*5482*.

Sequence analysis of the left end of Tn*5482*. Restriction analyses of the donor and transconjugants suggested that the left end of Tn*5482* lay between the *Eco*RV and *Cla*I sites 3′ to ORF1 in *E. faecalis* JG-7 (Fig. 2). In order to characterize this end of Tn*5482*, the 7-kb *Eco*RV fragment of JG-7 that includes approximately 4.9 kb of Tn*1546*::IS*1251* and 2.1 kb 3′ to ORF1 was cloned into LambdaZapII.

The DNA sequence of a portion of this fragment, extending from the *Eco*RV site to within ORF1 (2,266 nucleotides [nt]), is shown in Fig. 3. An ORF (nt 233 to 919) corresponding to a deduced sequence of 228 amino acids was identified. The deduced amino acid sequence of this ORF was similar (82% identity) to that of the putative transposase of the *Lactococcus lactis* element ISS*1* (32) and was nearly identical (98%, 224 of 228 amino acids) to that of IS*1216*, an IS described in *Enterococcus hirae* (GenBank accession no. X81654 [11a]). The ORF is flanked by two inverted repeats (IRs) with sizes of 18 bp that are identical to those of ISS*1* and IS*1216*. An apparently interrupted version of this 809-bp element, designated IS*1216V*,

