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Two Structurally Distinct VanA Resistance Elements at Different Locations in a Glycopeptide-Resistant Strain of *Enterococcus faecalis*

Glycopeptide-resistant enterococci (GRE) can carry more than one VanA element (3, 5), but we are unaware of previous evidence for separate transfer of such elements. *Enterococcus faecalis* JS3B was examined as one of 46 GRE isolated from fecal screens of a hematology patient at Addenbrooke's Hospital, Cambridge, United Kingdom (7). Overlapping PCR (1) showed that this strain contained a group H element (11). VanA resistance appeared to be associated with a 35-MDa plasmid, but Palepou et al. (6) suggested that group H elements reside on the chromosome. Strain JS3B was therefore investigated for carriage of multiple VanA elements.

Transfer of vancomycin resistance was performed by cross-streak conjugation (10). The recipient strains were *E. faecalis* JH2-2 and *E. faecium* GE-1, which are resistant to fusidic acid and rifampin and lack pheromone response genes *prgA* and *prgB* (see below). After incubation at 37°C for 72 h, the transconjugants were selected on brain heart infusion agar (Oxoid) containing 100 μg of rifampin per ml, 25 μg of fusidic acid per ml, and 10 μg of vancomycin per ml. Plates were incubated at 37°C and examined daily for 5 days. Eight to 12 individual colonies were subcultured onto Columbia horse blood agar. The colonial characteristics of selected transconjugants were noted, and plasmid profiles were examined following alkaline lysis and agarose gel electrophoresis (8).

Five transconjugants, representing each combination of plasmid profile, hemolysis reaction, and colonial morphology, were subjected to overlapping PCR (1, 11). Plasmids were resubjected to alkaline lysis, followed by Southern blotting onto a nylon membrane, and hybridized with a digoxigenin-labeled *vanA*-specific probe (2). Strain JS3B and the five transconjugants were also examined for the conserved pheromone response genes *prgA* (which encodes entry exclusion protein) and *prgB* (which encodes aggregation substance protein) with previously described PCR primers (4) and the same cycling conditions as for overlapping PCR.

Three transconjugants represented the *E. faecalis* JH2-2 host and contained group U elements. Two transconjugants represented *E. faecium* GE-1; one contained a group U element, whereas the other had a group H element (Table 1). Each of the four transconjugants with group U elements possessed a plasmid of ca. 35 MDa that hybridized with the *vanA* probe. These four transconjugants and JS3B all possessed *prgA* and *prgB*. The transconjugant with the group H element did not possess detectable plasmids, and the *vanA* probe hybridized only with residual chromosomal DNA. However, it is possible that the group H element may be carried on a large conjugative plasmid not recovered by the alkaline lysis technique employed here (9). The transconjugant with the group H element lacked pheromone response genes *prgA* or *prgB*, suggesting that transfer of its VanA resistance element did not depend on a pheromone-responsive conjugative plasmid.

In conclusion, strain JS3B contained two distinct VanA elements, a group U element carried on a pheromone-responsive plasmid of ca. 35 MDa and a group H element that appeared to be chromosomal. In overlapping PCR, the group H element, which yielded multiple amplicons, masked the group U element, which yielded fewer amplicons (11). These results stress the importance of using multiple molecular techniques to identify the structure and location of VanA elements in GRE.

TABLE 1. Characteristics of transconjugants derived from *E. faecalis* JS3B

Transconjugant	Recipient strain	Hemolysis reaction on Columbia horse blood agar	VanA element	Band or plasmid that hybridized with *vanA* probe	Result of PCR specific for:	
					prgA	*prgB*
1	*E. faecalis* JH2-2	β	U	35-MDa plasmid	+	+
2	*E. faecalis* JH2-2	No	U	35-MDa plasmid	+	+
3	*E. faecalis* JH2-2	β	U	35-MDa plasmid	+	+
4	*E. faecium* GE-1	No	H	Chromosomal band only	−	−
5	*E. faecium* GE-1	β	U	35-MDa plasmid	+	+

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