Biofilm formation by enterococci

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Enterococci are an important global cause of nosocomial infections, being increasingly associated with urinary tract infections, endocarditis, intra-abdominal and pelvic infections, catheter-related infections, surgical wound infections, and central nervous system infections. The two most common enterococci species are Enterococcus faecalis and Enterococcus faecium. Both are capable of producing biofilms, which consist of a population of cells attached irreversibly on various biotic and abiotic surfaces, encased in a hydrated matrix of exopolymeric substances. Many environmental and genetic factors are associated or have been proposed to be associated with the production of biofilm. This review discusses recent advances in knowledge about the biology and genetics of biofilm formation and the role of biofilms in enterococci pathogenesis.

Introduction

Enterococci, recognized as opportunistic pathogens, are natural inhabitants of the oral cavity, normal intestinal microflora, and female genital tract of both human and animals. They are common nosocomial agents that infect the urinary tract, bloodstream, intra-abdominal and pelvic regions, surgical sites and central nervous system (Murray & Weinstock, 1999; Richards et al., 2000). Enterococcus faecalis is the most common enterococci species and it is responsible for 80–90 % of human enterococcal infections (Jett et al., 1994; Jones et al., 2004). Enterococcus faecium accounts for the remainder of infections caused by enterococci spp. (Jett et al., 1994).

Biofilm is a population of cells attached irreversibly on various biotic and abiotic surfaces, and encased in a hydrated matrix of exopolymeric substances, proteins, polysaccharides and nucleic acids (Costerton, 2001). Biofilm formation is a complex developmental process involving attachment and immobilization on a surface, cell-to-cell interaction, microcolony formation, formation of a confluent biofilm, and development of a three-dimensional biofilm structure (O’Toole et al., 2000). Bacteria in a biofilm behave differently from their free-floating (planktonic) counterparts. The regulation of bacterial gene expression in response to cell population density, called quorum sensing, is accomplished through the production of extracellular signal molecules called autoinducers (Miller & Bassler, 2001). Biofilm production is regulated by quorum sensing systems in several bacterial pathogens. Biofilms are notoriously difficult to eradicate and are a source of many chronic infections. According to the National Institutes of Health, biofilms are medically important, accounting for over 80 % of microbial infections in the body (Lewis, 2001). A mature biofilm can tolerate antibiotics at concentrations of 10–1000 times more than are required to kill planktonic bacteria. Bacteria in biofilms are resistant to phagocytosis, making biofilms extremely difficult to eradicate from living hosts (Lewis, 2001). Bacteria in biofilms colonize a wide variety of medical devices, such as catheters, artificial cardiac pacemakers, prosthetic heart valves and orthopaedic appliances, and are associated with several human diseases, such as native valve endocarditis, burn wound infections, chronic otitis media with effusion and cystic fibrosis (Costerton et al., 1999). Enterococci in biofilms are more highly resistant to antibiotics than planktonically growing enterococci, thus the potential impact of biofilm formation could be significant.

Enterococci have also been reported as important organisms in periodontal infection (Molander et al., 1998; Peciuliene et al., 2000). The adherence (Joyanes et al., 1999, 2000) and production of a biofilm (Baldassarri et al., 2001; Distel et al., 2002; Mohamed et al., 2003, 2004; Toledo-Arana et al., 2001) by E. faecalis and E. faecium on different biomaterials have been demonstrated, and the capacity of enterococci to bind to various medical devices, such as ureteral stents (Keane et al., 1994), intravascular catheters (Sandoe et al., 2003), biliary stents (Dowidar et al., 1991) and silicone gastrostomy devices (Dautle et al., 2003), has been associated with the ability of enterococci to produce biofilms. Biofilm formation by E. faecalis on ocular lens materials, such as polymethacrylate, silicone and acrylic, has been documented (Kobayakawa et al., 2005). In this review, we discuss recent advances in the biology and genetics of biofilm formation by E. faecalis and E. faecium, and the role of the biofilm in enterococci pathogenesis.
The epidemiology of biofilm formation by *E. faecalis* and *E. faecium*

