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Influence of Origin of Isolates, Especially Endocarditis Isolates, and Various Genes on Biofilm Formation by *Enterococcus faecalis*

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Endocarditis isolates of *Enterococcus faecalis* produced biofilm significantly more often than nonendocarditis isolates, and 39% of 79 versus 6% of 84 isolates produced strong biofilm ($P < 0.0001$). *esp* was not required, but its presence was associated with higher amounts of biofilm ($P < 0.001$). Mutants disrupted in *dltA*, *efaA*, *ace*, *lsa*, and six two-component regulatory systems were largely unaltered, while disruptions in *epa*, *atn*, *gelE*, and *fsr* resulted in fewer attached bacteria, as determined using phase-contrast microscopy, and less biofilm ($P < 0.0001$).

Bacteria are frequently found as part of a complex of organisms known as biofilm (15). Although biofilm formation by enterococci has been reported (1, 3, 28), there has not been a systematic study of endocarditis isolates and there has been little published relating to the genetics of biofilm formation by *Enterococcus faecalis*. This previous study found that 93.5% of *esp*-positive isolates formed biofilm while no *esp*-lacking isolate produced biofilm; *esp* disruption in two strains resulted in decreased biofilm formation, while *esp* disruption had no significant effect on the strong biofilm phenotype of a third strain (28). In the present work, we studied the occurrence of *esp* and biofilm formation among isolates of *E. faecalis* and evaluated mutants of an *esp*-lacking strain in an effort to unravel the role played by, and the genesis of, biofilm formation by this organism.

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Bacterial strains. A total of 163 *E. faecalis* isolates (51 from sources outside the United States) were evaluated. Control strains (28) were kindly provided by I. Lasa. OG1RF (*esp* negative) (12) and mutants of OG1RF that had been previously generated (14, 17, 18, 22–25, 27, 33) were also evaluated.

Genetic methods. An intragenic fragment of *esp* was amplified by PCR using previously described primers (19) and used as a probe for colony hybridization, as described elsewhere (23). A disruption mutant (TX5427) of a homologue of *Streptococcus agalactiae dltA* was generated and confirmed, as described previously (27).

Biofilm formation. Bacteria that had been grown overnight were diluted 1:100 in 200 μl of tryptic soy broth–0.25% glucose and inoculated onto polystyrene microtiter plates (Falcon, Franklin Lakes, N.J.). After 24 h of static incubation at 37°C, plates were processed (2, 28), fixed with Bouin's fixative for 30 min, stained with 1% crystal violet (CV) for 30 min, and rinsed with distilled water. CV was solubilized in ethanol-acetone (80:20, vol/vol), and optical density at 570 nm (OD_{570}) was determined. Each assay was performed in quadruplicate on at least three occasions. For phase-contrast microscopy, bacteria were grown as described above except in polystyrene petri dishes (Falcon). After removal of planktonic bacteria, biofilm was directly examined by phase-contrast microscopy (magnification, ×600) with an Eclipse TE2000-E (Nikon Corp., Tokyo, Japan).

For primary adherence, 5 ml of a diluted overnight culture (OD_{600}, 0.1) was added to polystyrene petri dishes (Falcon) and incubated for 2 h for mutants, as described previously (28), and 30 min for clinical isolates (5) (greater adherence of clinical isolates made counting difficult at 2 h). Bacteria in five different fields were subjected to light microscopy and counted (magnification, ×1,000) after Gram staining.

Statistical analysis. Statistical analysis was performed using the Mann-Whitney test for continuous variables and Fisher's exact test (NCSS/PASS 2000 edition; NCSS Statistical Software, Kaysville, Utah) or the chi-square test for categorical variables. Median OD_{570} and interquartile range (IQR) values were calculated using GraphPad Prism 4 software.

Biofilm formation by clinical isolates. OD_{570} readings after CV staining ranged from 0.2 to 3.5 (Fig. 1), and isolates were categorized (Table 1) based on the approach of others (1, 10, 28) as strong (OD_{570}, >2; 36 isolates [22%]), medium (OD_{570}, 1 to 2; 92 isolates [56%]), or weak (OD_{570}, greater than 0.5 but less than 1; 23 isolates [14%]) biofilm formers or as non-biofilm formers (OD_{570}, ≤0.5; 12 isolates [7%]). The median OD_{570} values for controls (28) were 3.5 for *E. faecalis* strain 54 (categorized as a strong biofilm former in reference 28), 1.72 for strain 11279 (medium [28]), 0.85 for strain 11262 (weak [28]), and 0.61 for strain 23 (categorized as a non-biofilm former in reference 28). The ca. 93% of 163 *E. faecalis* isolates classified as biofilm producers is lower than the percentage reported in one study (20) that classified any samples with ODs

