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NOTES

Identification and Characterization of IS*1476*, an Insertion Sequence-Like Element That Disrupts VanY Function in a Vancomycin-Resistant *Enterococcus faecium* Strain

MARTIN G. MACKINNON,[1] MICHAEL A. DREBOT,[1,2]† AND GREGORY J. TYRRELL[1,3,4]*

Department of Microbiology and Immunology, Dalhousie University,[1] National Centre for Enteroviruses,[2] Division of Microbiology, Department of Pathology and Laboratory Medicine,[3] and Department of Medicine,[4] QEII Health Sciences Centre, Halifax, Nova Scotia B3H 2Y9, Canada

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The *vanY* gene of vancomycin-resistant enterococci encodes a D,D-carboxypeptidase. By using a PCR detection strategy, a VanA *Enterococcus faecium* clinical isolate was found to have an insertion sequence (IS)-like element designated IS*1476* in *vanY*. The activity of the VanY D,D-carboxypeptidase in this isolate was decreased in a fluorometric fluoraldehyde *o*-phthalaldehyde assay with diacetyl-L-Lys-D-Ala-D-Ala as the substrate. This, to our knowledge, is the first report of an IS-like element in a vancomycin resistance gene.

Enterococci have developed resistance to almost all antimicrobial agents used to treat infections caused by these organisms, with the most recent being the glycopeptide vancomycin (12). In VanA enterococci, resistance is conferred by a gene cluster carried on transposon Tn*1546* or closely related elements or as large conjugative elements (3, 10). This gene cluster contains *vanR*, *vanS*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ* (3). In addition to the aforementioned genes, two insertion sequence (IS)-like elements have been found in the VanA gene cluster of some isolates of enterococci resistant to vancomycin. These are IS*1251*, which has been found in the intergenic region of *vanSH*, and IS*1216V*-like, which has been found in the intergenic region of *vanXY* (5, 9). We report here an IS-like element located within the coding region of a vancomycin resistance gene. This IS-like element is located in *vanY* of a clinical isolate of *Enterococcus faecium* resistant to vancomycin.

A collection of 124 VanA *E. faecium* isolates collected in 1991 from hospitals in New York City were screened for differences in the *van* gene cluster. This was done by comparing the *van* gene cluster of these VanA isolates to the previously reported *van* gene cluster of Tn*1546* (3). All clinical VanA enterococcal isolates have been described previously (14, 15). These organisms were screened by PCR with oligonucleotides

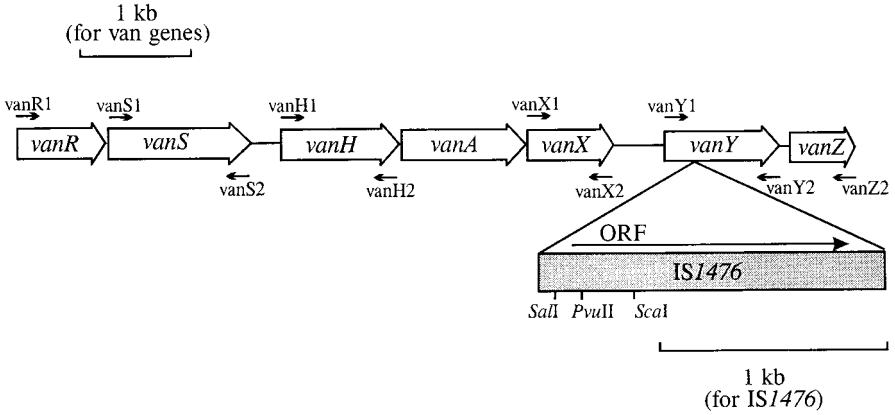

FIG. 1. Schematic representation of the VanA gene cluster showing the insertion site of IS*1476* in the *vanY* gene. The locations of primers used for PCR of *van* genes are indicated as small arrows. The large arrow indicates the direction of transcription of the putative transposase.

* Corresponding author. Present mailing address: Department of Microbiology and Public Health, 2B3.12 Walter Mackenzie Centre, University of Alberta Hospital, 8440-112 Street, Edmonton, Alberta T6G 2B7, Canada. Phone: (403) 492-8949. Fax: (403) 492-3864. E-mail: gjt@bugs.uah.ualberta.ca.