The prevalence of biofilm production varies worldwide. In Rome, Italy, 80% of *E. faecalis* and 48% of *E. faecium* isolates from infected patients were able to form biofilms (Baldassarri et al., 2001). In Pamplona, Spain, 57% of *E. faecalis* isolates derived from various clinical isolates produced biofilms (Toledo-Arana et al., 2001). In Sardinia, Italy, biofilm production was identified among 87% of *E. faecalis* clinical isolates and 16% of *E. faecium* clinical isolates (Dupre et al., 2003). In the UK, among 109 enterococcal bloodstream isolates studied, 100% of *E. faecalis* and 42% of *E. faecium* isolates produced biofilms. *E. faecalis* isolates from intravascular catheter-related bloodstream infections (CRBI) have been found to produce more biofilm than enterococcal isolates that cause non-CRBI (Sandoe et al., 2003). In the United States, Mohamed et al. (2004) reported that 93% of *E. faecalis* strains (51 isolates from outside the United States) identified from clinical and faecal isolates produced biofilms. In the same study, *E. faecalis* endocarditis isolates were found to produce more biofilm than non-endocarditis isolates (Mohamed et al., 2004). Biofilm-producing enterococcal isolates were characterized by the quantity of biofilm produced (i.e. strong, medium, weak or non-biofilm producer) with an optical density (OD₅70) classification (Mohamed et al., 2004; Toledo-Arana et al., 2001). In Okayama, Japan, Seno et al. (2005) reported that all of 352 *E. faecalis* isolates derived from urinary tract infections were capable of producing biofilms. In Poland, 59% of *E. faecalis* isolates collected from clinical specimens produced biofilms (Dworniczek et al., 2005). A study from a tertiary care hospital in India showed that 44 of the 171 isolates (26%) of *E. faecalis* and none of the 25 *E. faecium* isolates produced biofilms (Prakash, 2005). In Rome, Italy, among a collection of 52 *E. faecalis* isolates from orthopaedic infections 96% produced biofilms (Baldassarri et al., 2006). Other investigators have reported similar results and suggest that *E. faecalis* (95%) isolates produce a biofilm more often than *E. faecium* (29%) (Di Rosa et al., 2006). Collectively, these data suggest that *E. faecalis* produces biofilm more often than *E. faecium*, and that biofilm formation may be an important factor in the pathogenesis of enterococcal infection.

Factors influencing biofilm production

Nutrient contents of the growth medium, such as glucose, serum, availability of iron and CO₂, osmolarity, pH, and temperature, influence biofilm production among different bacteria. Carbohydrate metabolism regulates biofilm production among various Gram-positive bacteria, including *E. faecalis* (Pillai et al., 2004). One study has shown that tryptic soy broth (TSB) medium with 1% glucose supplementation enhances biofilm production in *E. faecalis* compared to TSB without glucose (Baldassarri et al., 2001). Another study found a reduction in biofilm production by *E. faecalis* as the glucose concentration increased from 0 to 0.2% in the culture medium (Kristich et al., 2004). The same study also observed greater biofilm production in media supplemented with 0.5% glucose compared to that with 0.2% glucose. Increased biofilm formation by *E. faecalis* OG1RF was also observed in TSB medium with 1% glucose compared to TSB alone (Pillai et al., 2004). Glucose-mediated intensification of biofilm also occurs in *E. faecalis* OG1RF, but not in the fsr mutant or the gelE mutant (Pillai et al., 2004). It has been suggested that a glucose-dependent transcriptional regulator may directly or indirectly control fsr, and that fsr mediates catabolite control of biofilm production through the downstream protease(s), gelatinase and serine protease (Pillai et al., 2004).