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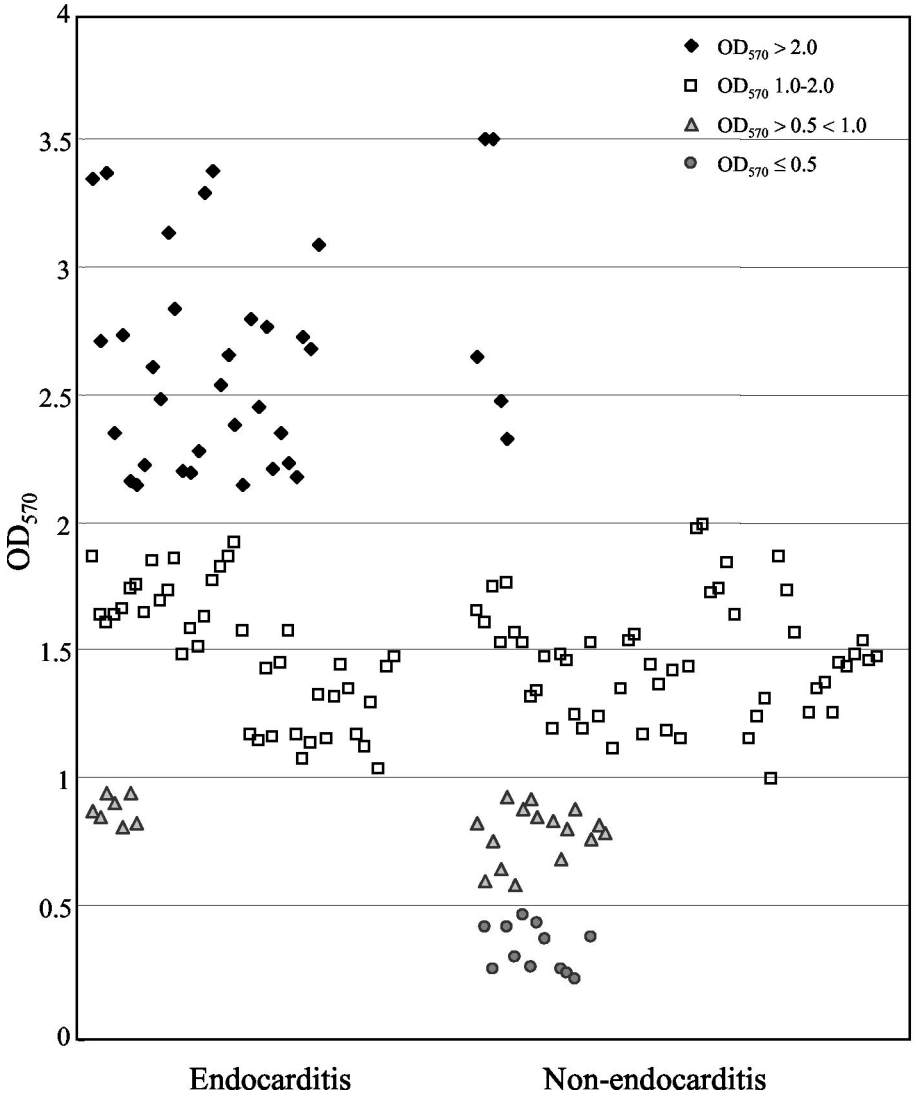

FIG. 1. Biofilm formation by *E. faecalis* isolates derived from different sources. Biofilm formation on a polystyrene surface was assessed after CV staining. Each dot indicates the median OD_{570} value from 12 determinations (three independent experiments, each performed in quadruplicate). The medians (and IQRs) for endocarditis isolates and those from other sources were 1.74 (IQR, 1.32 to 2.35) and 1.31 (IQR, 0.82 to 1.53), respectively ($P < 0.0001$).

of >0 as positive for biofilm formation and higher than that found by others (with slightly different methodologies) who reported 57% (28) and 80% (1) of *E. faecalis* isolates as positive for biofilm formation. If we consider only strong and medium production as positive (OD > 1.0), 78.5% of our isolates would be classified as biofilm formers (Table 1).

All 79 endocarditis isolates formed biofilm versus 86% of 84 isolates from other sources ($P < 0.001$), and 31 of 79 (39%) were strong biofilm formers (Table 1 and Fig. 1) versus only 5 (6%) of the isolates from other sources ($P < 0.0001$; median OD_{570}, 1.74 versus 1.31; $P < 0.0001$). To our knowledge, this is the first report to show that endocarditis isolates are associated with greater biofilm formation, but it would be premature to speculate whether biofilm contributes to or perhaps results from endocarditis. Results for primary adherence were generally, but not absolutely (e.g., TX0034 and TX0291), correlated with an organism's level of biofilm formation (Fig. 2A).

