Molecular Epidemiology of the fsr Locus and of Gelatinase Production among Different Subsets of Enterococcus faecalis Isolates

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We examined 215 Enterococcus faecalis isolates and found that neither the two-component regulatory locus fsr (E. faecalis regulator) nor gelatinase production was more common in disease-associated isolates than in isolates colonizing healthy individuals (ca. 60 to 65%). The majority of gelatinase-negative isolates, including 14 endocarditis isolates (of 80 isolates tested), contained the previously described 23.9-kb deletion and lacked fsrA and fsrB. While these findings indicate that neither fsr nor gelatinase is required for E. faecalis to cause infection, this study did not address whether fsr or gelatinase affects the severity of disease, as it does in animal models.

Enterococci are normally commensal flora, but they can cause a wide range of diseases, including urinary tract infections, bloodstream infections, wound infections, and endocarditis (12, 13). Although there are over 20 species of enterococci (8), Enterococcus faecalis isolates cause the majority of infections (22). Despite the increasing number of cases of enterococcal infection and the remarkable ability of these organisms to resist antibiotics, relatively little about their pathogenesis is known compared to what is known about some other gram-positive cocci.

Despite its role as a possible virulence factor, gelatinase is not produced by all clinical isolates, and a number of studies have attempted to correlate gelatinase activity with disease caused by E. faecalis (3, 5, 7, 17, 20, 24, 28). Nakayama et al. recently reported that a 23.9-kb chromosomal deletion involving the fsr locus was commonly found in urine isolates and was also found in most gelatinase-negative strains (17). In contrast, another study reported that fsr was present in 100% of 12 endocarditis isolates (20). In the present study, we compared clinical isolates of E. faecalis from patients with endocarditis, urinary tract infections, or blood-borne infections to fecal isolates from healthy volunteers with respect to the presence of fsr and gelatinase production.

A total of 215 E. faecalis isolates from clinical samples and from feces of healthy, community-based volunteers, collected between 1974 and 2003, were used in this study. The identification of all isolates as E. faecalis was initially done by biochemical tests and was confirmed by using an intragenic portion of the ace gene (18) as a probe for colony hybridization (data not shown). E. faecalis OG1RF (15), E. faecalis OG1Sp (6), E. faecalis V583 (23), and Enterococcus faecium TX0016 (2) are well-studied strains and were used in this study as controls. Brain heart infusion agar (Difco Laboratories, Detroit, Mich.) or brain heart infusion broth was used for growth. Gelatinase production was measured as previously described (21) after overnight incubation at 37°C and again after 7 days.

Genomic DNA was isolated as previously described (11, 29), and PCRs were performed with the Optimized Buffer B kit (Invitrogen, San Diego, Calif.) with primers listed in Table 1. The 23.9-kb deletion (17) was detected by using primer pair delsizeF1 and delsizeR1, located in open reading frame EF1841 in the V583 genome database (www.tigr.org) and in the 3’ end of the partially deleted fsrC gene (Fig. 1), respectively.

Colonies hybridized with E. faecalis was detected by using the RadPrime DNA labeling system (GIBCO/BRL, Gaithersburg, Md.). Probes consisting of intragenic gelE, fsrB (21), and ace (19) PCR products were generated by using OGI1RF genomic DNA templates and the primers given in Table 1, while the aac(6’)-Ib probe (4) (to rule out E. faecium) was generated by using TX16 genomic DNA (http://www.hgsc.bcm.tmc.edu/microbial/Efaecium/). Pulsed-field gel electrophoresis was performed as previously described (11, 14).

A number of investigators have studied gelatinase and E. faecalis isolates from clinical and community sources. While some of these studies measured gelatinase activity by plate assay (1, 5, 7, 10, 28), others used DNA-based techniques to determine whether the gelE gene was present (25), and such techniques are now known to not reliably predict the gelatinase phenotype. In our study, we used the gelatinase plate assay, hybridization, and PCR to correlate the observed gelatinase phenotype with the underlying fsr and gelE genotypes. Gelatinase activity was observed for 59.5% of 215 E. faecalis isolates (Table 2). With the exception of the slow-gelatinase-producing mutant OG1Sp (6), all isolates were gelatinase positive after 16 h of incubation. Previously, we reported that 27% of 30 healthy community E. faecalis fecal isolates, identified biochemically, produced gelatinase (5). However, we recently

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found by genetic methods that some of our original community fecal isolates were actually *E. faecium* isolates, leading to an underestimation of the percentage of *E. faecalis* isolates that were gelatinase positive. In the present study, we found that 66.6% of fecal *E. faecalis* isolates were gelatinase positive, compared to 58.7% of clinical isolates (endocarditis, blood, and urine isolates). Furthermore, we recently tested 15 additional fecal *E. faecalis* isolates from healthy volunteers and found that 87% were gelatinase producers. Gelatinase production in our clinical isolates correlates with that seen in other studies (1, 7, 16, 28).