The involvement of enterococcal surface protein in biofilm formation in the presence of a higher glucose concentration has been reported (Tendolkar et al., 2004). Two *E. faecalis* esp-positive strains FA2-2 (pEESP) and OG1RF (pESP) produce significantly more biovolume and thickness of biofilm than their controls, esp-negative FA2-2 (pAT28) and OG1RF (pAT28), respectively. In the same study, the presence of ≥0.5% glucose in the growth medium influenced the biofilm production by *E. faecalis* strain E99 (Tendolkar et al., 2006). A putative sugar-binding transcriptional regulator, *bopD* (*bopABCD* operon), that shows sequence homology with various proteins responsible for the regulation of maltose metabolism, was found to be essential for biofilm production (Hufnagel et al., 2004). The transposon insertion mutant *bopB* reduced the biofilm while the non-polar deletion mutant produced more biofilm than wild-type when grown in medium containing 1% glucose. However, the transposon mutant was able to produce more biofilm than wild-type, while the deletion mutant did not produce biofilm, when grown in medium containing 1% maltose (Creti et al., 2006).

Changes in the osmotic strength also affect biofilm formation in *E. faecalis*. A study showed that biofilm production was abolished by exposure to a medium to high osmolarity (2–3% sodium chloride) without affecting the growth of the bacteria, suggesting that *E. faecalis* monitors the environment and modulates biofilm formation in response to specific conditions (Kristich et al., 2004). Biofilm production by different strains of *E. faecalis* has been evaluated in various media. Biofilm accumulation by *E. faecalis* OG1RF in TSB, M17 and M9YE media slows and plateaus after 6 to 8 h of growth. In contrast, biofilm production in Todd–Hewitt yeast extract and brain heart infusion media abruptly stops after 4 h of growth and after that the density of emerging biofilm decreases (Kristich et al., 2004). These results suggest that certain environmental conditions promote long-term biofilm formation and maintenance, while other conditions only support short-term biofilm maintenance.

The effect of human serum on *E. faecalis* adhesion has been examined (Gallardo-Moreno et al., 2002). The
supplementation of 10% human serum to the culture medium increased the adhesion of *E. faecalis* ATCC 29212 to glass and silicone surfaces. Serum-induced biofilm production in an *E. faecalis* salB mutant has also been examined (Mohamed et al., 2006). Although the salB mutant showed decreased biofilm production in TSB medium +0.25% glucose (TSBG), enhanced biofilm formation was noticed in a salB mutant, but not by the wild-type *E. faecalis* OG1RF, when grown in TSBG +10% serum and TSBG +50 μg fibronectin ml−1. The same mutant failed to form biofilms in TSBG +50 μg collagen type I ml−1 (Mohamed et al., 2006). Biofilm-producing *E. faecalis* isolates survive better in macrophages than non-biofilm producers (Baldassarri et al., 2004). Such isolates expressing extracellular polysaccharide were found to survive within rat peritoneal macrophages (>24 h) for a longer period of time than polysaccharide-negative strains (Baldassarri et al., 2004). Collectively, these observations suggest that environmental signals regulate biofilm formation. It is also of interest to establish how these environmental signals regulate biofilm formation essentially from initiation to mature biofilm.

**Role of Esp in biofilm formation**

*E. faecalis* Esp has been implicated as a contributing factor in colonization and persistence of infection within the urinary tract (Shankar et al., 1999, 2001). An esp homologue has been identified in *E. faecium* (Eaton & Gasson, 2002). Conflicting outcomes have been published regarding the role of the esp gene product in biofilm formation. Toledo-Arana and colleagues reported that 93.5% of *E. faecalis* esp-positive isolates form biofilms on an abiotic surface and none of the esp-negative *E. faecalis* isolates produced biofilms (Toledo-Arana et al., 2001). In that study, the investigators also found that the insertional inactivation of esp in two mutants of *E. faecalis*, but not in a third, resulted in impaired biofilm production. They suggested that Esp promotes biofilm formation; however, additional determinants may contribute to biofilm formation in *E. faecalis* (Toledo-Arana et al., 2001).