Presence of *esp* and biofilm. *esp* was present in 74 (45%) of 163 isolates and 49% of biofilm producers (Table 1). Among endocarditis isolates, 48% were *esp* positive versus 59% of urine isolates, 48% of other clinical isolates, 33% of nosocomial fecal isolates, and 19% of community fecal isolates. The incidence of *esp* has been reported by others as 29 to 45% among *E. faecalis* blood isolates (4, 21, 29, 31), 42% among 33 endocarditis isolates (21), and 3 to 40% among fecal isolates (21, 31).

All 74 *esp*-positive isolates produced biofilm, and 77 of 89 *esp*-negative isolates also produced biofilm. This is in contrast to results from one study (28) in which none of the *esp*-negative isolates formed biofilm, but it is consistent with those of another study (20) reporting no association between *esp* and biofilm formation. However, we did find that 69% of strong, 46% of medium, and 30% of weak biofilm producers and 0 of 12 non-biofilm producers were *esp* positive ($P < 0.001$) and

TABLE 1. Occurrence of biofilm formation and presence of the *esp* gene in *E. faecalis* isolates

Clinical source[b] (total no. tested)	No. (%) of isolates with indicated biofilm phenotype[a]/no. (%) of isolates which were also *esp* positive							% of total isolates that were *esp* positive
	Strong	Medium	Strong or medium	Weak	Non-biofilm former	Weak or non-biofilm former	Positive for biofilm formation	
Endocarditis (79)	31 (39)/23 (74)	41 (52)/14 (34)	72 (91)/37 (51)	7 (9)/1 (14)	0 (0)/0 (0)	7 (9)/1 (14)	79 (100)/38 (48)	48
Urine (22)	0 (0)/0 (0)	17 (77)/12 (71)	17 (77)/12 (71)	3 (14)/1 (33)	2 (9)/0 (0)	5 (22)/1 (20)	20 (91)/13 (65)	59
Other[c] (31)	2 (7)/0 (0)	17 (55)/12 (71)	19 (61)/12 (71)	6 (19)/3 (50)	6 (19)/0 (0)	12 (39)/3 (25)	25 (81)/15 (60)	48
Hospital fecal specimens (15)	1 (7)/1 (100)	7 (47)/2 (29)	8 (53)/3 (38)	4 (27)/2 (50)	3 (20)/0 (0)	7 (47)/2 (29)	12 (80)/5 (42)	33
Community fecal specimens (16)	2 (13)/1 (50)	10 (63)/2 (20)	12 (75)/3 (25)	3 (19)/0 (0)	1 (6)/0 (0)	4 (25)/0 (0)	15 (94)/3 (20)	19
Total (163)	36 (22.1)/25 (69)[d]	92 (56.4)/42 (46)[d]	128 (78.5)/67 (52)	23 (14.1)/7 (30)[d]	12 (7.4)/0 (0)[d]	35 (21.5)/7 (20)	151 (92.6)/74 (49)	45

[a] Strong, OD_{570} of >2 (median, 2.57; IQR, 2.25 to 2.81); medium, OD_{570} of 1 to 2 (median, 1.47; IQR, 1.27 to 1.64); weak, OD_{570} of greater than 0.5 but less than 1 (median, 0.82; IQR, 0.76 to 0.88); non-biofilm former, OD_{570} of ≤0.5 (median, 0.33; IQR, 0.25 to 0.41).

[b] Median ODs were 1.74 (IQR, 1.32 to 2.35) for endocarditis isolates, 1.39 (IQR, 1.01 to 1.55) for isolates from urine, 1.18 (IQR, 0.64 to 1.64) for other clinical isolates, 0.99 (IQR, 0.68 to 1.57) for hospital fecal isolates, and 1.40 (IQR, 1.03 to 1.48) for community fecal isolates ($P, \leq 0.005$ for endocarditis isolates versus each of the other groups of isolates).

[c] Other clinical isolates were derived from ascites fluid, blood, catheters, cerebrospinal fluid, cervix, endometrium, penis, placenta, trachea, and wounds.

