Chapter

Mechanism Involved in Biofilm Formation of *Enterococcus faecalis*

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Abstract

Enterococci are commensal bacteria in the gastrointestinal flora of animals and humans. These are an important global cause of nosocomial infections. A Biofilm formation constitutes an alternative lifestyle in which microorganisms adopt a multi-cellular behavior that facilitates and prolongs survival in diverse environmental niches. The species of enterococcus forms the biofilm on biotic and abiotic surfaces both in the environment and in the healthcare settings. The ability to form biofilms is among the prominent virulence properties of enterococcus. The present chapter highlights the mechanisms underlying in the biofilm formation by enterococcus species, which influences in causing development of the diseases.

Keywords: biofilm, *Enterococcus faecalis*, pathogenesis, microcolony, quorum sensing

1. Introduction

Gram Positive bacterium has been renowned as a pathogen of hospitals acquired infectious. One among these bacteria is *Enterococcu*s species. *Enterococcus* species are ubiquitous, commensally inhabitants of the gastrointestinal tract of humans and animals. These can be frequently isolated from the environmental sources such as soil, surface water, raw plant and animal products. Even these can screen from female genital tract, oropharynx and skin. *Enterococcus sps* belongs to the gram positive, facultative anaerobic cocci with an optimum growth temperature of 35°C [1]. There are around 36 species of enterococci have been reported; conversely 26 species are associated with human infection. The most predominant human pathogen is *Enterococcus faecalis*, even *Enterococcus faecium* is one of the important pathogen which is prevalent increasing as hospital acquired infections. The other remaining enterococci species only accounts 5% of infections [2–4]. Some few examples of enterococcus species which are associated with human infections, *E. avium, E. cecorum, E. cassseliflavus, E. durans, E. gallinarum, E. raffinosus* [5, 6].

E. faecalis has now become the most common nosocomial pathogen and its virulence is increasing in clinical isolates. The presence and function of different suggested characteristics related virulence have been reported [7, 8]. The factor which influences the virulence is mediated through gelatinase production, enterococcus surface protein (ESP), aggregation substance (AS), and biofilm formation [9]. It

cause the following infections such as pelvic and abdominal infections, infections in the mouth especially after root canal surgery, infections in open wounds, a lesser known form of meningitis called enterococcal meningitis, infections in the blood called bacteremia and urinary tract infections.

Biofilms are surface attached, organized microbial communities made up of sessile cells (bacteria and /or fungi) embedded in an extracellular matrix composed of polysaccharides, DNA and other components.

2. Chronological background on biofilm

Generally bacterial cell grow in two modes; biofilm formation through aggregate and planktonic cell. It associated with microorganism in which cells stick to each other on a surface encased within matrix of extracellular polymeric substance produced by bacteria itself [10]. Antoni van Leeuwenhoek, the Dutch research, who discovered the simple microscope and observed 'animalcule' on surfaces of tooth and this event is known as discovery of biofilm. Characklis, in the year 1973 phrase that biofilms are not only tenacious but even resist to disinfectants (e.g. chlorine). In 1978, Costerton, defined the term biofilm and explained the importance of biofilm. Biofilms can be found in nature in all places like waste water, labs, and hospital settings. It forms as floating mat on the surface of liquid on both living and non-living surfaces [11].

3. Components of biofilm

Biofilm are produced from different group of organisms, the microbes cells produces the extracellular polymeric substances (EPS) such as DNA <1%, Polysaccharides 1–2%, proteins(includes enzymes) with <1–2%, RNA <1% and water with 97% are the major part of biofilm which is responsible for the flow of nutrients inside biofilm matrix [12]. The main two components of the biofilm that is water channel for nutrients transport and a region of densely packed cells having no prominent pores in it [12]. Another way microbial cells in which biofilms are arranged with significant different physiology and physical properties. They will access of antibiotics and human immune system. The organism that produces biofilm has capability to bear and neutralize antimicrobial agents and result in prolonged treatment. The bacteria which produces the biofilm, switch on the genes that can activate the expression of stress genes which in turn switch to resistant phenotypes due to certain changes examples are as follows cell density, nutritional, temperature, pH and osmolarity. When the biofilm water channels are compared with system of circulations showed that biofilms are considered primitive multi-cellular organism [13, 14]. The compositions of biofilms like DNA, proteins, polysaccharides and water will signify the biofilm integrity and making it resistant against different environmental factors [15].