The role of Esp in biofilm formation has been studied by another genetic approach. Two esp-lacking *E. faecalis* strains, FA2-2 and OG1RF, produced increased amounts of biofilm after successful introduction and expression of the esp gene (Tendolkar et al., 2004). In a parallel study, the same investigators tested the expression of in-frame deletion mutant forms of Esp lacking specific domains versus wild-type Esp in an isogenic background. The investigators identified that a mutant lacking the N-terminal domain region of Esp produced less biofilm than wild-type, suggesting that the N-terminal domain of Esp is sufficient for biofilm enhancement by *E. faecalis* (Tendolkar et al., 2005). In addition, the expression of Esp in two different heterologous hosts, *E. faecium* and *Lactococcus lactis*, had no effect on biofilm production, suggesting that their own factors act synergistically with this surface protein to enhance biofilm development (Tendolkar et al., 2005). Levels of Esp expression on the surface of *E. faecium* are quantitatively correlated with primary adherence and biofilm formation under different growth conditions, and its expression varies considerably among esp-positive isolates (Van Wamel et al., 2007).

A genetically defined *E. faecalis* OG1RF produces robust biofilms, not only in the absence of esp, but also in the absence of the entire pathogenicity island that harbours the esp coding sequence (Kristich et al., 2004). In a study of clinical enterococci, all 74 esp-positive isolates produced biofilms, and 77 of 89 esp-negative isolates also produced biofilms (Mohamed et al., 2004). Among the enterococci isolates producing biofilms, 69% were strong, 46% medium and 30% were weak producers of biofilm, and none of 12 non-biofilm producers were esp positive. The authors concluded that esp is not required for biofilm production, but a strong association between the presence of an *esp* gene and greater levels of biofilm production in *E. faecalis* existed with esp-positive isolates (Mohamed et al., 2004).

Other studies suggest that the *esp* gene does not appear to be necessary nor sufficient for the production of biofilm in *E. faecalis* and *E. faecium* (Dworniczek et al., 2005; Ramadhan & Hegedus, 2005). The presence of the *esp* gene in 15 *E. faecalis* isolates and 32 *E. faecium* clinical isolates was not associated with the ability to produce biofilms (Dupre et al., 2003). No association between the presence of *esp* and biofilm-forming ability was found among 108 enterococcal isolates from bloodstream infections (Sandoe et al., 2003). A report of esp-positive vancomycin-resistant *E. faecium* isolates not associated with heavy biofilm production was recently published (Raad et al., 2005).

The initial adhesion and production of biofilm are independent of the existence of esp. An esp-negative isolate was found to produce biofilm, and two esp-positive isolates did not form biofilm (van Merode et al., 2006). Di Rosa et al. (2006) have also shown that *E. faecalis* (36 out of 83) and *E. faecium* (9 out of 43) esp-positive isolates were not associated with biofilm formation. However, the same authors reported that some esp-positive strains produced thicker biofilms than esp-negative biofilm producers (Di Rosa et al., 2006). The exact factors, including Esp, and mechanisms involved in biofilm production by enterococci are still unknown and are an area of active investigation.

**Gelatinase in biofilm formation**

The gelatinase (GelE) of *E. faecalis* is an extracellular zinc metalloprotease that can hydrolyse gelatin, collagen and casein. Gelatinase influences full virulence in a mouse model of peritonitis, endocarditis (Singh et al., 1998, 2005) and endophthalmitis (Engelbert et al., 2004), in a nematode (Sifri et al., 2002) and in *in vitro* translocation (Zeng et al., 2005). Gelatinase and serine protease (SprE)
are encoded in an operon, gelE–sprE, whose expression is positively regulated by a quorum sensing system encoded by the fsr locus (Qin et al., 2001).

Two gelE mutants of E. faecalis OG1RF, TX5128, a gelE insertion mutant (GelE+, SprE-) (Singh et al., 1998) and TX5264 (AgeIE), a non-polar deletion mutant (GelE-, SprE+) (Qin et al.), displayed a 46 and 37% decrease in biofilm production, respectively, relative to wild-type OG1RF (Mohamed et al., 2003, 2004). The relative importance of gelE downstream on a co-transcribed gene, sprE, on biofilm formation has also been examined. The sprE (GelE+, SprE+) insertion mutant formed similar amounts of biofilm to the wild-type OG1RF, while the gelE insertion (GelE-, SprE-) and deletion (GelE-, SprE+) mutants decreased biofilm. These results indicate that gelatinase rather than serine protease is important for biofilm formation (Mohamed et al., 2004). A subsequent study found no difference in biofilm production between gelatinase-positive and gelatinase-negative E. faecalis isolates derived from clinical and faecal sources, suggesting that there was no correlation of gelatinase production and biofilm formation (Mohamed & Murray, 2005). In a subgroup analysis of esp-lacking isolates, the median biofilm optical density of gelatinase-positive isolates was higher, although not significantly so, than that of gelatinase-negative isolates, suggesting that gelatinase may contribute to biofilms in an esp-lacking background (Mohamed & Murray, 2005). No significant difference was found between gelatinase-producing isolates and gelatinase non-producing isolates for biofilm formation among a larger collection of E. faecalis isolates (Seno et al., 2005).