[d] $P, < 0.001$ for correlation between percentage of *esp*-positive isolates and strong, medium, weak, or no biofilm production (median ODs, 1.62 [IQR, 1.25 to 2.29] for *esp*-positive isolates and 1.37 [IQR, 0.85 to 1.71] for *esp*-negative isolates; $P < 0.001$).

that there was a significant difference in median OD values ($P < 0.001$) (Table 1), indicating a strong association between the presence of *esp* and greater levels of biofilm production.

Analysis of *E. faecalis* OG1RF mutants. The absence of *esp* in 51% of biofilm formers motivated us to look for other genes that might influence biofilm formation. Among mutants of *E. faecalis* OG1RF (an *esp*-negative medium biofilm former) that were previously generated, seven were defective in biofilm formation (P, <0.0001 for each mutant) compared to OG1RF (Fig. 2B). In particular, our *epa* (enterococcal polysaccharide antigen) gene cluster mutant, TX5179 (*orfde4*) (26, 33), showed ~73% reduction in biofilm formation, suggesting that this gene (encoding a putative glycosyltransferase, often involved in polysaccharide synthesis) (32, 33) and/or the cotranscribed *orfde5* is important for biofilm formation. We have no evidence to indicate a surface location for Epa (33) or a direct role of the Epa polysaccharide in attachment or biofilm accumulation, and there may be other effects, such as alteration of the overall cell wall layer, as suggested by others (7).

All three of our *fsr* mutants (17) also showed decreased biofilm formation, with reduction ranging from ~28 to 32% relative to that by OG1RF (Fig. 2B); *fsr*, a homologue of staphylococcal *agr* loci, positively regulates expression of gelatinase (GelE) and serine protease (SprE) genes and is involved in quorum sensing (13, 16, 17). This decrease was not as great as the 46% decrease seen for TX5128, a *gelE* insertion mutant ($GelE^-$ $SprE^-$) (24) ($P < 0.0001$) or for TX5264, a nonpolar *gelE* deletion mutant ($GelE^-$ $SprE^+$) (22) ($P < 0.01$) (Fig. 2B). A recent study also demonstrated that GelE enhances biofilm formation by *E. faecalis* (9). Since the *fsr* mutants also have the $GelE^-$ $SprE^-$ phenotype, it is possible that biofilm reduction is due to loss of protease production. Biofilm formation by an *agr* mutant of *Staphylococcus aureus* (30) was enhanced compared to that by the wild type, in contrast to our results with *fsr* mutants; however, the *fsr* mutants formed slightly (but significantly) more biofilm than the gelatinase mutants, suggesting an additional effect(s) of *fsr* which influences biofilm formation in the same direction as *agr*. Future studies will be needed to address what additional role *fsr* may have on biofilm production. We also examined a *gelE* in-frame-deletion mutant and TX5243, a *sprE* insertion mutant ($GelE^+$ $SprE^-$) (17); the *sprE* mutant formed as much biofilm as the wild type, while the *gelE* insertion (Gel^- $SprE^-$) and deletion (Gel^- $SprE^+$) mutants showed decreased biofilm formation (Fig. 2B), indicating that gelatinase rather than the serine protease is important for biofilm formation.

Our autolysin (*atn*) mutant, TX5127, previously shown to display increased chaining and decreased autolysis (18), showed ~39% reduction in biofilm formation (Fig. 2B). A similar finding was seen in a *Lactococcus lactis* autolysin (*acmA*) mutant, which exhibited long chains of cells, adhered less efficiently than the wild type, and was unable to form biofilm (11). A *Staphylococcus epidermidis* autolysin (*atlE*) mutant also showed decreased primary attachment to polystyrene (8).

Among the previously described two-component regulatory system mutants (27), five were unaltered in biofilm formation, although the *etaR* mutant (TX10293) showed a small (~8%) but significant ($P < 0.02$) reduction in biofilm (Fig. 2B). Mutants TX5132 (*efaA*, encoding an *E. faecalis* antigen A) (23)

