Signal Transduction, Quorum-Sensing, and Extracellular Protease Activity in Enterococcus faecalis Biofilm Formation

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GUEST COMMENTARY

Signal Transduction, Quorum-Sensing, and Extracellular Protease Activity in *Enterococcus faecalis* Biofilm Formation

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Biofilms are surface-attached communities of bacteria, encased in an extracellular matrix of secreted proteins, carbohydrates, and/or DNA, that assume phenotypes distinct from those of planktonic cells. These phenotypes include a slower growth rate, increased antibiotic resistance, and elevated frequency of lateral gene transfer (15, 20, 33, 38). The ability of certain bacterial strains to form biofilms has been associated with virulence in a number of pathogens, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans* (21). An association between biofilm formation and virulence has also been reported for *Enterococcus faecalis*, a gram-positive bacterium that has recently emerged as a leading cause of nosocomial infections (7, 26). *E. faecalis* biofilms on dental root canals (6), urethral catheters (36), ureteral stents (28), and heart valves (8) have been observed. While it is not clear that the ability of *E. faecalis* to form biofilms is essential for virulence, it appears that a majority of clinical isolates do possess the ability to form a biofilm in vitro (18, 35). Efforts to identify the molecular entities critical for development into this insidious and persistent mode of existence have been recently undertaken. In a previous issue of this journal, Hancock and Perego provided strong evidence that the activity of a single enzyme controlled by a single signal transduction pathway plays a key role in the formation of *E. faecalis* biofilms (11). This finding establishes a new focus for investigating the molecular mechanisms of biofilm development and raises the possibility for development of a targeted therapeutic agent to prevent the establishment of biofilms in vivo.

The culprit enzyme is a secreted zinc metalloprotease, gelatinase, a thermolysin-like M4 protease similar to those found in other bacterial pathogens (2). Gelatinase cleaves a broad range of substrates in vitro, including Azocoll, casein, gelatin, hemoglobin, plasmid conjugation factors, collagen, fibrin, and an autolysin (17, 30, 37). The expression of the gene encoding gelatinase, *gelE*, is dependent on the *fsr* genes that encode a two-component signal transduction system (24, 25). Hancock and Perego determined a role for *fsr* and *gelE* in biofilm formation after carrying out a systematic inactivation of each of the 18 genes predicted by a homology search of the fully sequenced genome of *E. faecalis* strain V583 to encode response regulator proteins (10). Only one of the mutants generated, *fsrA*, impaired the ability of *E. faecalis* strain V583A to form biofilms in vitro. Further analysis demonstrated that mutations in the gene encoding FsrA's cognate sensor kinase, *fsrC*, also disrupted biofilm formation. Disrupting the *gelE* gene phenocopied the poor biofilm-forming ability of *fsr* mutants, and driving the expression of a plasmid-borne copy of *gelE* from a constitutive promoter completely rescued biofilm formation in an *fsr* mutant. Moreover, the addition of an *fsr* mutant to the culture medium of enzymatically active GelE purified from *E. faecalis* culture supernatants restored the ability of the *fsr* mutant to form biofilms. These results confirm and extend other recent findings of the involvement of *fsr* and *gelE* biofilm formation in different strain backgrounds (14, 18, 22), thus building a solid case for the importance of gelatinase activity in biofilm development.

While the physiological substrate(s) of gelatinase required for biofilm development is unknown, three properties of the enzyme are gratifyingly consistent with properties of biofilms. First, surface attachment seems to be facilitated by hydrophobic nonpolar substrata (7), and biofilm formation presumably involves changes in cell-surface adhesion properties. Gelatinase is a secreted protease that appears to cleave its substrates primarily at hydrophobic residues (17). Might gelatinase activity increase the hydrophobicity of the cell surface? Second, biofilm formation has been shown in some cases to require the activation of density-dependent gene expression mediated through cell-cell signaling or quorum sensing (5, 16, 39). The *fsr* two-component system that controls expression of gelatinase is a member of the *agr*-like subfamily of two-component signal transduction systems that includes and depends upon a secreted signaling peptide thought to endow the system with a quorum-sensing property (13). For the *fsr* system, the secreted signaling peptide is encoded by *fsrB*, and *gelE* expression has indeed been shown to be cell density dependent in a manner that depends on intact *fsrA*, *fsrB*, and *fsrC* genes (19, 24, 25). Thus, gelatinase is well poised to be involved in a process that is cell density dependent and requires alterations of cell surface proteins and/or host matrix proteins. Lastly, like biofilms, gelatinase has been associated with virulence. The disruption of the *gelE* gene was shown to cause attenuated virulence in a mouse peritonitis model (32), a rabbit endophthalmitis model (9), and a nematode model (31).

