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Mobilization of the Penicillinase Gene in *Enterococcus faecalis*

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***Enterococcus faecalis* SF4855 is a β-lactamase-producing isolate resistant to high levels of gentamicin, with determinants for these resistances on the chromosome. SF4855 transferred both determinants into *E. faecalis* FA2-2 and UV202 at a frequency of 10^{-9} in the presence of the MLS plasmid pYN120. β-Lactamase and gentamicin resistance probes hybridized to three locations on the chromosome of FA2-2 transconjugants on contour-clamped homogeneous electric field electrophoresis. The study results suggest mobilization of the β-lactamase determinant.**

Enterococcus faecalis strains producing β-lactamase are becoming more prominent and have been associated with serious infections (8, 12, 14, 17, 24). A recent study has suggested transposition of the β-lactamase gene (18, 21). We attempted to confirm that enterococcal β-lactamase genes can be mobilized and provide further evidence that they are transposon related.

E. faecalis SF4855 is a urine isolate from a patient in North Carolina. β-Lactamase production was evaluated with nitrocephin (Glaxo Laboratories, Middlesex, England). Antimicrobial susceptibilities were determined by a standard microdilution method (13). All antibiotics used were from Sigma Chemical Co. (St. Louis, Mo.) except for erythromycin (Abbott Laboratories, North Chicago, Ill.) and gentamicin (Elkins, Cherry Hill, N.J.).

Overnight filter matings were performed as previously described (5). *E. faecalis* FA2-2, JH2SS, and UV202 were used as plasmid-free recipient strains. FA2-2 has chromosomal mutations conferring resistance to rifampin and fusidic acid and is derived from *E. faecalis* JH2 (1). JH2SS has chromosomal mutations conferring resistance to streptomycin and spectinomycin and is derived from *E. faecalis* JH2 (23). UV202 has chromosomal mutations conferring resistance to rifampin and fusidic acid, is an UV radiation-sensitive derivative of *E. faecalis* JH2-2, and is recombination deficient (25). Transfer frequencies were expressed as the number of tranconjugants per recipient cell present at the time of plating on selective medium. Plasmid pYN120 is a 43-kb conjugative plasmid that transfers into FA2-2 and UV202 recipients at a frequency of 10^{-2} and mediates only erythromycin resistance. This plasmid has shown in preliminary experiments the ability to accept insertion and mobilization of a coresident, chromosome-borne gentamicin transposon (22). Plasmid DNA was isolated by previously outlined procedures (2, 3). DNA was digested with *Eco*RI and *Hin*dIII (BRL Life Technologies, Gaithersburg, Md.) and analyzed by agarose gel electrophoresis. Contour-clamped homogeneous electric field electrophoresis of genomic DNA digested with *Sma*I and *Apa*I (BRL) was performed as previously published (3, 11, 20). Chromosomal fragment sizes were approximated based on comparison with a bacteriophage lambda ladder standard (Bio-Rad Laboratories, Richmond, Calif.). A probe specific for the 6′-aminoglycoside acetyltransferase 2″-phosphotransferase bifunctional enzyme in *E. faecalis* constructed by Ferretti et al. (4) was used to determine the relatedness of the gentamicin resistance determinants. The β-lactamase probe used was derived from a previously described 840-bp *Hin*dIII-*Xba*I fragment of pJM13 containing the leader sequence and 80% of the structural staphylococcal β-lactamase gene (7, 10). Probes were labeled with biotin with a nick translation kit (BRL). DNA was transferred to nitrocellulose by Southern blot hybridization, and probes were detected with a Blugene kit (BRL).

The MIC of penicillin for the β-lactamase-producing *E. faecalis* SF4855 was 4.0 μg/ml. Other MICs were as follows: gentamicin, >2,000 μg/ml; streptomycin, 64 μg/ml; tetracycline, 50 μg/ml; and erythromycin, 1.5 μg/ml. No plasmids were detected in SF4855. DNA-DNA hybridization of gentamicin and β-lactamase probes with SF4855 showed that these two determinants localized to the same 115-kb *Sma*I and 290-kb *Apa*I restriction chromosomal fragments on CHEF electrophoresis. In mating studies with SF4855 as the donor and FA2-2 as the recipient, with selection for gentamicin resistance, no transconjugants were obtained. The low MIC of penicillin for SF4855 relative to recipient strains (MIC = 2.0 μg/ml) limited direct selection for penicillin resistance. Isolates of SF4855 containing pYN120 were then constructed and used as donors, with FA2-2 and UV202 as the recipient cells. With selection for gentamicin resistance, and both recipient markers, transconjugants producing β-lactamase were obtained at a frequency of 10^{-9} per recipient. There was no change in the transfer frequency of pYN120. Of 100 transconjugants examined, in only 1 isolate was the determinant for gentamicin resistance transferred without the determinant encoding β-lactamase. Analysis of transconjugants showed transfer of pYN120. Restriction enzyme analysis of plasmid content with *Eco*RI and *Hin*dIII indicated that the genes encoding β-lactamase production and gentamicin resistance were not inserted into pYN120 in the transconjugants. In secondary matings, FA2-2 and UV202 transconjugants containing pYN120 were used as donors. JH2SS was used as the recipient, and selecting for gentamicin, streptomycin, and spectinomycin resistance, the transfer frequency was again 10^{-9}. Transconjugants con-