4. Epidemiology of biofilm formation by *Enterococcus faecalis*

In the worldwide, the prevalence of production of biofilm varies to different part. The study reported in Rome, Italy, 80% of *E. faecalis* isolates have ability to form biofilms in the infected patients [16]. In India, a study has showed that 52% of *E. faecalis* isolated screened from clinical samples has showed the biofilm formation [17]. In China, Shenzhen Nanshan Hospital, the prevalence of *E. faecalis* biofilm formation

has showed 50.4% (57/113) in urinary tract infection isolates [18]. The biofilm formation in case of food isolates were less with 60% non-biofilm producers. The major ability in formation of biofilm was endodontic isolates with 73.7% was observed in the Department of Operative Dentistry and Periodontology, University of Freiburg Medical Center, Germany [19].

A study carried out Ahvaz teaching hospital, Iran demonstrated that high frequency 63% of biofilm formation in clinical isolates [20]. The *E. faecalis* bacterial isolated from patient with complicated UTI from department of Urology, Okayama University, Japan has showed the biofilm formation 64 (18.2%) and 156 (44.3%) exhibited strong and medium respectively [21]. A study reported at Malaysia, the *E. faecalis* isolates has showed the biofilm formation of 49% [22]. In the United Kingdom, 100% *E. faecalis* isolates produced biofilms, these isolates were from intravascular catheter-related bloodstream infections (CRBI) found to produce more biofilm than enterococcal isolates that cause non-CRBI [23]. A 93% of *E. faecalis* strains isolated from clinical samples especially fecal isolates have showed more biofilm formation in the United States [24]. In Spain, 57% of *E. faecalis* clinical isolates represent the biofilm production [25]. Tertiary care hospital in India showed 26% isolates of *E. faecalis* having capability in forming biofilm [26].

5. Pathogenesis of biofilm in causing disease

Generally infectious is connected with biofilm primarily confine to particular location and though time detachment may occur. Further, the detached biofilms may result in bloodstream or urinary tract infections or in the production of blockage of blood flow [26]. In another side cells in biofilms are mostly resistant to antimicrobial agents and the host immune system. *E. faecalis* isolates which produces biofilms is 1000 times more resistant to antibodies, antimicrobial agents and phagocytosis process than non-biofilm producers. Consequently, infections caused from *E. faecalis* associated with biofilm aggravated in this case [27, 28].

In endocarditis infection a complex biofilm formed by *E. faecalis* and host components will be formed on cardiac valve. These biofilms causes disease is through three basic mechanisms. Firstly, the biofilms physically disrupts valve function and may cause leakage. Second, detachment of biofilm can be carried to a terminal point in the circulation and formation of emboli (blockage of the blood vessel). Finally, the biofilm provides continuous infection of the bloodstream even during antibiotic treatment. These can cause recurrent fever, chronic systemic inflammation and lead to other infection also [27, 29].

6. Mechanism steps involved in *E. faecalis* biofilm formation

It comprises of four stages; initial attachment, microcolony formation, biofilm maturation (which is in part governed by quorum sensing) and dispersal.

7. Initial attachment

A surface adhesion is the first step in establishing a biofilm, and a number of surface adhesions, proteases, and lipids are involved. The endocarditis and

biofilm-associated pilus (Ebp), which is composed of subunits A, B, and C, mediates the adherence of biofilms on surface *in-vitro* and *in-vivo* [30–35]. The deletion of ebpABC attenuates binding to platelets, fibrinogen and collagen, reduces initial attachment, and thus impairs biofilm formation *in-vitro* [30, 32, 33].

In addition, Ebp contributed to early biofilm formation in *in-vivo* models of urinary tract infection (UTI), catheter associated UTI (CAUTI), and infectious endocarditis, in which bacteria with deletions of pilus components were substantially attenuated [30, 32, 33, 36]. Additionally, the absence of surface adhesions, such as aggregation substance (Agg), enterococcol surface protein (ESP), and adhesion to collagen from *E. faecalis* (Ace), reduced adhesion to cultured human cells and prevented biofilm formation *in-vivo* [37–41]. Bacteria deficient for Esp showed reduced initial attachment and decreased bladder colonization in a UTI ascending model, which is not unexpected since Esp binds fibrinogen and collagen, and these ligands are present in the bladder because Esp binds fibrinogen and collagen, and these ligands are present in the bladder [41, 42].