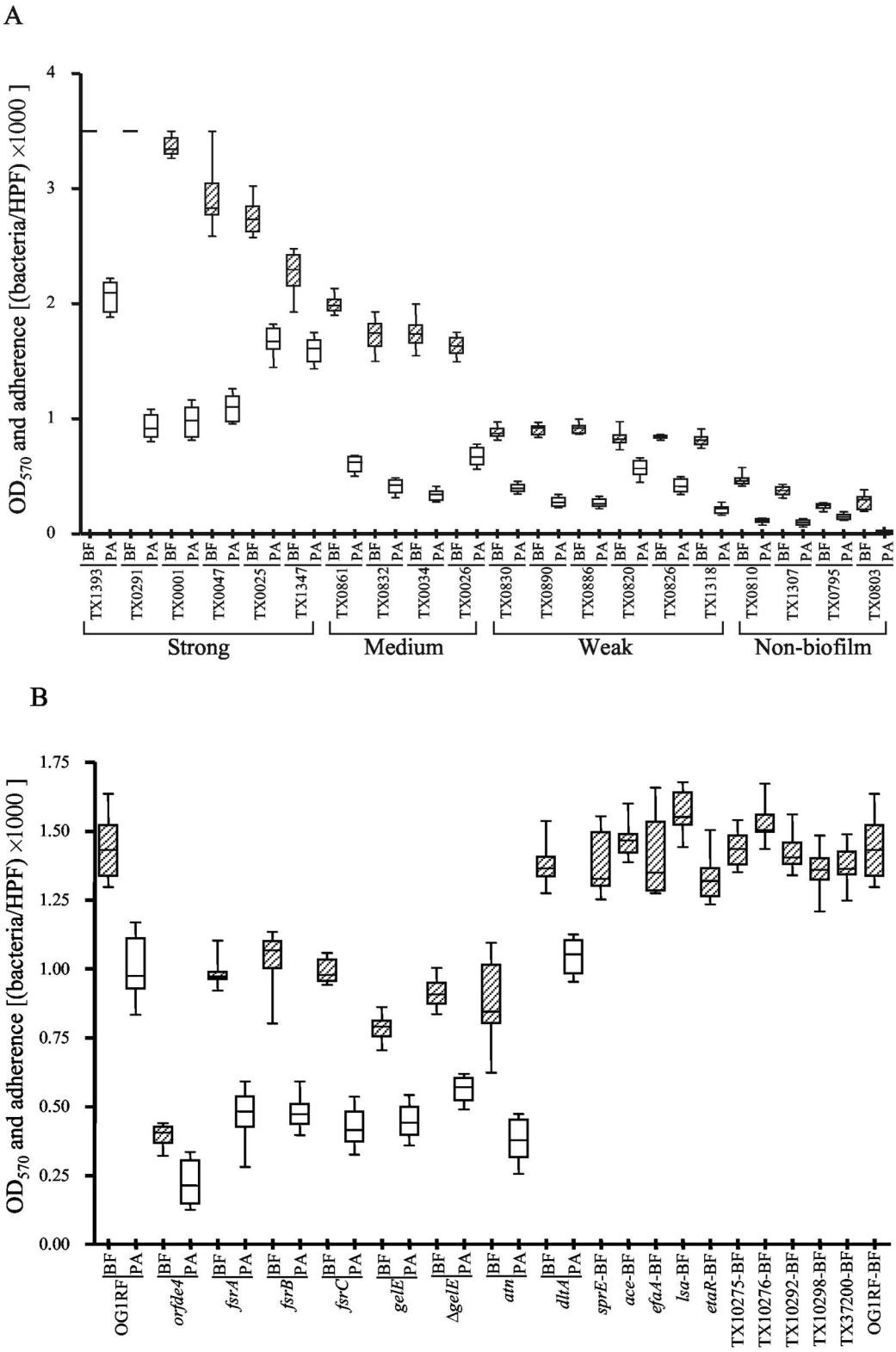

FIG. 2. Biofilm formation (BF) and primary adherence (PA) by representative *E. faecalis* clinical isolates (A) and mutants (B). Median and IQR values are shown. Values for the biofilm assays are from 12 determinations (three independent experiments, each performed in quadruplicate). All readings for TX1393 and TX0291 were 3.5, the maximum OD detectable by our microplate reader. Primary adherence was assessed after incubation on a polystyrene surface for 30 min for clinical isolates and 2 h for mutants. Bacteria in five different fields from two independent plates were subjected to light microscopy (HPF, high-power field; magnification, ×1,000) and counted after Gram staining. TX10275, TX10276, TX10292, TX10298, TX37200, and *etaR* are two-component regulatory system mutants. The other 10 mutants which showed no change in biofilm were not tested for primary adherence.