† Present address: Zoonotic Diseases, NLSP, B.O.M., L.C.D.C., Tunneys Pasture, PL.:1001B, Ottawa, Ontario K1A 0L2, Canada.

TABLE 1. Oligonucleotides used in this study

Primer pair	Amplicon	Nucleotide sequence[a]	Size of amplicon (bp)	Reference
vanR1 vanS2	vanRS	5′-ATGAGCGATAAAATACTT 5′-TTAGGACCTCCTTTTATC	1,827	3
vanS1 vanH2	vanSH	5′-TTGGTTATAAAATTGAAAATT 5′-CTATTCATGCTCCTGTCT	2,337	3
vanH1 vanX2	vanHAX	5′-ATGAATAACATCGGCATTAC 5′-TTATTTAACGGGGAAATC	2,606	3
vanX1 vanY2	vanXY	5′-ATGGAAATAGGATTTACTTT 5′-TTACCTCCTTGAATTAGTAT	1,947	3
vanY1 vanZ2	vanYZ	5′-ATGAAGAAGTTGTTTTTTTTA 5′-CTTACACGTAATTTATTC	1,550	3
IS*1476* *Eco*RI *Hin*dIII	IS*1476*	 5′-CGTgaattcATCTAAACATGA 5′-CTATCaagcttCCCGTATCC	 1,563	 This study
LPCR1 LPCR2	vanR-Z	5′-TATAAAATATTAACAgcatgcTAGCGATGCCG 5′-CCGGTTTTCCCCTCACTTggtaccTACGGG	6,907	This study

[a] The lowercase letters in the oligonucleotide sequences represent engineered restriction endonuclease cleavage sites.

(Gibco BRL, Burlington, Ontario, Canada) specific for regions flanking the *vanRS*, *vanHAX*, and *vanYZ* genes (Fig. 1 and Table 1). *E. faecium* 228 has the plasmid pHKK100 containing the VanA gene cluster, as reported previously, and was used as the positive control (8). PCR screening resulted in the generation of amplicons referred to as vanRS, vanSH, vanHAX, vanXY, and vanYZ from the positive control strain. All clinical isolates gave rise to vanHAX amplicons, indicating that they are VanA isolates. Fifty-five VanA *E. faecium* isolates possessed IS-like element IS*1251*, located between *vanS* and *vanH*. We did not find the IS*1216V*-like element in the *van* gene cluster of organisms examined. One isolate, *E. faecium* W14-91, gave rise to a vanYZ amplicon larger than the vanYZ amplicon generated from *E. faecium* 228. Amplification of *vanY* from *E. faecium* W14-91 identified an insertion in this gene of 1.5 kb. The insertion location within *vanY* was determined by restriction endonuclease mapping. Oligonucleotides specific for regions flanking the 5′ and 3′ ends of the 1.5-kb DNA insertion in *vanY* were then designed (Table 1), and amplification of the insertion was carried out by PCR. Three representative PCR products were cloned into pTZ18R and sequenced in both directions (13). The 1.5-kb DNA element was found to be inserted 290 bp downstream of the start site of the 911-bp *vanY* gene (Fig. 2) and to possess the characteristics of an IS-like element now designated IS*1476*.

IS*1476* is characterized by (i) its size of 1,500 bp, (ii) a 1,272-bp open reading frame (ORF) encoding a putative 424-amino-acid protein, (iii) 25-bp terminal imperfect inverted repeated sequences, (iv) 8-bp duplication of target DNA, and (v) significant homology with other IS-like elements from gram-positive bacteria. This homology was shown by a search of the GenBank database for similar amino acid sequences with the BLAST program (1). This search yielded the highest scores of identity with the ORFs in IS*1181* from *Staphylococcus aureus* (36%), IS*1251* from *E. faecium* (35%), ISL*3* from *Lactobacillus delbruckei* (32%), and IS*1165* from *Leuconostoc mesenteroides* (28%) (6, 7, 9, 11).