Ace is also involved in interacting with collagen, laminin, and dentin and deletion of Ace resulted in reduced colonization in rat endocarditis and UTI models [43–47]. As a result, Ace deletion in the peritonitis model did not reduce bacterial burden suggesting Ace-mediated biofilm formation is not relevant to peritoneal infection. By disparity, deletion of Agg reduced adherence to renal epithelial cells [38, 39], binding to lipoteichoic acid (LTA) of other *E. faecalis* cells (and therefore inter-bacterial clumping) and bacterial titers recovered from endocarditis vegetation on aortic heart valves. Agg cannot colonize the urinary tract, suggesting that Agg-mediated biofilms aren't necessary for ascending UTI's [48, 49].

In-vitro, biofilm associated glycolipid synthesis A (BgsA) contributes to initial adhesion and biofilm development, but its role *in-vivo* is unknown [50]. The extracellular secreted protein encoded by salB (Saga-Like Protein B) increased fibronectin and collagen binding but decreased biofilm formation paradoxically, which has hypothesized to be owing to the salB mutant cells decreased hydrophobicity. These investigations suggest that a variety of variables play a role in the initial attachment of bacteria, and that their contribution is likely to vary depending on the surface to which the bacteria adhere. As a result, focusing on a single component as anti-adherence or anti-biofilm strategy is unlikely to totally prevent enterococcal biofilm formation [37].

8. Microcolony formation

Bacteria proliferate and produce modest amounts of biofilm matrix to form aggregates known as microcolonies after first adhesion [51]. However, the enterococcal mechanisms that drive the establishment of microcolonies are unknown, and no transcriptome data from early-stage biofilms or microcolonies is available. The importance of microcolonies for gut colonization has been demonstrated. *E. faecalis* colonization of the stomach of germ free mice resulted in discrete microcolonies covered in a fibrous sweater-like matrix within a week, rather than the largely 2D biofilm sheets (2–3 cells high) that are normally observed in biofilm models *in-vitro* [52].

Despite the fact that microcolonies are commonly assumed to be a temporary stage of early biofilm production, these data imply that microcolonies may represent a mature biofilm stage in this niche that is particularly crucial for gut colonization. In addition, *in-vitro* enterococcal microcolonies emerge in response to antibiotic

therapy [53, 54]. Biofilms treated with sub-inhibitory levels of daptomycin began to restructure extensively into microcolonies as early as 8 hours after drug exposure, in contrast to typical biofilm sheets. Even in the absence of antibiotics, deletion mutants of eapOX, which encodes a glycosyl-transferase involved in the formation of cell wall associated rhamnopolysaccharide (Epa), developed microcolonies *in-vitro.* In contrast to the monolayer biofilms, these epaOX microcolonies had lower structural integrity, as shown by their facile separation following washing.

9. Biofilm growth and maturation

Active growth and synthesis of extracellular matrix components such as extracellular DNA (eDNA), polysaccharides, LTA, and extracellular proteases are required for biofilm development. eDNA is the best studied matrix component of enterococcal biofilms:eDNA can be found at the bacterial septum, as part of intercellular filamentous structures, and as part of the larger biofilm matrix, and its release from cells is controlled by autolysin Atla [55–57].

eDNA-associated cells showed no significant cell lysis and had a membrane potential [55], implying that eDNA is liberated from metabolically active cells. As a result, DNase treatment decreased biofilm stability and increased detachment [58, 59], whereas atlA deletion decreased eDNA release and biofilm formation [56]. Despite the lack of evidence that eDNA influences the spatial organization of enterococcal biofilms (as has been postulated for other bacterial species), eDNA remains a potential therapeutic target.

Biofilm production is also aided by non-proteinaceous cell surface components such as glycoproteins, polysaccharides, and modified lipids. The dltABCD operons are involved in the production of D-alanine esters of LTA, which are an important component of Gram-positive bacteria's cell wall, and deletion of this operons decreased biofilm formation *in-vitro*, decreased adherence to epithelial cells, and increased susceptibility to antimicrobial peptides [60]. Biofilm on plastic D (BopD), a potential sugar-binding transcriptional regulator, also promotes to biofilm development *in-vitro* [61].