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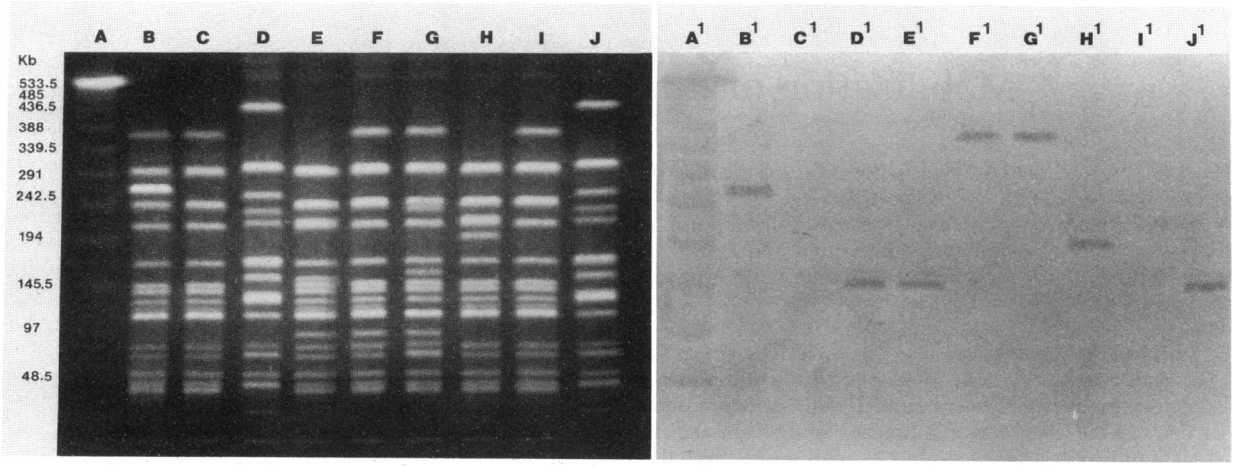

FIG. 1. Lanes A to J, CHEF electrophoresis of chromosomal DNA digested with *Sma*I. Approximate molecular sizes are shown on the left. Lanes: A, bacteriophage lambda ladder; B, UV202 transconjugant; C, UV202; D and J, 4855; E, F, G, and H, FA2-2 transconjugants; I, FA2-2. DNA-DNA hybridization with chromosomal DNA shown in lanes A^1 to J^1. Lanes: A^1, hybridization of the probe for bacteriophage lambda ladder; B^1 to J^1, DNA-DNA hybrization of the probe for β-lactamase.

tained pYN120 with gentamicin resistance and β-lactamase determinants located on the chromosome. CHEF electrophoresis of donors, plasmid-free recipients, and transconjugants resulting from matings of SF4855 containing pYN120 as donor with FA2-2 and UV202 as the recipients are shown in Fig. 1. For UV202 (lane C) matings, there was one *Sma*I-digested chromosomal DNA pattern in the transconjugants (lane B). For FA2-2 (lane I) matings, four *Sma*I-digested chromosomal DNA patterns of transconjugants (lanes E, F, G, and H) were observed. The first pattern (lane E) had loss of the first (360-kb) fragment and the addition of approximately 150- and 100-kb fragments. The second pattern (lane F) had the addition of one (100-kb) fragment. The third pattern (lane G) had the apparent addition of three fragments (approximately 230, 160, and 100 kb) and loss of a 120-kb fragment. The fourth pattern (lane H) had loss of the first (360-kb) fragment and addition of a 190-kb fragment. DNA-DNA hybridization of *Sma*I-digested transconjugant chromosomal DNA (Fig. 1, lanes B^1 to J^1) showed hybridization of the β-lactamase probe to three different locations on the chromosomes of the FA2-2 transconjugants and to one location on the chromosome of the UV202 transconjugant. Hybridization to the bacteriophage lambda ladder standard with a probe for the lambda ladder was used as a control (lane A^1). The gentamicin resistance probe hybridized to the same *Sma*I- and *Apa*I-digested chromosomal fragments on FA2-2 and UV202 transconjugants as the β-lactamase probe (data not shown).