Biofilm-promoting activity has been found in gelatinase-producing OG1RF-conditioned media with gelatinase-deficient E. faecalis JH2, a poor biofilm producer. An isogenic mutant of E. faecalis OG1RF, TX5264 (AgeIE mutant), and another mutant, TX5243 (sprE mutant), were tested for biofilm-promoting activity. Conditioned media from the sprE mutant and wild-type OG1RF possessed essentially 100% of biofilm-promoting activity, but conditioned media from gelE mutant lacked biofilm-promoting activity (Kristich et al., 2004). These results indicate that gelatinase is responsible for the biofilm-promoting activity found in the OG1RF-conditioned media. To test whether gelatinase enhanced biofilm formation by E. faecalis, the authors cloned gelE into a plasmid, pMSP3614 (JH2/pMSP3614) under the control of a nisin-inducible promoter to express gelE in E. faecalis JH2 (esp, gelE+, but GelE-). A partial restoration of biofilm production by this strain was observed, suggesting that gelatinase enhances biofilm formation (Kristich et al., 2004). Inactivation of the fsr-controlled gelE gene (JM104/gelE) of a different strain, E. faecalis V583 (a clinical isolate), was found to impair biofilm formation (Hancock & Perego, 2004). Two inactive forms of gelatinase expressed in E. coli did not promote biofilms. However, the purified active gelatinase from E. faecalis culture supernatant induced biofilm production by strain FA2-2. This study suggests that the enzymic activity of gelatinase is required for its role in biofilm production (Hancock & Perego, 2004). A study supporting the role of gelatinase in biofilm production, with complementation experiments introducing a plasmid pTEX5249 (a 6 kb fragment containing fsrA, fsrB, fsrC and the first 395 bp of gelE cloned into pAT18) into E. faecalis JH2-2, found a 53% increase in biofilm production compared with the respective controls (Mohamed & Murray, 2006). These results corroborate the findings with E. faecalis FA2-2 (Hancock & Perego, 2004).

Two recent studies attempted to look for the association of gelatinase and biofilm production in enterococcal isolates collected in Italy (Baldassarri et al., 2006; Di Rosa et al., 2006). No such correlation was found among E. faecalis isolates from orthopaedic infections (Baldassarri et al., 2006). In another study, gelatinase was not required for biofilm production among 83 E. faecalis and 45 E. faecium isolates examined (Di Rosa et al., 2006). Although genetic manipulation studies have confirmed that gelatinase is essential for biofilm formation, epidemiological studies have not supported the link between gelatinase and biofilm production among the E. faecalis clinical isolates tested.

Role of fsr locus in biofilm formation

The fsr locus (E. faecalis regulator) in E. faecalis, which contains the fsrA, fsrB and fsrC genes, and is a homologue of staphylococcal agrBCA loci, has been characterized (Qin et al., 2000). fsrB contains the signalling peptide liberating the gelatinase biosynthesis activating pheromone (GBAP) peptide probably by auto-processing (Nakayama et al., 2001) and a quorum sensing system. When GBAP accumulates at the transition from exponential to stationary phase, the gelE and sprE genes are induced (Nakayama et al., 2001); these genes are located immediately downstream from the fsr regulon, and encode a gelatinase and serine protease, respectively.