The deletion of bopABC, which is located upstream of bopD, boosted biofilm growth in glucose but decreased biofilm growth and colonization levels in the murine gut, implying that the ability to utilize maltose is required for biofilm growth in the gut. MprF2, a paralogue of multiple peptide resistance factor (MprF), was likewise found to promote eDNA release and biofilm formation [61–63]. MprF2 reduces the net positive charge of the membrane via aminoacylating phosphatidylglyceroal to mediate electrostatic repulsion of cationic antimicrobial peptides.

While deletion of MprF2 had no effect on biofilm persistence in a mouse bacteremia model, deletion of both MprF1 and MprF2 reduced biofilm persistence in a wound infection model, suggesting that cell membrane charge may play a role in biofilm formation and pathogenicity *in-vivo* [63, 64]. These findings back up the theory that cell surface glycoproteins, membrane phosphatidylglycerol, and polysaccharides all play a role in biofilm development.

The quorum sensing response regulator FsrA regulates matrix remodeling by upregulating the expression of gelE, SprE, and altA [57, 58, 65–67]. The proteases gelE and sprE were found to diminish biofilm formation *in-vitro* and bacterial load in numerous *in-vivo* models [68–71]. However, in a rabbit endocarditis model, loss of gelE alone increased fibrinous matrix formation in aotic vegetation, leading to endocarditis as shown in the **Table 1** [70].

Name of the Gene	Gene code	Role
D-alanine- d-alanine ligase	*ddl*	It involved in metabolism process (d-ala) especially for bacterial peptidoglycan biosynthesis. Its role in cell wall integrity and biofilm formation.
Cytolysin	*cyl*	It a secreted toxin expressed in response to pheromones, contributes to the pathogenicity of *E. faecalis* by causing blood hemolysis.
Gelatinase	*gelE*	It hydrolyzes the gelatin and ability to damage host tissues plays a vital role in spreading of enterococci in their host. It promotes the aggregation of the cells in microcolonies which constitutes the initial step of biofilm formation.
Serine protease	*sprE*	It hydrolyzes the casein, quorum sensing and autolysis (release of eDNA)
Fecal streptococci regulator locus genes	*fsrA, fsrB, fsrC*	It the major quorum sensing in *E. faecalis*, the *fsr* regulator locus, is encoded by *fsrA*, *fsrB* and *fsrC* genes which regulate the expression of both gelatinase and serine protease. It controls biofilm development through regulating the production of gelatinase.
Biofilm associated pili	*ebp*	It is the protein organelles, anchored to the surface of the bacterium, that interact with the external environment. It role in biofilm formation, initial attachment and IE.
Adhesion to collagen of *E. faecalis*	*ace*	A surface protein that facilitates the bacterial adherence to collagen is the adhesion to collagen of *E. faecalis*. It play key role in adherence and colonization process.
Aggregation substance	*agg*	A surface protein expressed in response to pheromone induction that mediates the adherence of *E. faecalis* to renal epithelial cells. It plays important role in adherence to and colonization of host tissues.
Enterococcal fibronectin-binding protein A	*efbA*	It is an adhesin, localized on the outer surface of *E. faecalis* that confers adhesion to immobilized fibronectin.
Enterococcal surface protein	*esp*	It promotes primary attachment and biofilm formation.
LuxS/autoincuder –2 (AI-2) quorum sensing system	*luxS*	It plays role in interspecies communication and involved in bacterial virulence, persistence infections and biofilms

Table 1.
Different quorum sensing genes signaling molecules involved in Enterococcus quorum sensing system and virulence factors production.

In-vitro, sprE deletion increased autolysis and eDNA release and accelerated biofilm development, but gelE deletion inhibited eDNA releaseand elevated ace expression, which may increase surface attachment but make the biofilm detachable [71, 72].

10. Quorum sensing

Population density-dependent signaling influences biofilm formation [73, 74]. Despite the fact that quorum sensing and peptide pheromone signaling are known to coordinate gene expression and direct enterococcus biofilm growth, there have been few research on these tiny signaling molecules and secondary messengers in

enterococci. The cCF10 peptide pheromone, which facilitates the transfer of the conjugative plasmid pCF10, is an exception. This plasmid has the ability to transfer antibiotic resistance genes as well as virulence determinants like Agg across cells [75–79]. The buildup of cCF10, which stimulates conjugation proteins, is required for pCF10 transfer. The mechanism underpinning peptide pheromone-mediated gene regulation and plasmid transfer has been well documented, and it was recently demonstrated in mice to promote pCF10 transmission between *E. faecalis* cells in the gut [79, 80]. The immature peptide pheromones cAD1 and cCF10 are processed by the membrane protease Eep. Eep also facilities the proteolytic processing of RsiV, the anti-sigma factor for sigV, resulting in improved stress resistance. A sigV mutant showed similar symptoms, indicating that Eep is involved in the regulation of sigV production [81–83].