In the studies that have comparatively evaluated the plasmids of β-lactamase producing enterococcal isolates, plasmids were conjugative, transferred at a high frequency in mating experiments, and had different restriction enzyme patterns but were structurally related in DNA-DNA hybridization experiments (6, 9, 10, 16). β-Lactamase has been shown to transfer with high-level gentamicin resistance when transferred by conjugative plasmids in all but one isolate (MIC of gentamicin = 12 μg/ml) (15).

This study confirms earlier reports that the β-lactamase determinant can reside on the chromosome (17, 18). Rice and colleagues (18) demonstrated by cross-streak mating transfer of the β-lactamase resistance determinant into one recipient, with the determinant transferring in conjunction with resistances to erythromycin, gentamicin, streptomycin, and tetracycline in varying combinations. We extend these observations by showing possible mobilization of the β-lactamase determinant and its apparent insertion into three separate locations on the chromosomes of other enterococci. Rice and Marshall (19) have also provided evidence for the incorporation of the chromosomal β-lactamase gene of *E. faecalis* into a transposon derived from staphylococci. There is an apparent close relationship between the gentamicin resistance and β-lactamase determinants. In this study, gentamicin resistance and β-lactamase probes hybridized to the same *Sma*I- and *Apa*I-digested chromosomal fragments on CHEF electrophoresis. The two determinants were also transferred together with selection with gentamicin. The large chromosomal fragment sizes limit determination of whether the two determinants are encoded by two distinct genetic elements.

CHEF electrophoresis of donors, recipients, and transconjugants resulting from matings of SF4855 as donor and FA2-2 and UV202 as recipients showed four different DNA patterns in transconjugants for FA2-2 and one DNA pattern in transconjugants for UV202. When the molecular weights of the DNA fragments in each lane were summed, there were differences not only among the four FA2-2 transconjugants, but also between the transconjugants and FA2-2. These results may be explained by DNA fragments that are similar in size and cannot be easily separated from each other by CHEF electrophoresis (doublets or triplets). For example, in one FA2-2 transconjugant, the apparent addition of approximately 230-, 160-, and 100-kb fragments and loss of a 120-kb fragment would seem to indicate the transconjugant had gained about 370 kb of DNA. The insertion of even a very large transposon would not account for this large apparent increase in amount of DNA. A similar situation may have occurred with UV202, where the transconjugant apparently gained a 250-kb fragment, too large to be explained by the addition of a transposon. Another explanation may be chromosomal duplication or rearrangement between donor and recipient cells. The transfer of a large cryptic plasmid from the donor to the transconjugants, the integration of a large plasmid into the chromosome of transconjugants, and/or the transfer of an erythromycin transposon from pYN120 into

the chromosome of the transconjugants that was not detectable by our methods would be other conceivable explanations for these data.

Further studies are needed to elaborate the relationship of the β-lactamase and gentamicin resistance determinants, since they are transferred together. Since plasmid pYN120 was needed for possible mobilization, studies of the relationship of this plasmid to transfer of gentamicin resistance and β-lactamase determinants would also be of interest in understanding the mechanism for transfer.