Carniol & Gilmore (2004) in a thoughtful commentary discussed the role of signal transduction, quorum sensing and extracellular protease activity in biofilm formation by E. faecalis. Murray’s group found that all three fsr mutants (fsrA, fsrB, fsrC) showed a reduction in biofilm formation ranging from ~28 to 32% compared to E. faecalis OG1RF (Mohamed et al., 2003, 2004). These results were confirmed by another study showing the involvement of fsr in biofilm formation in the same strain (Pillai et al., 2004). Hancock & Perego (2004) showed that E. faecalis V853 fsr quorum sensing system controls biofilm development through the production of gelatinase (Hancock & Perego, 2004). The fsrA, fsrB, fsrC and gelE insertion mutants, obtained by single cross-over recombination, were significantly impaired in their ability to produce biofilms. The complementation of these mutants restored biofilm formation (Hancock & Perego, 2004). In the case of agr system, the agr mutants of Staphylococcus aureus
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(Vuong et al., 2000) and Staphylococcus epidermidis (Vuong et al., 2004) have been shown to enhance biofilm production compared to the isogenic wild-type. Additional roles of fsr in the biofilm formation were recently reported (Mohamed & Murray, 2006). The effect of fsr on biofilm production by E. faecalis, independent of activation of its gelatinase production, was tested by a microtitre plate biofilm assay, primary adherence and phase-contrast microscopy (Mohamed & Murray, 2006). After introduction of an fsr locus containing plasmid, pTEX5249, into a strong biofilm producer, TX0014 (fsr, gelE, esp), pTEX5249 (TX5454) showed a 41% reduction in biofilm production compared with wild-type and a plasmid only control. The same trend has been noted with a medium biofilm producer, TX0006 (fsr, gelE, esp). These results suggest that fsr has an effect independent of gelatinase on biofilm formation in E. faecalis, and that this effect is in the same direction as that of agr of staphylococci. E. faecalis also contains a luxS homologue of unknown significance (Schauder et al., 2001), and the role of this system in virulence and in biofilm formation has not been investigated thus far.

Contribution of other genes in biofilm formation

Several groups have attempted to identify additional factors that may influence the process of biofilm formation in E. faecalis (Table 1). The first report of involvement of multiple genes, such as fsr, gelE, epa, atn, in biofilm formation by E. faecalis was published in 2004 (Mohamed et al., 2004). Polysaccharides have been implicated in biofilm formation. These molecules are associated with the cell surface as a capsular polysaccharide or secreted as an exopolysaccharide into the environment. An epa (enterococcal polysaccharide antigen) gene cluster mutant of E. faecalis, orfde4 (TX5179) (Xu et al., 2000), showed a 73% reduction in biofilm formation, suggesting that this gene encodes a putative glycosyltransferase that is involved in polysaccharide synthesis and biofilm production (Mohamed et al., 2004). An E. faecalis autolysin (atn) mutant showed a 30% reduction in biofilm formation (Mohamed et al., 2004). The two-component regulatory system mutant, etaR, showed a small reduction (Mohamed et al., 2004).

The abnormal shape and cell surface of the salB mutant of E. faecalis has been demonstrated by an electron microscopy study (Breton et al., 2002). The disruption of the salB (secretory antigen-like) gene in E. faecalis OG1RF grown in TSBG resulted in a 54% reduction in biofilm production. The salA mutant exhibited a small reduction in biofilm compared to wild-type. Biofilm formation was restored to the salB mutant after complementation (Mohamed et al., 2006).

Inactivation of dltA (d-alanine lipoteichoic acid) of the dlt operon, encoding a D-alanine-D-alanyl carrier protein ligase, leads to a lack of D-alanine esters on teichoic acid that causes a stronger negative net charge on the bacterial surface. The dltA mutant produced significantly less biofilm compared to wild-type E. faecalis 12030 (Fabretti et al., 2006). However, biofilm produced by a dltA mutant isolate of a different strain of E. faecalis (i.e. OG1RF) was equal to that of wild-type (Mohamed et al., 2004).