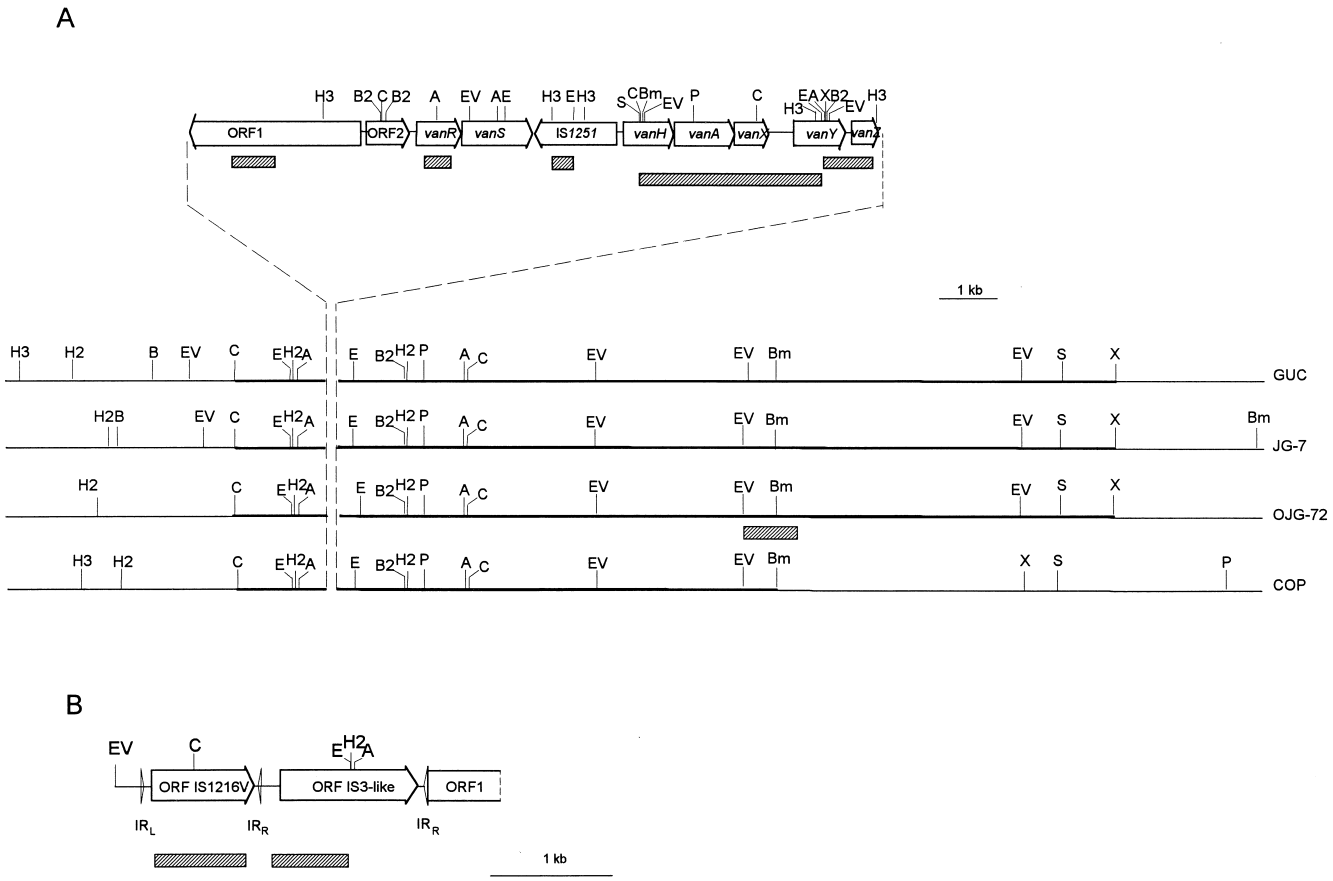

FIG. 2. (A) Restriction maps of the *van* cluster and flanking regions from genomic DNA of *E. faecium* GUC, the transconjugants *E. faecalis* JG-7 and OJG-72, and *E. faecium* COP. The inset at the top represents Tn*1546*::IS*1251*, and the lower four maps represent the flanking regions in each of the strains. The locations of the ORF1, *vanR*, IS*1251*, *vanHAXY*, *vanYZ*, and 0.9-kb *Sal*I-KpnI probes are indicated by hatched rectangles. Sites that lie outside the region illustrated and that are not shown are the leftmost *Eco*RV sites of OGJ-72 and COP and *Hin*dIII sites of OJG-72 and JG-7 and the rightmost *Bam*HI sites of GUC and OJG-72 and *Pvu*II sites of GUC, JG-7, and OJG-72. The locations of the leftmost *Hin*cII restriction sites were deduced from hybridizations with the IS*3*-like probe (not shown) and of the rightmost *Eco*RV, *Bam*HI, and *Pvu*II sites from the 0.9-kb *Sal*I-*Kpn*I probe; other restriction sites outside Tn*1546*::IS*1251* were determined on the basis of hybridization with the ORF1 and *vanYZ* probes. Thick lines indicate the minimum size of Tn*5482* as determined by restriction mapping. A, *Ava*I; B, *Bgl*I; B2, *Bgl*II; Bm, *Bam*HI; C, *Cla*I; E, *Eco*RI; EV, *Eco*RV; H2, *Hin*cII; H3, *Hin*dIII; P, *Pvu*II; S, *Sal*I; X, *Xho*I. (B) Enlarged schematic diagram of the region 3′ to ORF1 in strain JG-7, corresponding to the sequence shown in Fig. 3. ORFs of the IS-like elements are indicated by arrows. The right and left IRs of IS*1216V* (IR_R and IR_L, respectively) are shown by triangles, as is the putative IR of the IS*3*-like element (IR_R). The ORF1 region of Tn*1546*::IS*1251*, indicated by a rectangle, is a truncated version of that present in Tn*1546* (3), lacking the first 120 nt. Hatched rectangles indicate the locations of the IS*1216V* and IS*3*-like probes.

has also been recently identified on pHKK702, a vancomycin resistance plasmid isolated from *E. faecium* (22).

A second ORF (nt 1108 to 2040 [Fig. 3]) that was transcribed in the same direction as IS*1216V* and that corresponded to a protein with a size of 310 amino acids was identified. The deduced amino acid sequence of this ORF showed homology to those of the longer second ORFs (the putative transposases) of several members of the IS*3* family. The greatest degree of similarity was identified with ORFB of the *E. coli* IS*150* (37.2% amino acid identity overall [40]) and ORF2 of *Streptococcus agalactiae* IS*861* (36.2% identity overall [38]). The 7-bp sequence AAAAAAG (nt 1134 to 1141 [Fig. 3]) present near the start of the ORF identified is characteristically found near the overlap between the ORFs of IS*3* family elements and has been implicated in translational frameshifting (5). A 26-bp sequence, CTGTCTACTTGACAGGGGGC AGTTCA, that is similar (16 of 26 nt) to the right IR of IS*861* (38) is present 73 nt downstream from the stop codon of the IS*3*-like ORF. The sequence of Tn*1546*::IS*1251* begins immediately at the end of this putative IR. Restriction analysis had suggested that the ORF1 region of Tn*1546*::IS*1251* was identical to that of Tn*1546* (21). However, the sequence of Tn*1546*::IS*1251* differs from that of Tn*1546* (3) in that the initial 120 nt are absent, including the left IR of Tn*1546* and the 45 nt at the 3′ end of the coding region of the ORF1 gene (Fig. 2B and 3).