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REFERENCES

1. **Clewell, D. B., P. K. Tomich, M. C. Gawron-Burke, A. E. Franke, Y. Yagi, and F. Y. An.** 1982. Mapping of *Streptococcus faecalis* plasmids pAD1 and pAD2 and studies relating to transposition of Tn*917*. J. Bacteriol. **152:**1220–1230.
2. **Clewell, D. B., Y. Yagi, G. M. Dunny, and S. K. Schultz.** 1974. Characterization of three plasmid deoxyribonucleic acid molecules in a strain of *Streptococcus faecalis*: identification of a plasmid determining erythromycin resistance. J. Bacteriol. **117:** 283–289.
3. **Donabedian, S. M., J. W. Chow, J. M. Boyce, R. E. McCabe, S. M. Markowitz, P. E. Coudron, A. Kuritza, C. L. Pierson, and M. J. Zervos.** 1992. Molecular typing of ampicillin-resistant, non-β-lactamase-producing *Enterococcus faecium* from diverse geographic areas. J. Clin. Microbiol. **30:**2757–2761.
4. **Ferretti, J. J., K. S. Gilmore, and P. Courvalin.** 1986. Nucleotide sequence analysis of the gene specifying the bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. J. Bacteriol. **167:**631–638.
5. **Forbes, B. A., and D. R. Schaberg.** 1983. Transfer of resistance plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: evidence for conjugative exchange of resistance. J. Bacteriol. **153:**627–634.
6. **Markowitz, S. M., V. D. Wells, D. S. Williams, C. G. Stuart, P. E. Coudron, and E. S. Wong.** 1991. Antimicrobial susceptibility and molecular epidemiology of β-lactamase producing, aminoglycoside-resistant isolates of *Enterococcus faecalis*. Antimicrob. Agents Chemother. **35:**1075–1080.
7. **McLaughlin, J. R., C. L. Murray, and J. C. Rabinowitz.** 1981. Unique features of the ribosomal binding site sequence of the gram-positive *Staphylococcus aureus* beta-lactamase gene. J. Biol. Chem. **256:**11283–11291.
8. **Murray, B. E.** 1992. β-Lactamase-producing enterococci. Antimicrob. Agents Chemother. **36:**2355–2359.
9. **Murray, B. E., D. A. Church, A. Wanger, K. Zscheck, M. E. Levison, M. J. Ingerman, E. Abrutyn, and B. Mederski-Samoraj.** 1986. Comparison of two beta-lactamase-producing strains of *Streptococcus faecalis*. Antimicrob. Agents Chemother. **30:** 861–864.
10. **Murray, B. E., and B. Mederski-Samoraj.** 1983. Transferable β-lactamase: a new mechanism for in-vitro penicillin resistance in *Streptococcus faecalis*. J. Clin. Invest. **72:**1168–1171.
11. **Murray, B. E., K. V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock.** 1990. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. **28:**2059–2063.
12. **Murray, B. E., K. V. Singh, S. M. Markowitz, H. A. Lopardo, J. E. Patterson, M. J. Zervos, E. Rubeglio, G. M. Eliopoulos, L. B. Rice, F. W. Goldstein, S. G. Jenkins, G. M. Caputo, R. Nasnas, L. S. Moore, E. S. Wong, and G. Weinstock.** 1991. Evidence for clonal spread of a single strain of β-lactamase-producing *Enterococcus* (*Streptococcus*) *faecalis* to six hospitals in five states. J. Infect. Dis. **163:**780–785.
13. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. Approved standard. M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **Patterson, J. E., S. M. Colodny, and M. J. Zervos.** 1988. Serious infection due to β-lactamase-producing *Streptococcus faecalis* with high-level resistance to gentamicin. J. Infect. Dis. **158:** 1144–1145.
15. **Patterson, J. E., B. L. Masecar, and M. J. Zervos.** 1988. Characterization and comparison of two penicillinase-producing strains of *Streptococcus* (*Enterococcus*) *faecalis*. Antimicrob. Agents Chemother. **32:**122–124.
16. **Patterson, J. E., A. Wanger, K. K. Zscheck, M. J. Zervos, and B. E. Murray.** 1990. Molecular epidemiology of beta-lactamase-producing enterococci. Antimicrob. Agents Chemother. **34:**302–305.
17. **Rhinehart, E., N. E. Smith, C. Wennersten, E. Gorss, J. Freeman, G. M. Eliopoulos, R. C. Moellering, Jr., and D. A. Goldman.** 1990. Rapid dissemination of β-lactamase producing, aminoglycoside-resistant *Enterococcus faecalis* among patients and staff on an infant-toddler surgical ward. N. Engl. J. Med. **323:**1814–1818.
18. **Rice, L. B., G. M. Eliopoulos, C. Wennersten, D. Goldmann, G. A. Jacoby, and R. C. Moellering, Jr.** 1991. Chromosomally mediated β-lactamase production and gentamicin resistance in *Enterococcus faecalis*. Antimicrob. Agents Chemother. **35:**272–276.
19. **Rice, L. B., and S. H. Marshall.** 1992. Evidence of incorporation of the chromosomal β-lactamase gene of *Enterococcus faecalis* CH19 into a transposon derived from staphylococci. Antimicrob. Agents Chemother. **36:**1843–1846.
20. **Smith, C. L., and C. R. Cantor.** 1987. Purification, specific fragmentation, and separation of large DNA molecules. Methods Enzymol. **155:**449–467.
21. **Smith, M. C., and B. E. Murray.** 1992. Comparison of enterococcal and staphylococcal β-lactamase-encoding fragments. Antimicrob. Agents Chemother. **36:**273–276.
22. **Thal, L. A., J. Chow, J. E. Patterson, and M. J. Zervos.** 1992. Abstr. Annu. Meet. Am. Soc. Microbiol. 1992, A-28, p. 5.
23. **Tomich, P. K., F. Y. An, and D. B. Clewell.** 1980. Properties of erythromycin-inducible transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **141:**1366–1374.
24. **Wells, V. D., E. S. Wong, B. E. Murray, P. E. Coudron, D. S. Williams, and S. M. Markowitz.** 1992. Infections due to beta-lactamase producing high-level gentamicin-resistant *Enterococcus faecalis*. Ann. Intern. Med. **116:**285–292.
25. **Yagi, Y., and D. B. Clewell.** 1980. Recombination-deficient mutant of *Streptococcus faecalis*. J. Bacteriol. **143:**966–970.