In-vitro, Eep, together with AhrC and the ArgR family transcriptional regulators, leads to biofilm formation, and deletion of the genes encoding either protein lowered bacterial burden in UTI and endocarditis models [84–86]. Furthermore, eep deletion mutants develop tiny aggregates unlike wild-type biofilms. FsrABC is another quorum-sensing system. FsrC is a membrane sensor kinase that detects density-dependent accumulation of the FsrB peptide and triggers a signal to the FsrA response regulator [87]. Because this system controls multiple biofilm-related genes and operons (such as bopABCD, ebpABC, GelE, and SprE), knocking down fsrABC entirely eliminates biofilm formation [88]. FsrD, a precursor for the cyclic peptide gelatinase biosynthesis activating pheromone (GBAP), is also controlled by the Fsr quorum sensing system as shown in the **Table 1** [89]. Finally, autoinducer 2 (Al-2) is involved in *E. faecalis* biofilm formation and is produced by S-ribosylhomocysteinelyase (LuxS). *In-vitro* biofilm development of *E. faecalis* is increased by Al-2 supplementation, while luxS deletion causes aberrant biofilm production with aggregation a dense structure, in contrast to the confluent monolayers of wild type *in-vitro* biofilms [90, 91].

11. Factors influencing for the formation of biofilms in *E. faecalis*

11.1 Dlt gene

A Lipoteichoic Acid, component of *E. faecalis*, the most common organism in root canals, develops colonies on the dentin surface (LTA). LTA is a biofilm-forming component of *E. faecalis* that functions as a receptor molecule on receptor cells during the aggregation process. *E. faecalis* antigen recognizes immune cells via pattern recognition receptors (PRRs) and induces the release of proinflammatory cytokines like TNF alpha (TNFα), interleukin 1 beta (IL-1β), IL-6, and IL-8 [92]. LTA causes cells to produce cytokines, which is followed by the activation of Nuclear Factors kβ (NF-kβ), which promotes cytokines release as shown in the **Table 2** [93].

The release of these cytokines causes the dlt gene in LTA to fabricate D-alanine instantly, causing other bacteria to assist in the formation of biofilms [94, 95]. The D-Ala-LTA gene is triggered by the surface protein of Gram-Positive bacteria. Cationic homeostasis and autolytic activity are controlled by this gene. Additionally, it is involved in the assimilation of metal cations as well as the electromechanical repair of bacterial cell walls [94]. These capabilities will enhance bacterial cell system transfer while even increasing autolytic activity. The host's defense system will be weakened by the modified tick.

Factors	Function
dlt gene	It as acts biofilm forming component during aggregation process. It causes cells to produce cytokines. It controls cationic homeostasis and autolytic activity
Cytolysin lytic enzymes	It is the virulence factors, play role in lysing erythrocytes and collagen fragmentation. The *cylLL* and *cylLS* genes on cytolysin promoted for longer survive of *E. faecalis.*
Hyaluronidase	It acts as toxin protein for the progression of host tissue increase damage and inflammation. It beneficial protein for the development of *E. faecalis.*
Dentine Matrix	It increases the enhancement of biofilm formation through dentin. It also resists the antimicrobial treatment by delay penetration of the drug through the biofilm matrix by altering/changing the physiological shaper of biofilm growth in dentin.
Nutrients	Glucose is the major determinate in the formation of *E. faecalis.* It utilizes as the carbon source and hydrolyzes the substrate for its survival.
Environmental	Physicochemical properties of the surface may exert a strong influence on the rate and extent of attachment. Temperature, cations, and presence of antimicrobial agents influence the attachment. The optimum temperature 37°C, pH -8.5 increase the production biofilm formation.

Table 2.
Factors influencing for the formation of biofilms in E. faecalis.