DNA hybridization of the IS*1216V* and IS*3*-like probes. The presence of IS*1216V* near one putative terminus suggested that Tn*5482* might be a composite transposon, a class of mobile element characterized by copies of an IS at each end (15). To determine whether additional copies of IS*1216V* or the IS*3*-like element were present on Tn*5482*, DNA probes containing these elements were synthesized by PCR amplification (Fig. 2B and 3). Genomic DNAs from OJG-72 each yielded one *Eco*RV fragment hybridizing with the IS*3*-like probe and three *Eco*RV fragments hybridizing with the IS*1216* probe; no hybridization was obtained with DNA from the recipient OG1X. These results suggest that the transconjugants acquired three copies of IS*1216V*-like elements during mating (there are no *Eco*RV sites in IS*1216V*). Restriction mapping of genomic DNA from OJG-72 located two of the hybridizing fragments at least 7 kb

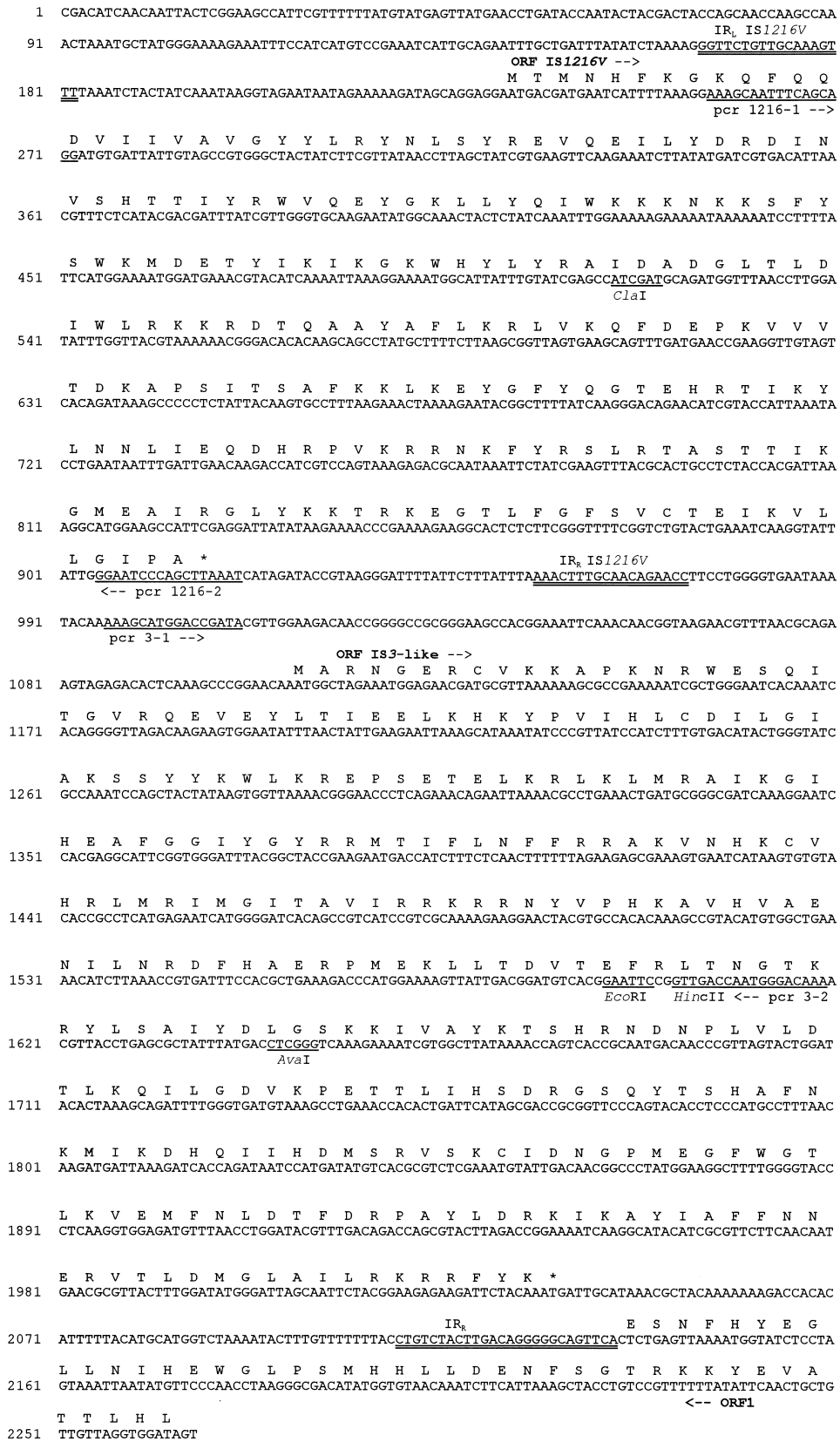

FIG. 3. Partial DNA sequence of the 7-kb *Eco*RV fragment from *E. faecalis* JG-7. Double underlines indicate the right and left IRs (IR_R and IR_L, respectively) of IS*1216V* and the putative IR_R of the IS*3*-like element. Single underlines indicate restriction sites and locations of PCR primers used for synthesis of the IS*1216V* (pcr 1216-1 and pcr 1216-2) and IS*3*-like (pcr 3-1 and pcr 3-2) probes. Translation is shown above the nucleotide sequence. Stop codons are indicated by asterisks.

3′ to *vanZ*. The third fragment, as expected, corresponded to the left end of Tn*5482*.

To more precisely determine the extent of Tn*5482*, a 4.5-kb *Eco*RV fragment lying downstream of *vanZ* was identified from a LambdaZapII library of OJG-72 genomic DNA by hybridization with the IS*1216* probe. A 0.9-kb probe was prepared from the excised plasmid (Fig. 2A). Mapping with this probe showed that the restriction maps (e.g., *Pvu*II and *Bam*HI sites) of GUC, JG-7, and OJG-72 downstream from the rightmost *Xho*I site differed among the three strains, suggesting that Tn*5482* is approximately 26 to 30 kb in size.

DISCUSSION

Although outbreaks of disease caused by vancomycin-resistant enterococci due to clonal spread have been described (6, 20, 27), recent epidemiologic data indicate that dissemination of VanA phenotype resistance commonly occurs via horizontal transfer of the *van* gene cluster (4, 8, 30). The identification of Tn*1546* and related elements on conjugative plasmids (3, 14, 19, 21), or plasmids that are conjugatively mobilized (22a), coupled with the isolation of vancomycin resistance plasmids from enterococcal strains with a variety of genetic backgrounds (6, 8, 30, 46), suggests that conjugal plasmid transfer is one mechanism by which horizontal transmission of resistance occurs.

