Journal of Medical Microbiology (2007), **56**, 1581–1588 **DOI** 10.1099/jmm.0.47331-0

Review

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 polymethymethacrylate, 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 (OD_{570}) 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 CO_2, 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 (pESPF) and OG1RF (pESPF) 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 $\geqslant 0.5$ % 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 45) *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 (Δ*gelE*), 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 s*prE* (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 showed 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 (Δ*gelE* 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 nicin-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* V583 *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*

(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

Gene/locus	Protein/function	Reference
atn	Autolysin	Mohamed *et al.* (2004)
bee	Biofilm enhancer in *Enterococcus*/a putative cell wall-anchored protein	Tendolkar *et al.* (2006)
bop	Biofilm on plastic surface/a putative sugar-binding transcriptional regulator	Hufnagel *et al.* (2004)
dltA	D-alanine lipoteichoic acid/D-alanine-D-alanyl carrier protein ligase	Fabretti *et al.* (2006)
ebpA, ebpB, ebpC	Endocarditis and biofilm-associated pili	Nallapareddy *et al.* (2006)
ebpR	Transcriptional regulator of *ebpABC*	Bourgogne *et al.* (2007)
epa (*orfde4*)	Enterococcal polysaccharide antigen/a putative glycosyltransferase involved in polysaccharide synthesis	Mohamed *et al.* (2004)
esp	Enterococcal surface protein	Toledo-Arana *et al.* (2001); Tendolkar *et al.* (2004, 2006)
etaR	Enterococcal two-component system regulator	Mohamed *et al.* (2004)
fsrA, fsrB, fsrC	*E. faecalis* regulator/two-component quorum-sensing signal transduction system, regulates the expression of gelatinase and serine protease	Mohamed *et al.* (2004, 2006); Pillai *et al.* (2004); Hancock & Perego (2004)
gelE	Secretory metalloprotease gelatinase E	Mohamed *et al.* (2004); Kristich *et al.* (2004); Hancock & Perego (2004)
salA	Secretory antigen-like A	Mohamed *et al.* (2006)
salB	Secretory antigen-like B/cell-shape determinant	Mohamed *et al.* (2006)
srtC	Sortase C/an enzyme that anchors surface proteins to the cell wall	Nallapareddy *et al.* (2006)

mutagenesis. The gene cluster involved was named *bee* (biofilm enhancer in enterococcus) (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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