The *vanY* gene encodes a D,D-carboxypeptidase which cleaves D-Ala from the pentapeptide UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala, thus preventing this peptidoglycan precursor from being incorporated into the enterococcal cell wall (2, 4, 16). To determine if insertion of IS*1476* into *vanY* resulted in the loss of VanY D,D-carboxypeptidase activity, measurements of this enzyme activity were done in *Escherichia coli* membranes as described by Wright et al. (16). Plasmids pMGM2 and pMGM3 were constructed by PCR with oligonucleotides LPCR1 and LPCR2 and the Expand High Fidelity PCR system (Boehringer Mannheim, Laval, Quebec, Canada) (Tables 1 and 2). These plasmids were transformed into *E. coli* XL2-Blue MRF′ (Stratagene, La Jolla, Calif.) (Table 2). Transformed *E. coli* cells were grown in Luria-Bertani broth to a density of an optical density of 0.55 at 600 nm after which isopropyl-β-D-thiogalactopyranoside (Boehringer Mannheim) was added to a final concentration of 1 mM. Cultures were incubated at 37°C for 4 h. The cells were harvested by centrifugation at 3,000 × *g* for 10 min and washed with 0.8% NaCl, extracted, and assayed for D,D-carboxypeptidase activity. The extraction procedure is similar to that of Wright et al. (16). Briefly, the

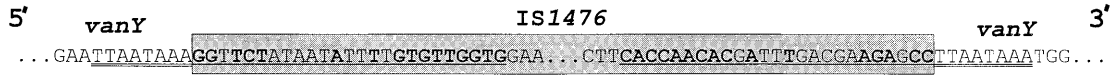

FIG. 2. Schematic representation of the IS-like element IS*1476* located in the *vanY* gene of *E. faecium* W14-91. IS*1476* is shaded. Imperfect inverted repeats of 25 bp each are underlined. Repeated nucleotides are indicated in boldface type. The 8-bp direct repeats generated by target duplication are double underlined. The first nucleotide of the direct repeats corresponds to nucleotide 282 of *vanY*. The complete nucleotide sequence of IS*1476* can be found in GenBank under accession no. U63997.

TABLE 2. Bacterial strains and plasmids

Strain or plasmid	Relevant properties	Reference or source
Strains		
E. coli XL2-Blue MRF′	Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac*[F′*proAB lacI*^{q} *Z*Δ*M15* Tn*10* (Tet^r Amy Cam^r)]	Stratagene (La Jolla, Calif.)
E. faecium 228	Vm^r plasmid pHKK100	8
E. faecium W14-91	Vm^r clinical isolate	14
Plasmids (pUC derivatives)		
pTZ18-R	Cloning vector, Amp^r	Pharmacia Biotech (Baie D'Urfe, Quebec, Canada
pMGM1	*Eco*RI-*Hin*dIII 1.5-kb IS*1476* amplicon cloned into pTZ18-R	This study
pMGM2	*Sph*I-*Kpn*I *vanR vanS vanH vanA vanX vanY vanZ* amplicon from pHKK100 cloned into pTZ18-R	This study
pMGM3	*Sph*I-*Kpn*I *vanR vanS vanH vanA vanX vanY*::IS*1476 vanZ* amplicon from W14-91 cloned into pTZ18-R	This study

pellets were resuspended in 5 ml of 50 mM HEPES–5 mM $MgCl_2$–0.5 mM EDTA–1 mM dithiothreitol (pH 7.5) and sonicated for 3 min with a Vibra cell sonicator (Sonics and Materials, Danbury, Conn.). This suspension was centrifuged at 1,000 × *g* for 10 min. The supernatant was then centrifuged at 110,000 × *g* for 10 min, after which the pellet (cell membrane fraction) was suspended in 2 ml of 50 mM HEPES–5 mM $MgCl_2$–5mM EDTA–1 mM dithiothreitol–1% (wt/vol) 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS; pH 7.5) and the contents were mixed at 4°C for 60 min. The suspension was then centrifuged at 110,000 × *g* for 30 min. The supernatant was collected and used as the source of enzyme activity for the D,D-carboxypeptidase assay with fluoraldehyde *o*-phthalaldehyde (Pierce, Rockford, Ill.) (16). *E. coli* containing pMGM2 (*vanY*) was approximately twofold more active against the substrate diacetyl-L-Lys-D-Ala-D-Ala than pMGM3 (*vanY*::IS*1476*). Specifically, *E. coli* XL2-Blue MRF′(pMGM2) (*vanRSHAXYZ*) and XL2-Blue MRF′(pMGM3) (*vanRSHAXY*::IS*1476*, *Z*) had mean ± standard deviation D,D-carboxypeptidase activities of 26 ± 9 and 10 ± 3 μmol of D-Ala per mg of protein, respectively. These data suggest that the insertion of IS*1476* into the *vanY* gene has led to decreased levels of functional *vanY* gene product.

