

Pathogenicity Biofilm formation of *Enterococcus faecalis*

Nur ASMAH

Department of Dentistry Conservative, Dentistry Faculty, Universitas Muslim Indonesia, Makasar, Sulawesi Selatan, Indonesia. Corresponding Author. E-mail: asmahnurg@gmail.com

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ABSTRACT Biofilm formation is closely related to the pathogenicity of *E. faecalis* in persistent root canal infections. Limited information is available about the ability and characteristics of *E. faecalis* biofilm-forming in the persistent pathogenicity of root canal infections. Based on these problems, the purpose of this paper is to improve the molecular understanding of *E. faecalis* on virulence factors associated with biofilm formation against persistent root canal infections to support laboratory diagnosis and therapy of oral *E. faecalis*. In conclusion, the release of cytokines triggers the *dlt* gene of LTA to improving: homeostasis, autolytic activity, and bacterial envelope properties. The role of cytolysin activated by the *cytLL* and *cytLS* genes improving the survival ability of *E. Faecalis*. Hyaluronidase will facilitate other bacteria to migrate from the root canal to periapical lesions resulting in the triggering of toxin production, which will increase damage and inflammation in the host. *Enterococcus faecalis*, through dentine matrix formation, hydrolyze collagen and induce apatite deposition in developing biofilms. Besides, these bacteria can also increase their tolerance to antimicrobials by blocking the inflammatory response's acid reaction. Alkaline conditions will neutralize the lactic acid secreted by osteoclasts to absorb hard tissue

KEYWORDS: biofilm, cytolysin, dentine, *Enterococcus faecalis*, Virulence factors

INTRODUCTION

Many reports state that *Enterococcus faecalis* is the dominant bacteria causing periradicular lesions.^{1,2} The pathogenicity of *E. faecalis* to persistent root canal infections is related to its virulence factors. Virulence factor is the degree of ability of an opportunistic pathogen to cause disease to the host.³ Virulence factors are extracellular products secreted by bacterial cells and are involved in the pathogenesis of root canal infections.

The virulence factor of *Enterococcus faecalis*, which plays an essential role in biofilm formation, is Lipoteichoic Acid (LTA). The LTA constituent *E. faecalis* acts as a receptor for aggregation and forming biofilms. The formation of the biofilm structure can provide adequate defense for microbes, both defense against the host and root canal drugs.³

The biofilms formation are adaptable to bad environments and metabolize even in conditions of nutrient deficiency actively. This condition is related

to the nature of *E. faecalis*, which can adapt and survive in root canals with minimal nutrition and access to avoid root canal medicaments.⁴ Biofilm matrix, changing the growth rate of biofilm microorganisms, and other physiological changes depend on biofilm's growth model.^{5,6}

Biofilm formation is an essential factor for *E. faecalis* in the pathogenicity of persistent root canal infections. The conditions under which biofilms can occur in infected root canals in vivo are not well understood.⁷

Information about the capabilities and characteristics of *E. faecalis* in biofilm formation in order to the persistent pathogenicity of root canal infections is still limited. Based on this problem, the aim of this paper is to improve the molecular understanding of *E. faecalis* related to biofilm formation against persistent root canal infections to support laboratory diagnosis and therapy of oral *E. faecalis*.

DISCUSSION

Morphology and Pathogenicity

The *Enterococcus faecalis* (*E. faecalis*) is a positive gram, facultatively anaerobic, and cocci.⁸ *Enterococcus faecalis* is commonly found in root canals and can survive at pH 4-11 and temperature 10-45°C. The ability to survive in such an environment allows these bacteria to stay for a long time in the root canals and invade the dentinal tubules.⁹⁻¹³ Prolonged root canal infection allows *E. faecalis* to enter the entire root canal system, including ramifications and dentinal tubules. 63% of root canal treatment failures were reinfected due to *Enterococcus faecalis*.^{14,15}

Biofilm and *E. faecalis* Virulence

The information currently available on the relationship between endodontics and microbial biofilms is obtained from observing bacteria's condensation in the root canal system.¹⁶ One of the factors that cause *Enterococcus faecalis* to survive in root canals is the formation of biofilms.¹ Four important factors influence the formation of biofilms for can increase the pathogenicity of *E. faecalis*, namely: *dlt* gene; cytolysin lytic enzymes, hyaluronidase; dentine Matrix Structurization, and increases tolerance to antimicrobials.

1. *dlt* gene

Enterococcus faecalis contaminates root canals and forms colonies on the dentin surface with Lipoteichoic Acid (LTA). Lipoteichoic Acid (LTA) is a constituent of *E. faecalis*, which acts as a receptor on receptor cells for aggregation, crucial in biofilm formation. *E. faecalis* antigen will be recognized by pattern recognition receptors (PRRs) from immune cells to induce the production of proinflammatory cytokines such as TNF α , interleukin 1 beta (IL-1 β), interleukin 6 (IL-6), and interleukin 8 (IL-8).⁶

Lipoteichoic Acid (LTA) stimulates cells to express cytokines followed by activation of Nuclear factor κ B (NF- κ B), promoting the rapid release of cytokines.¹⁷ The release of these cytokines will trigger the *dlt* gene in LTA to immediately produce D-alanine, which will trigger other bacteria to assist in forming biofilms.^{18,19} The surface protein of gram-positive bacteria triggers the coding for the D-Ala-LTA gene. This gene is responsible for cationic homeostasis and autolytic activity. Besides, it is involved in the process of assimilation of metal cations and the electromechanical repair of bacterial cell walls.¹⁸ These functions will increase the system transport in bacterial cells and, on the other hand, the autolytic activity. The modulated tick will weaken the host defense system.