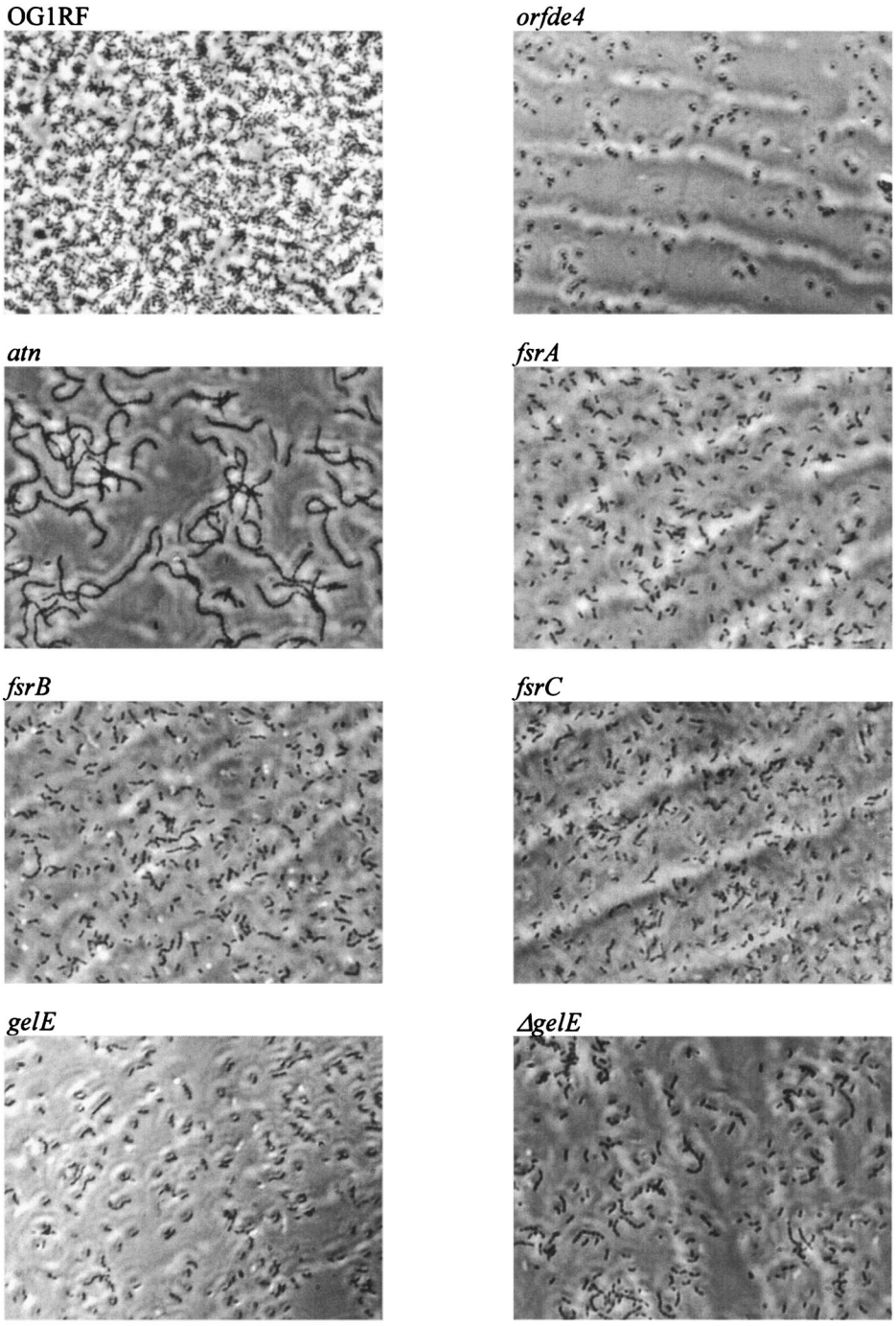

FIG. 3. Phase-contrast photomicrographs of biofilms on a polystyrene surface. Images are representative of what was observed in multiple fields (magnification, ×600).

and TX5256 (*ace*, encoding a collagen adhesin) (14) were also unchanged relative to OG1RF, while TX5332 (*lsa*, encoding an ATP-binding cassette transporter required for lincosamide and streptogramin A resistance) (25) showed a small (~9%) but significant ($P < 0.02$) increase (Fig. 2B). Biofilm formation by TX5427 (this study) disrupted in a *dltA* homologue was approximately equal to that by OG1RF, unlike that by an *S. aureus dlt* mutant (6).

In a primary attachment assay, OG1RF attached to polystyrene more efficiently than the seven mutants with reduced biofilm formation (P, <0.001 for each mutant) (Fig. 2B). As determined by phase-contrast microscopy, OG1RF formed a more confluent layer, with dark clusters of bacteria in microcolonies interspaced with areas of less densely packed bacteria, whereas these seven mutants showed fewer attached bacteria without microcolonies (Fig. 3). This indicates that *epa*, *atn*, *gelE*, and the *fsr* locus influence primary attachment, although additional effects on biofilm accumulation are also possible.

The *atn* mutant was again (18) noted to exhibit long chains of cells whereas mutants disrupted in *gelE*, *fsrA*, *fsrB*, and *fsrC* showed short chains and the *orfde4* mutant showed no chain formation (Fig. 3).