However, several studies have identified clinical isolates in which the *vanA* gene cluster apparently resides in the chromosome rather than on a plasmid. Green and coworkers characterized three isolates of *E. faecium* in which genomic, but not plasmid, DNA preparations hybridized with a *vanA* gene probe (17). We have identified *E. faecium* isolates in which the *van* genes are present on a modified Tn*1546*-like element, Tn*1546*::IS*1251*, which also appears to reside in the chromosome, as determined by differential hybridization of plasmid and genomic DNA (21). These observations suggest that dissemination of vancomycin resistance may also be occurring via transfer of elements that are integrated into the chromosome.

In this study, DNA hybridization of PFGE-separated genomic DNA from the clinical isolate *E. faecium* GUC and its vancomycin-resistant transconjugants located the *vanA* gene cluster on large *Sma*I fragments that differed in size between the strains, providing more conclusive evidence for a chromosomal location. Furthermore, the *van* gene cluster was transferred from the chromosome of the donor strain to different chromosomal locations in the recipients, including a recombination-deficient recipient. Together, our observations suggest that in *E. faecium* GUC, the *van* gene cluster resides on a transposon with a size of at least 26 kb. This element has been tentatively designated Tn*5482*.

Sequence analysis revealed that Tn*5482* contains several IS-like elements. One of these, IS*1216V*, begins at or near the left end of Tn*5482*, i.e., within the 0.5-kb region that was predicted by restriction analysis to contain the left end. DNA hybridizations with the IS*1216V* probe indicated that two additional copies of IS*1216V* or a closely related element were transferred during filter matings. Although their precise location was not determined, these elements lay downstream of *vanZ*, toward the right end of Tn*5482*. IS*1216V* appears to be closely related to ISS*1*, an element that promotes chromosomal integration in *L. lactis* (16). Rice has recently identified an ISS*1*-like element in the chromosome of *E. faecalis* (36). It is thus tempting to speculate that IS*1216V* promotes chromosomal integration of Tn*5482*.

