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Restriction Mapping and Hybridization Studies of a β-Lactamase-Encoding Fragment from *Streptococcus (Enterococcus) faecalis*

KAREN K. ZSCHECK,[1] RICHARD HULL,[2] AND BARBARA E. MURRAY[1,3]*

Program in Infectious Diseases and Clinical Microbiology[1] and Department of Medicine,[3] University of Texas Medical School, and Department of Microbiology, Baylor College of Medicine,[2] Houston, Texas 77030

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A restriction map of a 5.1-kilobase *Eco*RI fragment encoding an enterococcal β-lactamase was prepared and compared with the restriction map of a cloned staphylococcal β-lactamase gene and with the published maps of several other staphylococcal β-lactamase genes. Comparison and hybridization studies showed that there were identical restriction sites in the region of the *bla* structural gene but not in the region surrounding this gene.

Enzyme inactivation as a mechanism of penicillin resistance in enterococci was first reported in 1983, when Murray et al. described a clinical isolate of *Streptococcus (Enterococcus) faecalis* which produced a β-lactamase (6, 9). Hybridization studies with this strain showed that a staphylococcal β-lactamase gene probe hybridized to enterococcal plasmid DNA, suggesting that the enterococcal enzyme was of staphylococcal origin (10). The present study was undertaken to generate a restriction endonuclease map of the 5.1-kilobase β-lactamase-encoding enterococcal fragment and to localize and compare the homologous sequences of the enterococcal and staphylococcal penicillinase genes by DNA hybridization.

S. faecalis HH22 is a gentamicin- and penicillin-resistant, β-lactamase-producing enterococcal strain that has been previously described (10). pBEM1 is a Bla^+ recombinant plasmid constructed by cloning a 5.1-kilobase *Eco*RI fragment from HH22 into pACYC184 (8). pJM13 is a recombinant plasmid consisting of a 7.0-kilobase *Eco*RI fragment containing the β-lactamase gene of a constitutive mutant of the naturally occurring plasmid pI258 of *Staphylococcus aureus* cloned into pMB9 (4).

Plasmid DNA was isolated as previously described (3). Restriction endonuclease digestions were carried out according to the instructions of the manufacturer (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The molecular size standards used were lambda DNA digested with *Hin*dIII and *Eco*RI (Boehringer Mannheim Biochemicals), the 1-kilobase ladder, and ϕX174 replicative form digested with *Hae*III (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The digested DNA was electrophoresed in 0.7% agarose gels for 10 h at 40 V. In addition, some DNA bands were excised from the agarose, electroeluted, digested by a second enzyme, and then electrophoresed in a 12% polyacrylamide gel at 200 V for 5 h.

The DNA probe used in the hybridization studies was the 840-base-pair (bp) *Hin*dIII-*Xba*I fragment of pJM13 previously described (10). This fragment consists of 75 bp upstream of the promoter, the promoter, the leader sequence, and 80% of the structural β-lactamase gene; the *Hin*dIII site is upstream of the promoter, and the *Xba*I site is within the structural gene (5). The probe was prepared and used in hybridizations as described previously (10).

The restriction map of pACYC184 has been previously published (1); by using this information and performing various additional restriction digestions, a restriction map of the enterococcal insert of pBEM1 was generated (Fig. 1). Enzymes which were tested but had no sites within the insert include *Bgl*II, *Hpa*I, *Pvu*II, and *Sal*I. Hybridizations with the 840-bp probe of pJM13 were performed in order to identify the homologous sequences within the map of pBEM1. The enterococcal β-lactamase gene was localized within the region of the *Hin*dIII-*Xba*I sites shown in Fig. 1.

The restriction map of the staphylococcal β-lactamase-encoding fragment of pJM13 is shown in Fig. 1 with published restriction enzyme sites and the *Eco*RV sites that we determined. A comparison of the pBEM1 enterococcal insert map with the pJM13 staphylococcal insert map reveals that the restriction sites between the *Eco*RV sites are very similar. To further compare the staphylococcal and enterococcal Bla^+ inserts, digested DNAs from both pJM13 and pBEM1 were hybridized with the staphylococcal *Hin*dIII-*Xba*I *bla* probe. With the *Eco*RI-*Eco*RV-*Xba*I triple digestion of pBEM1 and pJM13 (Fig. 2, lanes e and l, respectively), the probe hybridized with two bands of the same size from each of the plasmids (Fig. 2, lanes e′ and l′). Digestion of both plasmids with *Eco*RI, *Cla*I, *Hin*dIII, and *Eco*RV also resulted in the production of several bands of the same size (pBEM1 is shown in lane d and pJM13 is shown in lane k in Fig. 2), and these bands showed homology with the probe (Fig. 2, lanes d′ and k′). With the *Eco*RI-*Hin*dIII-*Xba*I triple digestion, a single fragment from both plasmids (pBEM1 in lane a′ and pJM13 in lane h′ in Fig. 2) hybridized with the probe. Digestion of both plasmids with *Taq*I, *Hin*fI, and *Mnl*I generated many fragments of different sizes on the gel, but the autoradiograph again showed that fragments of the same size in both pBEM1 and pJM13 hybridized with the probe (data not shown).