In conclusion, our results agree with other reports that biofilm formation is very common among *E. faecalis* clinical as well as fecal isolates. We also found that the percent and degree of biofilm formation are significantly greater among endocarditis isolates than among isolates from other sources. Although *esp* was not required for biofilm formation, its presence showed a significant association with the degree of biofilm production. Our study also identified several other genes that influenced primary attachment and biofilm formation by *E. faecalis* OG1RF.

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REFERENCES

1. **Baldassarri, L., R. Cecchini, L. Bertuccini, M. G. Ammendolia, F. Iosi, C. R. Arciola, L. Montanaro, R. Di Rosa, G. Gherardi, G. Dicuonzo, G. Orefici, and R. Creti.** 2001. *Enterococcus* sp. produces slime and survives in rat peritoneal macrophages. Med. Microbiol. Immunol. (Berlin) **190:**113–120.
2. **Christensen, G. D., W. A. Simpson, J. J. Younger, L. M. Baddour, F. F. Barrett, D. M. Melton, and E. H. Beachey.** 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. **22:**996–1006.
3. **Distel, J. W., J. F. Hatton, and M. J. Gillespie.** 2002. Biofilm formation in medicated root canals. J. Endod. **28:**689–693.
4. **Eaton, T. J., and M. J. Gasson.** 2001. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. Appl. Environ. Microbiol. **67:**1628–1635.
5. **Fournier, B., and D. C. Hooper.** 2000. A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of *Staphylococcus aureus*. J. Bacteriol. **182:**3955–3964.
6. **Gross, M., S. E. Cramton, F. Gotz, and A. Peschel.** 2001. Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. Infect. Immun. **69:**3423–3426.
7. **Hancock, L. E., and M. S. Gilmore.** 2002. The capsular polysaccharide of *Enterococcus faecalis* and its relationship to other polysaccharides in the cell wall. Proc. Natl. Acad. Sci. USA **99:**1574–1579.
8. **Heilmann, C., M. Hussain, G. Peters, and F. Gotz.** 1997. Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. Mol. Microbiol. **24:**1013–1024.
9. **Kristich, C. J., Y. H. Li, D. G. Cvitkovitch, and G. M. Dunny.** 2004. Esp-independent biofilm formation by *Enterococcus faecalis*. J. Bacteriol. **186:** 154–163.
10. **Loo, C. Y., D. A. Corliss, and N. Ganeshkumar.** 2000. *Streptococcus gordonii* biofilm formation: identification of genes that code for biofilm phenotypes. J. Bacteriol. **182:**1374–1382.
11. **Mercier, C., C. Durrieu, R. Briandet, E. Domakova, J. Tremblay, G. Buist, and S. Kulakauskas.** 2002. Positive role of peptidoglycan breaks in lactococcal biofilm formation. Mol. Microbiol. **46:**235–243.
12. **Murray, B. E., K. V. Singh, R. P. Ross, J. D. Heath, G. M. Dunny, and G. M. Weinstock.** 1993. Generation of restriction map of *Enterococcus faecalis* OG1 and investigation of growth requirements and regions encoding biosynthetic function. J. Bacteriol. **175:**5216–5223.
13. **Nakayama, J., Y. Cao, T. Horii, S. Sakuda, A. D. Akkermans, W. M. de Vos, and H. Nagasawa.** 2001. Gelatinase biosynthesis-activating pheromone: a peptide lactone that mediates a quorum sensing in *Enterococcus faecalis*. Mol. Microbiol. **41:**145–154.
14. **Nallapareddy, S. R., X. Qin, G. M. Weinstock, M. Hook, and B. E. Murray.** 2000. *Enterococcus faecalis* adhesin, Ace, mediates attachment to extracellular matrix proteins collagen type IV and laminin as well as collagen type I. Infect. Immun. **68:**5218–5224.
15. **O'Toole, G., H. B. Kaplan, and R. Kolter.** 2000. Biofilm formation as microbial development. Annu. Rev. Microbiol. **54:**49–79.
16. **Qin, X., K. V. Singh, G. M. Weinstock, and B. E. Murray.** 2001. Characterization of *fsr*, a regulator controlling expression of gelatinase and serine protease in *Enterococcus faecalis* OG1RF. J. Bacteriol. **183:**3372–3382.
17. **Qin, X., K. V. Singh, G. M. Weinstock, and B. E. Murray.** 2000. Effects of *Enterococcus faecalis fsr* genes on production of gelatinase and a serine protease and virulence. Infect. Immun. **68:**2579–2586.