2. Cytolysin lytic enzymes

One of the virulence factors of *E. faecalis* bacteria is a lytic enzyme acted on by cytolysin. Apart from lysing erythrocytes, this enzyme can cause tissue damage at the inflammation site due to collagen fragmentation. This role is activated by the *cytLL* and *cytLS* genes on cytolysin to increase the survival ability of *E. faecalis*. As it is known, *Enterococcus faecalis* is the dominant microorganism in root canals.^{6,20} *E. faecalis* cytolysin will inhibit the growth of other bacteria. The *cytLL* and *cytLS* genes in cytolysin *E. faecalis* encode structural subunits of cytolysin. They have produced cytolysin under anaerobic conditions also respond to oxygen changes depleting root canals.

3. Hyaluronidase

The *Enterococcus faecalis* have hyaluronidase that facilitates the bacteria and toxins spread to the host tissue. Hyaluronidase will continue to promote the migration of other bacteria from the root canal to the periapical lesions. Besides, hyaluronidase triggers other bacteria to produce toxins that increase damage and inflammation.^{22,23} This condition is very beneficial for the development of *E. faecalis*

4. Dentine Matrix Structurization

At the leading site of *E. faecalis* invasion, especially dentin, *Enterococcus faecalis* will increase resistance to antimicrobial agents by strengthening the biofilm structural properties. For this reason, *Enterococcus faecalis* is known to delay the penetration of antimicrobial agents through the biofilm matrix formed in two ways, namely changing the growth rate of other microorganisms in biofilm formation and by stimulating changes in the physiological shape of biofilm growth in dentin.

Enterococcus faecalis also reported to form thicker biofilms when cultured under nutrient-poor conditions than nutrient-rich media.²⁴ *E. faecalis* biofilms profitably regenerate themselves under stress-inducing systems in other bacteria that can induce a more resilient *E. faecalis* biofilm. Besides, *Enterococcus faecalis* will obtain essential carbon by hydrolyzing the substrate needed to maintain its existence.²⁵

Enterococcus 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.¹ The biofilm's pH balance is always maintained by bacteria through the assimilation of protons into the cell so that a lower internal cell pH

is obtained. As a result, the dentin buffer capacity is unable to maintain pH in the dentinal tubule, and *Enterococcus faecalis* remain alive.⁸

Other research has shown that the persistence of *E. faecalis* following root canal treatment can be attributed to its ability to induce apatite re-deposition in the developing biofilm. Besides, the dentin apatite that makes up the dentin matrix is chlorapatite $\text{Ca}_5(\text{PO}_4)_3$.²⁶ There are differences in dissolution tolerances of various types of apatite. Until now, from the nanostructure side, chlorapatite is a weaker apatite than hydroxyapatite and fluorapatite.^{26,27} Therefore, although it is known that calcium hydroxide can stimulate the formation of hard tissue by increasing the Ca^{2+} ion to promote defense through dentin mineralization^{28,29} will vary depending on the type of apatite that makes up the host dentin. However, no further studies have provided information on this drug resistance concerning this inorganic dentin material's nanostructures. Besides, dentin degradation does not depend on inorganic materials alone. As it is known, 20% of the 85% organic dentin is composed

of collagen.²⁷ *E. faecalis* virulence factor, namely gelatinase, is an essential factor in hydrolyzing host collagen. High gelatinase is known to contribute to the degradation of the dentin organic matrix.³⁰

5. Increased Tolerance to Antimicrobials

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.¹ Currently, the primary choice of practitioners is calcium hydroxide as a root canal drug. It is known, *Enterococcus faecalis* is resistant to calcium hydroxide. This is a severe clinical condition. As is well known, every root canal treatment failure, associated continuously with *Enterococcus faecalis*.⁸ Calcium hydroxide is known to block the acid reaction produced by the inflammatory response that occurs. The alkaline pH conditions will neutralize the lactic acid secreted by osteoclasts to absorb hard tissue.^{26,27}

CONCLUSION

Four important factors influence the biofilms formation to increase the pathogenicity of *E. faecalis*, namely: *dlt* gene; cytolysin lytic enzymes, hyaluronidase; structuring the dentin matrix, and increases tolerance to antimicrobials. Biofilm formation is closely related to the pathogenicity of *E. faecalis* in persistent root canal infections.

The release of cytokines triggers the *dlt* gene of LTA to improving: homeostasis, autolytic activity, and bacterial envelope properties. The role of cytolysin activated by the *cytLL* and *cytLS* genes improving the survival ability of *E. Faecalis*.

Hyaluronidase will facilitate other bacteria to migrate from the root canal to periapical lesions resulting in the triggering of toxin production, which will increase damage and inflammation in the host. *Enterococcus faecalis*, through dentine matrix formation, hydrolyze collagen and induce apatite deposition in developing biofilms. Besides, these bacteria can also increase their tolerance to antimicrobials by blocking the inflammatory response's acid reaction. Alkaline conditions will neutralize the lactic acid secreted by osteoclasts to absorb hard tissue

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