In this study, we have shown that the homologous regions in the enterococcal insert are contained in a *Hin*dIII-*Xba*I fragment equal in size to the probe for the staphylococcal structural β-lactamase gene (Fig. 2, lanes a and h). The two inserts also have a number of identical restrictions sites in and around the region of the *bla* structural gene but are more

* Corresponding author.

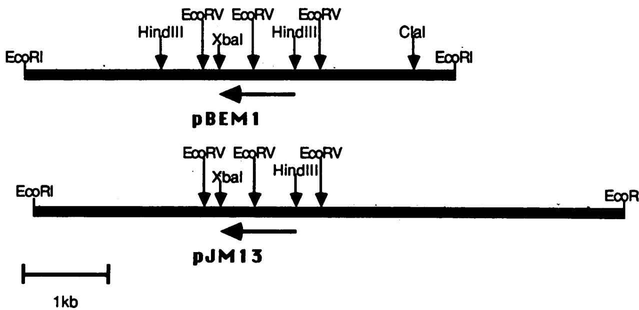

FIG. 1. Linear restriction maps of the enterococcal insert of pBEM1 and the staphylococcal insert of pJM13. Horizontal arrows indicate regions encoding the structural β-lactamase genes. kb, Kilobases.

dissimilar in the surrounding sequences. For example, the *Eco*RI sites are different, and the enterococcal insert contains a *Cla*I site and a *Hin*dIII site that are not present in the staphylococcal *Eco*RI insert (Fig. 1). A comparison of the restriction map of the enterococcal penicillinase gene with the published maps of the staphylococcal penicillinase plasmids pI524, pSK4 and pUW3626 (2, 7) also showed differences in several restriction sites, including *Bgl*II, *Eco*RI, *Hin*dIII, *Hpa*I, *Pvu*II, *Sal*I, and *Xba*I; these sites, some of which are contained in an invertible region, are located directly upstream of the staphylococcal β-lactamase structural gene. Weber et al. have recently shown that the staphylococcal penicillinase gene found on a large conjugative plasmid is located on a transposon (D. A. Weber, R. V. Goering, and D. E. Rohwer, submitted for publication). Although the β-lactamase-encoding *Eco*RI fragment from that staphylococcal plasmid also has a *Hin*dIII-*Xba*I fragment approximately 900 bp in size, there are several restriction sites that differ from those of the enterococcal Bla fragment, including *Eco*RI, *Hpa*I, *Xba*I, *Pvu*II, *Sal*I, and *Hin*dIII. This suggests that even if the enterococcal β-lactamase is on a transposon, the transposon is different or arranged differently from that in staphylococci.

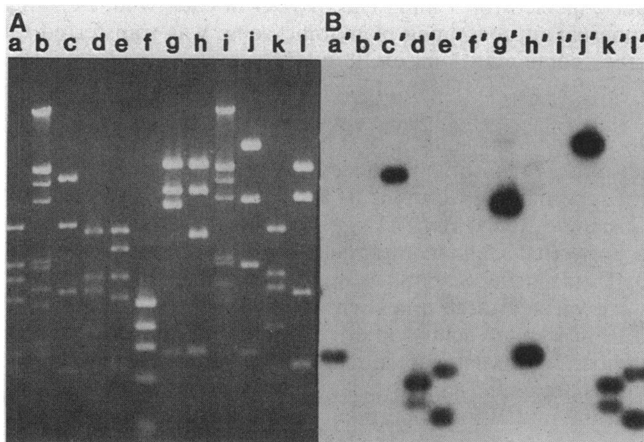

FIG. 2. Agarose gel (A) and corresponding autoradiograph (B) after hybridization to the staphylococcal β-lactamase gene probe from pJM13. Lanes b and i, Lambda DNA digested with *Hin*dIII and *Eco*RI; lane f, φX174 digested with *Hae*III. Other lanes: pBEM1 digested with *Eco*RI, *Hin*dIII, and *Xba*I (a and a'); *Eco*RI and *Cla*I (c and c'); *Eco*RI, *Cla*I, *Hin*dIII, and *Eco*RV (d and d'); and *Eco*RI, *Xba*I, and *Eco*RV (e and e'); and pJM13 DNA digested with *Eco*RI and *Hin*dIII (g and g'); *Eco*RI, *Hin*dIII, and *Xba*I (h and h'); *Eco*RI and *Cla*I (j and j'); *Eco*RI, *Cla*I, *Hin*dIII, and *Eco*RV (k and k'); and *Eco*RI, *Xba*I, and *Eco*RV (l and l'). The bands in lanes d' and k' are 645 and 500 bp, and those in lanes e' and l' are 760 and 420 bp.

In conclusion, we have presented further evidence that the enterococcal penicillinase structural gene originated from a staphylococcal strain but that there are differences outside the structural gene region that suggest that the entire region was not derived from *S. aureus* or has undergone significant rearrangement.

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