18. **Qin, X., K. V. Singh, Y. Xu, G. M. Weinstock, and B. E. Murray.** 1998. Effect of disruption of a gene encoding an autolysin of *Enterococcus faecalis* OG1RF. Antimicrob. Agents Chemother. **42:**2883–2888.
19. **Rice, L. B., L. Carias, S. Rudin, C. Vael, H. Goossens, C. Konstabel, I. Klare, S. R. Nallapareddy, W. Huang, and B. E. Murray.** 2003. A potential virulence gene, hyl_{Efm}, predominates in *Enterococcus faecium* of clinical origin. J. Infect. Dis. **187:**508–512.
20. **Sandoe, J. A., I. R. Witherden, J. H. Cove, J. Heritage, and M. H. Wilcox.** 2003. Correlation between enterococcal biofilm formation in vitro and medical-device-related infection potential in vivo. J. Med. Microbiol. **52:**547–550.
21. **Shankar, V., A. S. Baghdayan, M. M. Huycke, G. Lindahl, and M. S. Gilmore.** 1999. Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. Infect. Immun. **67:**193–200.
22. **Sifri, C. D., E. Mylonakis, K. V. Singh, X. Qin, D. A. Garsin, B. E. Murray, F. M. Ausubel, and S. B. Calderwood.** 2002. Virulence effect of *Enterococcus faecalis* protease genes and the quorum-sensing locus *fsr* in *Caenorhabditis elegans* and mice. Infect. Immun. **70:**5647–5650.
23. **Singh, K. V., T. M. Coque, G. M. Weinstock, and B. E. Murray.** 1998. In vivo testing of an *Enterococcus faecalis efaA* mutant and use of *efaA* homologs for species identification. FEMS Immunol. Med. Microbiol. **21:**323–331.
24. **Singh, K. V., X. Qin, G. M. Weinstock, and B. E. Murray.** 1998. Generation and testing of mutants of *Enterococcus faecalis* in a mouse peritonitis model. J. Infect. Dis. **178:**1416–1420.
25. **Singh, K. V., G. M. Weinstock, and B. E. Murray.** 2002. An *Enterococcus faecalis* ABC homologue (Lsa) is required for the resistance of this species to clindamycin and quinupristin-dalfopristin. Antimicrob. Agents Chemother. **46:**1845–1850.
26. **Teng, F., K. D. Jacques-Palaz, G. M. Weinstock, and B. E. Murray.** 2002. Evidence that the enterococcal polysaccharide antigen gene (*epa*) cluster is widespread in *Enterococcus faecalis* and influences resistance to phagocytic killing of *E. faecalis*. Infect. Immun. **70:**2010–2015.
27. **Teng, F., L. Wang, K. V. Singh, B. E. Murray, and G. M. Weinstock.** 2002. Involvement of PhoP-PhoS homologs in *Enterococcus faecalis* virulence. Infect. Immun. **70:**1991–1996.
28. **Toledo-Arana, A., J. Valle, C. Solano, M. J. Arrizubieta, C. Cucarella, M. Lamata, B. Amorena, J. Leiva, J. R. Penades, and I. Lasa.** 2001. The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. Appl. Environ. Microbiol. **67:**4538–4545.
29. **Vergis, E. N., N. Shankar, J. W. Chow, M. K. Hayden, D. R. Snydman, M. J. Zervos, P. K. Linden, M. M. Wagener, and R. R. Muder.** 2002. Association between the presence of enterococcal virulence factors gelatinase, hemolysin, and enterococcal surface protein and mortality among patients with bacteremia due to *Enterococcus faecalis*. Clin. Infect. Dis. **35:**570–575.
30. **Vuong, C., H. L. Saenz, F. Gotz, and M. Otto.** 2000. Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J. Infect. Dis. **182:**1688–1693.
31. **Waar, K., A. B. Muscholl-Silberhorn, R. J. Willems, M. J. Slooff, H. J. Harmsen, and J. E. Degener.** 2002. Genogrouping and incidence of virulence factors of *Enterococcus faecalis* in liver transplant patients differ from blood culture and fecal isolates. J. Infect. Dis. **185:**1121–1127.
32. **Xu, Y., B. E. Murray, and G. M. Weinstock.** 1998. A cluster of genes involved in polysaccharide biosynthesis from *Enterococcus faecalis* OG1RF. Infect. Immun. **66:**4313–4323.
33. **Xu, Y., K. V. Singh, X. Qin, B. E. Murray, and G. M. Weinstock.** 2000. Analysis of a gene cluster of *Enterococcus faecalis* involved in polysaccharide biosynthesis. Infect. Immun. **68:**815–823.

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