Tn*5482* also contains an IS*3*-like sequence, which lies just downstream from IS*1216V*. Members of the IS*3* family include the gram-negative elements IS*150* (40) and IS*600* (5), the *S. agalactiae* element IS*861* (38), and the *Lactobacillus* element IS*1223* (43). These elements are 1.2 to 1.5 kb in size and are characterized by overlapping ORFs: a short upstream ORF and a longer, more highly conserved downstream ORF (5, 43). Several observations suggest Tn*5482* contains a truncated version of an IS*3* family element. The ORF identified is homologous to the longer downstream ORFs of the IS*3* family, but no sequence corresponding to an upstream ORF was identified. Despite the absence of an upstream ORF, a 7-bp sequence associated with frameshifting between the two ORFs of IS*3* family elements (5) is present. In addition, a 26-bp sequence that shows similarities to the right IRs of the IS*3* family was present downstream of the ORF identified, but no complementary sequence corresponding to a left IR was present.

Transposons and IS elements may act as targets for insertion of other mobile elements (28, 40). The organization of the left end region of Tn*5482* is consistent with its evolution by sequential insertions of IS elements. The ORF1 region of Tn*1546*::IS*1251* lacks the left IR and a portion of ORF1 that is present in Tn*1546*. The right IR of the IS*3*-like element lies at the junction with Tn*1546*::IS*1251* DNA, suggesting that this interruption of the ORF1 region may have resulted from insertion of the IS*3*-like element. The apparent truncation of the upstream region of the IS*3*-like element is consistent with the subsequent insertion of the intact IS*1216V* element. Since sequences corresponding to the putative missing sequences of Tn*1546* or the IS*3*-like element were not identified upstream of IS*1216V* in *E. faecalis* JG-7, the remnants of these elements are unlikely to lie within Tn*5482*.

The mechanism by which Tn*5482* is transferred intercellularly is unknown. Although the presence of IS elements on Tn*5482*, including one at or near the predicted left end, is most consistent with the structure of a composite transposon, such elements do not direct their own conjugal transfer (31). The transfer of vancomycin resistance by filter mating in the absence of detectable plasmid DNA, e.g., from *E. faecalis* OJG-72C1 to JH2-2, suggests that Tn*5482* may be capable of independent conjugal transfer. Tn*5482* therefore might represent a conjugative transposon with atypical structural features. Although IS elements have not been described in the conjugative transposons of enterococci, they have been identified in those of *L. lactis*. For example, Tn*5276* contains IS*904* near one end (34). Tn*5482* also differs from previously characterized enterococcal conjugative transposons in that these uniformly encode tetracycline resistance (10, 31, 37), and *E. faecium* GUC is susceptible to tetracycline. Recent evidence suggests that the *vanB* gene cluster may reside on large conjugative elements which may be transferred from *E. faecium* to *E. faecalis* and that integrate into the chromosome, probably by illegitimate recombination (33). Tn*5482* may represent a similar element. Alternatively, Tn*5482* may be a nonconjugative transposon that is mobilized by an as-yet-undetected conjugal element.

PFGE analysis suggests that the transfer of Tn*5482* may be associated with events more complex than simple transposon insertion. Although only one fragment in each transconjugant hybridized with the *vanHAXY* probe, comparison of the *Sma*I restriction digestion patterns of the transconjugants showed changes in the electrophoretic mobility of at least two fragments present in the recipient strain. These results cannot be accounted for by the presence of *Sma*I sites in Tn*5482*, since only one fragment in the recipient would be expected to change in size. Possibilities include the transfer of an element in addition to Tn*5482* during mating or recombination within the chromosome. Extensive recombination between the genomes of the mating cells has been observed during transfer of

the conjugative transposon Tn*925*. The interaction of the donor and recipient cells has thus been likened to protoplast fusion (42).

In summary, evidence has been presented that in *E. faecium* GUC, the *van* genes reside on a mobile element, Tn*5482*, which contains the modified *vanA* gene cluster Tn*1546*::IS*1251*, a truncated IS*3*-like element, and the ISS*1*-like element IS*1216V*. Tn*5482* is transferred from the chromosome of one strain to that of another and may be able to direct its own conjugal transfer. Although the mechanism by which intercellular transfer of Tn*5482* occurs is unclear, the identification of similar elements among other *E. faecium* isolates suggests that transfer of Tn*5482* and related elements may be involved in the dissemination of vancomycin resistance.

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