11.2 Cytolisin lytic enzymes

A lytic enzyme operated on by cytolysin is the one of *E. faecalis* bacteria's virulence factors. Apart from lysing erythrocytes, collagen fragmentation caused by this enzyme can cause tissue injury at the site of inflammation. The cylLL and cylLs genes on cytolysin promote this role, allowing *E. faecalis* to survive longer. *E. f*aecalis is the most common microbe found in root canals [92, 96]. Other bacteria will be inhibited by *E. faecalis* cytolysin. The cylLL and cylLS genes in *E. faecalis* cytolysin encode structural cytolysin subunits. They create cytolysin in anaerobic circumstances and respond to oxygen depletion in root canals by producing cytolysin as shown in the **Table 2**.

11.3 Hyaluronidase

Hyaluronidase is a protein to be found in *E. faecalis* that helps the bacteria and toxins progress to the host tissue. Other bacteria will continue to migrate from the root canal to the periapical lesions as a result of hyaluronidase. Furthermore, hyaluronidase stimulates the production of toxins by other bacteria, which increases damage and inflammation. This stipulation is very beneficial for the development of *E. faecalis* [97, 98].

11.4 Dentine matrix structurization

E. faecalis will increase resistance to antimicrobial treatments by increasing the biofilm structural characteristics at the primary site of *E. faecalis* invasion, notably dentin. As a result, *E. faecalis* is known to delay antimicrobial agent penetration through the biofilm matrix by altering the growth rate of other microbes in biofilm development and encouraging changes in the physiological shape of biofilm growth in dentin.

When *E. faecalis* is cultivated in nutrient-poor media, it forms thicker biofilms than when cultured in nutrient-rich media [99]. Under stress inducing mechanism in

other bacteria that can cause a more resilient *E. faecalis* biofilm. Besides *E. faecalis* biofilms profitably renew themselves. Furthermore, *E. faecalis* will receive vital carbon by hydrolyzing the substrate required for survival [23].

E. faecalis will continue to grow and develop in environments with or without oxygen with extreme alkaline pH by penetrating cell membrane ions and increasing the cytoplasmic's buffer capacity [100]. The pH balance of the biofilm is always maintained by bacteria by assimilation of protons into the cell, resulting in a lower internal cell pH. As a result, the dentin buffer capacity is unable to keep the pH in the dentinal tubule constant, and *E. faecalis* survives [101].

Other investigations found in *E. faecalis* that the ability to promote apatite re-deposition in the forming biofilm is responsible for its persistence after root canal therapy. Besides this, the dentin matrix is composed of chlorapatite $Ca_5(PO_4)_3$ [102]. Different varieties of apatite have different dissolving tolerances. Till date, chlorapatite has been considered as a weaker apatite than hydroxyapatite and fluorapatite in terms of nanostructure [102, 103]. Although it is known that calcium hydroxide can stimulate the formation of hard tissue by raising the Ca^{2+} ion to increase defense through dentin mineralization, the type of apatite that makes up the host dentin will influence the results [104, 105].

However, no further research into the drug resistance of this inorganic dentin material's nanostructures has been done. Furthermore, dentin deterioration is not solely dependent on inorganic elements. Collagen makes up 20% of the organic dentin, which accounts for 85% of the total [103]. Gelatinase, an *E. faecalis* virulence component, is required for hydrolyzing host collagen, High gelatinase levels have been linked to dentin organic matrix degradation [106, 107].

11.5 Tolerance for antimicrobial therapy

Antimicrobial therapy is known to be limited to eliminating free microbes but not to remove cells bound to the biofilm so that re-infection can occur [100]. As a root canal medication, calcium hydroxide is currently the most popular option among dentists. *E. faecalis* is known to be resistant to calcium hydroxide. This is a serious clinical problem. Every root canal treatment failure, which is documented widely, has linked to *E. faecalis* [101]. Calcium hydroxide is known to prevent the acid reaction that happens as a result of the inflammatory response. This lactic acid generated by osteoclasts to absorb hard tissue will be neutralized by the alkaline pH [102, 103].

12. Conclusion

Enterococcus faecalis is one of the most predominant organism in nosocomial infection and also developed the drug resistance. The intrinsic virulence factors *E. faecalis* are associated in biofilm formation and other environmental factor and signals are alarming the biofilm formation. A genome wide study is required to know the role of genetic and environmental factors in development of biofilm and mounting the superior strategies for biofilm control in *E. faecalis* isolates.

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