The importance of gelatinase in biofilm-mediated human infections is far from clear-cut, however. Two studies reported

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an enrichment of *gelE* or gelatinase in infection-derived isolates versus isolates derived from the feces of healthy individuals (4, 23), but a separate study disputes this correlation (27). Additionally, three studies report that only about half or less of endocarditis-derived isolates were gelatinase producing (1, 4, 27), whereas a large majority of endocarditis-derived isolates in a separate study were shown to form biofilms in vitro (18).

Enterococcal infections invariably result from the complex interplay of multiple host and bacterial factors. In addition to *fsr*-regulated gelatinase, other enterococcal traits promote biofilm formation in vivo. One example is Esp, a protein found on the surface of *E. faecalis* cells (29). Esp unambiguously enhances biofilm formation in vitro and seems to be correlated with the presence of biofilms in vivo (18, 34, 35). Nutrient availability and osmolarity reportedly also influence *E. faecalis* biofilm formation in vitro (14). Glucose seems to affect biofilm formation, but in a manner that apparently depends on the specific glucose concentration and the genetic background of the strain (14, 22, 34). However, the analysis by Hancock and Perego indicates that, at least under conditions in which strain V583 was tested, the *fsr* two-component system is the only two-component system important for biofilm formation. If this is the case, then any environmental or extracellular factors influencing biofilm formation must either be sensed by *fsr*, be transported into the cell, contribute only extracellularly, or influence intracellular events by some unknown mechanism. The authors' analysis also indicates that gelatinase is the only entity downstream of *fsr* signaling that mediates biofilm formation. A separate study reported that *gelE* expression only partially rescued biofilm formation of an *fsr* mutant. This experiment was carried out with an OG1RF background driving *gelE* expression from an inducible promoter (14). The failure to achieve full complementation in these circumstances may be due to suboptimal transcription of *gelE*, differences in biofilm formation between OG1RF and V583, or both.

All things considered, gelatinase is clearly a key player in biofilm formation, and determining its physiological substrates will improve understanding of the process of biofilm development. A number of hypotheses for the physiological role of gelatinase may be constructed based on its in vitro substrates and a *gelE* mutant phenotype. For example, gelatinase can cleave fibrin. Could this ability facilitate host tissue colonization (37)? It can also activate a muramidase-1 autolysin and prevent cell chaining (30, 37). Could this activation be a prerequisite for biofilm formation? This hypothesis is consistent with the observation that a cell-surface autolysin from *Staphylococcus epidermidis* is involved in cell attachment to abiotic surfaces (12). Other possibilities include the processing of an extracellular signaling molecule or the processing of proteins that lead more directly to the creation of an attachable cell surface or the extracellular matrix.

One important question is how specific the role of gelatinase is in biofilm development. Does it have to be gelatinase, or could another protease do the trick? Hancock and Perego rule out a contribution from the serine protease gene, *sprE*, that lies just downstream of *gelE*, but what about a more general protease? Connelly et al. recently reported that *Bacillus subtilis* mutants lacking extracellular proteases were incapable of making biofilms, but biofilm formation can be restored by the addition of either proteinase K, dispase I, or subtilisin to the culture medium (3). Similarly, trypsin treatment enhanced the adhesion of *E. faecalis* cells to heart cell lines (1).

Finally, might protease inhibitors be employed to control enterococcal infections? Protease inhibitors have proven to be effective antiretrovirals, especially in the treatment of human immunodeficiency virus infection, and they also hold promise for tumor inhibition. The administration of protease inhibitors in combination with antibiotics is being explored for treatment of anthrax. Testing protease inhibitors in combination with antibiotics as treatment for other bacterial infections, including *E. faecalis*, is an obvious and exciting next step.

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