The sugar-binding transcriptional regulator, bopD, is a member of the bop (biofilm on plastic) operon. bopD is involved in biofilm formation by E. faecalis (Hufnagel et al., 2004). A recent study identified phenotypes linked to the strong biofilm formation of E. faecalis E99 by transposon

**Table 1. Genetic determinants involved in E. faecalis biofilm formation**

<table>
<thead>
<tr>
<th>Gene/locus</th>
<th>Protein/function</th>
<th>Reference</th>
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<tbody>
<tr>
<td>atn</td>
<td>Autolysin</td>
<td>Mohamed et al. (2004)</td>
</tr>
<tr>
<td>bee</td>
<td>Biofilm enhancer in Enterococcus/a putative cell wall-anchored protein</td>
<td>Tendolkar et al. (2006)</td>
</tr>
<tr>
<td>bop</td>
<td>Biofilm on plastic surface/a putative sugar-binding transcriptional regulator</td>
<td>Hufnagel et al. (2004)</td>
</tr>
<tr>
<td>ebpA, ebpB, ebpC</td>
<td>Endocarditis and biofilm-associated pilis</td>
<td>Nallapareddy et al. (2006)</td>
</tr>
<tr>
<td>ebpR</td>
<td>Transcriptional regulator of ebpABC</td>
<td>Bourgogne et al. (2007)</td>
</tr>
<tr>
<td>epa (orfde4)</td>
<td>Enterococcal polysaccharide antigen/a putative glycosyltransferase involved in polysaccharide synthesis</td>
<td>Mohamed et al. (2004)</td>
</tr>
<tr>
<td>esp</td>
<td>Enterococcal surface protein</td>
<td>Toledo-Arana et al. (2001); Tendolkar et al. (2004, 2006)</td>
</tr>
<tr>
<td>etaR</td>
<td>Enterococcal two-component system regulator</td>
<td>Mohamed et al. (2004)</td>
</tr>
<tr>
<td>fsrA, fsrB, fsrC</td>
<td>E. faecalis regulator/two-component quorum-sensing signal transduction system, regulates the expression of gelatinase and serine protease</td>
<td>Mohamed et al. (2004, 2006); Pillai et al. (2004); Hancock &amp; Perego (2004)</td>
</tr>
<tr>
<td>gelE</td>
<td>Secretory metalloprotease gelatinase E</td>
<td>Mohamed et al. (2004); Kristich et al. (2004); Hancock &amp; Perego (2004)</td>
</tr>
<tr>
<td>salA</td>
<td>Secretory antigen-like A</td>
<td>Mohamed et al. (2006)</td>
</tr>
<tr>
<td>salB</td>
<td>Secretory antigen-like B/cell-shape determinant</td>
<td>Mohamed et al. (2006)</td>
</tr>
<tr>
<td>srtC</td>
<td>Sortase C/enzyme that anchors surface proteins to the cell wall</td>
<td>Nallapareddy et al. (2006)</td>
</tr>
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</table>
mutagenesis. The gene cluster involved was named bee (biofilm enhancer in enterococci) (Tendolkar et al., 2006). The ebp operon (encoding endocarditis and biofilm-associated pili) and its downstream gene, sortase (srtC), are essential for biofilm formation by E. faecalis OG1RF. A series of mutants of ebpA, ebpB, ebpC and srtC have been generated in E. faecalis. These mutants are defective in primary adherence and biofilm formation (Nallapareddy et al., 2006). EbpR a transcriptional regulator of ebpABC, was found to reduce biofilm formation (Bourgogne et al., 2007).

Conclusions

Enterococci are recognized as a major cause of nosocomial infections and form biofilms that are dependent on multiple genetic factors. A number of environmental factors and signals also influence biofilm formation. Research into signal transduction proteins, and how they regulate biofilm formation and at what stage, is needed. Certain genetic determinants are required for biofilm formation in vitro and research into the relevance of these findings in vivo, using appropriate animal models that mimic the complex interaction between biofilm and host, is necessary. The number of genetic factors known to be involved in biofilm production has increased in recent years, due to the availability of genomic and proteomic approaches, but it is clear that much more research is needed to allow a better understanding of the regulation of biofilm production. A complete understanding of the role of genetic and environmental factors in the development of biofilm may lead to improved strategies for biofilm control among enterococci.

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