

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* OG1SSp (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.

Colony hybridization was performed as described (26) with probes labeled 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 OG1RF genomic DNA templates and the primers given in Table 1, while the *aac(6')-II* 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 OG1SSp (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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TABLE 1. Primers used in this study

Primer	Amplicon size (bp)	Reference	Sequence (5' to 3')
Primers used to make probes			
aceF	1,003	18	GAGCAAAAGTTCAATCGTTGAC
aceR		18	GTCTGTCTTTTCACTTGTCTTCT
aacF	320	4	GCGGTAGCAGCGCTAGACCAAG
aacR		4	GCATTTGGTAAGACACCTACG
gelEF	1,200	This study	GATGAAGGGAAATAAAAATTTTATAC
gelER		This study	AGTAAGTAACTGGTCTTGTCTAAG
fsrBF1	541	21	GGGAGCTCTGGACAAAAGTATTATCTAACCG
fsrBR2		21	TGGTACCCACACCATCACTGACTTTTGC
Primers used to determine size of deletion and for sequencing			
delsizeF1	1,800	This study	GAAACTTCACTTTCTGAATAAACTTG
delsizeR1		This study	GTTGGAGAGTATGCCTATCAACTCG

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*⁺, *fsrB*-lacking genotype (Fig. 1). To expand this observation, we included endocarditis, blood, and commu-

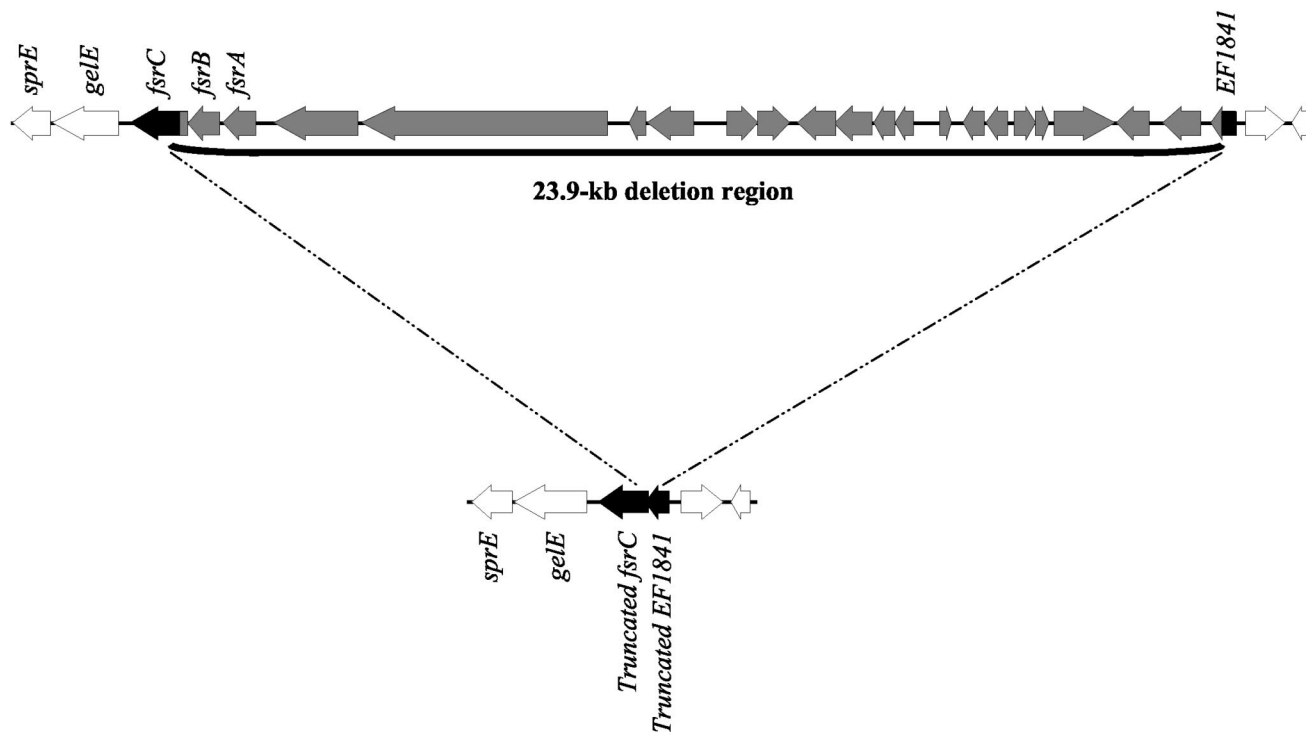
FIG. 1. The 23.9-kb deletion affecting the *fsr* locus.

TABLE 2. Summary of results for all isolates tested

Source of isolates	No. (%) of isolates:					Total no. of isolates from source (% of total)
	Gelatinase ^a positive	Gelatinase negative				
		<i>fsrB</i> ⁺ <i>gelE</i> ⁺	Lacking <i>fsrB</i> , <i>gelE</i> ⁺ ^b	Lacking <i>fsrB</i> and <i>gelE</i>	<i>fsrB</i> ⁺ , lacking <i>gelE</i>	
Endocarditis	49 (61.2)	7 (8.7)	14 (17.5)	10 (12.5)	0	80 (37.2)
Blood	24 (75)	1 (3.1)	5 (15.6)	2 (6.3)	0	30 (14.8)
Urine	41 (50)	2 (2.4)	36 (43.9)	3 (3.7)	0	82 (38.1)
Feces from healthy community-based volunteers	14 (66.6)	0	5 (23.8)	1 (4.8)	1 (4.8)	21 (9.7)
Total	128 (59.5)	10 (4.7)	60 (27.9) ^b	16 (7.4)	1 (0.5)	215 (100)

^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).

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 all 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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