In summary, we have found a vancomycin-resistant strain of *E. faecium* which has a new IS-like element designated IS*1476* in the coding region of the *vanY* gene. The presence of IS*1476* in *vanY* resulted in reduced levels of VanY D,D-carboxypeptidase activity produced by *E. coli* containing this gene versus the levels produced by an *E. coli* strain containing an intact copy of *vanY*. These results corroborate those of other investigators suggesting that *vanY* is not necessary for vancomycin resistance in vitro (2, 4).

Nucleotide sequence accession number. The nucleotide sequence of IS*1476* is deposited in GenBank under accession no. U63997.

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REFERENCES

1. **Altshul, S. F., F. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Arthur, M., C. Molinas, and P. Courvalin.** 1992. Sequence of the *vanY* gene required for production of a vancomycin-inducible D,D-carboxypeptidase in *Enterococcus faecium* BM4147. Gene **120:**111–114.
3. **Arthur, M., C. Molinas, F. Depardieu, and P. Courvalin.** 1993. Characterization of Tn*1546*, a Tn*3*-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J. Bacteriol. **175:**117–127.
4. **Arthur, M., F. Depardieu, P. Reynolds, and P. Courvalin.** 1996. Quantitative analysis of the metabolism of soluble cytoplasmic peptidoglycan precursors of glycopeptide-resistant enterococci. Mol. Microbiol. **21:**33–44.
5. **Arthur, M., F. Depardieu, G. Gerbaud, M. Galimand, R. Leclercq, and P. Courvalin.** 1997. The VanS sensor negatively controls VanR-mediated transcriptional activation of glycopeptide resistance genes of Tn*1546* and related elements in the absence of induction. J. Bacteriol. **179:**97–106.
6. **Derbise, A., K. G. H. Dyke, and N. El Solh.** 1994. Isolation and characterization of IS*1181*, an insertion sequence from *Staphylococcus aureus*. Plasmid **31:**251–264.
7. **Germond, J. E., L. Lapierre, M. Delley, and B. Mollet.** 1995. A new mobile genetic element in *Lactobacillus delbruecki* subsp. *bulgaricus*. Mol. Gen. Genet. **248:**407–416.
8. **Handwerger, S., M. J. Pucci, and A. Kolokathis.** 1990. Vancomycin resistance is encoded on a pheromone response plasmid in *Enterococcus faecium* 228. Antimicrob. Agents Chemother. **34:**358–360.
9. **Handwerger, S., J. Skoble, L. F. Discotto, and M. J. Pucci.** 1995. Heterogeneity of the *vanA* gene cluster in clinical isolates of enterococci from the northeastern United States. Antimicrob. Agents Chemother. **39:**362–368.
10. **Handwerger, S., and J. Skoble.** 1995. Identification of a chromosomal mobile element conferring high-level vancomycin resistance in *Enterococcus faecium*. Antimicrob. Agents and Chemother. **39:**2446–2453.
11. **Johansen, E., and A. Kibenich.** 1992. Isolation and characterization of IS*1165*, an insertion sequence of *Leuconostoc mesenteroides* subsp. *cremoris* and other lactic acid bacteria. Plasmid **27:**200–206.
12. **Leclercq, R., E. Derlot, J. Duval, and P. Courvalin.** 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N. Engl. J. Med. **319:**157–161.
13. **Sanger, F. S., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
14. **Tyrrell, G. J., R. N. Bethune, B. Willey, and D. E. Low.** 1997. Species identification of enterococci via intergenic ribosomal PCR. J. Clin. Microbiol. **35:**1054–1060.
15. **Willey, B. M., B. N. Kreiswirth, A. E. Simor, Y. Faur, M. Patel, G. Williams, and D. E. Low.** 1993. Identification and characterization of multiple species of vancomycin-resistant enterococci, including an evaluation of Vitek software version 7.1. J. Clin. Microbiol. **31:**2777–2779.
16. **Wright, G. D., C. Molinas, M. Arthur, P. Courvalin, and C. T. Walsh.** 1992. Characterization of VanY, a DD-carboxypeptidase from vancomycin-resistant *Enterococcus faecium* BM4147. Antimicrob. Agents Chemother. **36:** 1514–1518.