In an earlier paper analyzing *fsr* (21), it was shown that 62% of 95 isolates produced gelatinase but that 91% of the isolates hybridized to a *gelE* probe (very similar to the 92% found in the present study), while 71% hybridized to an *fsrB* probe; an intact *fsr* locus and *gelE* are both necessary for gelatinase production (21). Subsequent to our observation of a discrepancy between the presence of *gelE* and gelatinase production, a study of urine isolates obtained from a single Japanese hospital (17) identified a 23.9-kb deletion affecting the *fsr* locus, which explains the *gelE*/H11001, *fsrB*-lacking genotype (Fig. 1). To expand this observation, we included endocarditis, blood, and commu-

<table>
<thead>
<tr>
<th>Primer</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
<th>Sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>aceF</td>
<td>1,003</td>
<td>18</td>
<td>GAGCAAAAGTTCAATCGTGAC</td>
</tr>
<tr>
<td>aceR</td>
<td></td>
<td>18</td>
<td>GTCTGTCTTTTCACCTGTTC</td>
</tr>
<tr>
<td>aacF</td>
<td>320</td>
<td>4</td>
<td>GCGGTAAGCAGCGCTAGACCAAG</td>
</tr>
<tr>
<td>aacR</td>
<td></td>
<td>4</td>
<td>GCATTTGGTAAAGCACCTACG</td>
</tr>
<tr>
<td>gelEF</td>
<td>1,200</td>
<td>This study</td>
<td>GATGAAGGGAAAAATAAAAATTTTATAC</td>
</tr>
<tr>
<td>gelER</td>
<td></td>
<td>This study</td>
<td>AGTAAGTAACTGGCTTTGCTTAAG</td>
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<td>fsrBF1</td>
<td>541</td>
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<td>GGGGAGCTCTGGCACAAGTATTATCTAACC</td>
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<tr>
<td>fsrBR2</td>
<td></td>
<td>21</td>
<td>TTGTTACCACACCACATGACTTTTG</td>
</tr>
</tbody>
</table>

**FIG. 1.** The 23.9-kb deletion affecting the *fsr* locus.
nity fecal isolates collected from geographically diverse regions over a 29-year period. Using colony hybridization, we found 60 isolates (including 14 endocarditis isolates) that hybridized with the gelE probe but lacked fsrB, suggesting that they lacked the 23.9-kb region (Table 2). In contrast, a previous study involving a small number of endocarditis isolates suggested that fsr was present in all endocarditis isolates (20). The discrepancy between the two studies may have resulted from the small number of isolates used or possibly from the fact that the probe consisted of all three fsr genes, which may have resulted in a positive hybridization result because 1,081 bp of the fsrC gene is still present in strains with the deletion. While the 23.9-kb region was missing in all categories of isolates we tested (Table 2), 16 isolates lacked both gelE and fsrB, similar to the findings presented in the earlier report (21), suggesting an even larger deletion in these isolates, as was also reported by Nakayama et al. (17).

All 60 fsrB-lacking, gelE-containing isolates were confirmed by PCR to lack the 23.9-kb region. Sequencing of the 1.8-kb PCR deletion junctions from 10 isolates (4 endocarditis isolates, 2 other blood isolates, 2 urine isolates, and 2 community fecal isolates) revealed that they were identical for 300 bp on either side of the junction, indicating that the region is highly conserved and also suggesting that the deletion occurred by the same mechanism in all isolates (Fig. 1).

We also examined 35 isolates with the 23.9-kb deletion by pulsed-field gel electrophoresis. The observed banding patterns indicated at least 22 distinct strains (a difference of >7 bands) (27) among these 35 isolates from geographically diverse locations. Several urine isolates collected in the same location were related, while the majority of endocarditis, blood, and community fecal isolates were distinct strains. These results show that the deletion occurred in many distinct clinical strains, as well as in isolates from healthy volunteers.

In conclusion, the percentage of clinical isolates displaying gelatinase activity was similar to the percentage of healthy fecal isolates displaying gelatinase activity. In addition to urine isolates, the previously described 23.9-kb deleted region (17) was also lacking in endocarditis, blood, and healthy stool isolates. While fsr and gelatinase increase the severity of disease in animal models of _E. faecalis_ infection, they are not required for the organism to cause disease (9). Controlled clinical studies will be needed to determine whether the presence of the _fsr_ locus or gelatinase production affects the course of _E. faecalis_ infections in humans or the outcome of the treatment.

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New community fecal isolates were collected at the Clinical Research Center, University of Texas Medical School. This study was approved by the UTHSC Committee for the Protection of Human Subjects, and informed consent was obtained from participants.

### REFERENCES


### TABLE 2. Summary of results for all isolates tested

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Gelatinase positive</th>
<th>Lacking fsrB, gelE</th>
<th>Lacking fsrB and gelE</th>
<th>fsrB−, lacking gelE</th>
<th>Total no. of isolates from source (%) of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocarditis</td>
<td>49 (61.2%)</td>
<td>7 (8.7%)</td>
<td>14 (17.5%)</td>
<td>10 (12.5%)</td>
<td>80 (37.2%)</td>
</tr>
<tr>
<td>Blood</td>
<td>24 (75%)</td>
<td>1 (3.1%)</td>
<td>5 (15.6%)</td>
<td>2 (6.3%)</td>
<td>30 (14.8%)</td>
</tr>
<tr>
<td>Urine</td>
<td>41 (50%)</td>
<td>2 (2.4%)</td>
<td>36 (43.9%)</td>
<td>3 (3.7%)</td>
<td>82 (38.1%)</td>
</tr>
<tr>
<td>Feces from healthy community-based volunteers</td>
<td>14 (66.6%)</td>
<td>0</td>
<td>5 (23.8%)</td>
<td>1 (4.8%)</td>
<td>21 (9.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>128 (59.5%)</td>
<td>10 (4.7%)</td>
<td>60 (27.9%)</td>
<td>16 (7.4%)</td>
<td>215 (100%)</td>
</tr>
</tbody>
</table>

a Isolates that produced gelatinase had the _fsrB−_ gelE+ genotype.

b All isolates with this genotype appeared to have the 23.9-kb deletion as determined by PCR (see text).


