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Cinnamaldehyde inhibits *Enterococcus faecalis* biofilm formation and promotes clearance of its colonization by modulation of phagocytes in vitro

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Highlights

- Cinnamaldehyde inhibited biofilm formation *E.faecalis*.
- Expression of genes involved in biofilm formation are affected by Cinnamaldehyde.
- Cinnamaldehyde modulated macrophages in presence of *E.faecalis*.
- CA improved phagocytosis and clearance of *E.faecalis*.

Abstract

The nosocomial pathogen, Enterococcus faecalis plays a crucial role in the pathogenesis of variety of infections including endocarditis, urinary tract, and recurrent root canal infections. Primary virulence factors of *E. faecalis* such as biofilm formation, gelatinase production and suppression of host innate immune response can severely harm host tissue. Thus, novel treatments are needed to prevent *E. faecalis* biofilm development and pathogenicity due to the worrisome rise in enterococcal resistance to antibiotics. The primary phytochemical in cinnamon essential oils, cinnamaldehyde, has shown promising efficacy against a variety of infections. Here, we looked into how cinnamaldehyde affected the growth of biofilms, the activity of the enzyme gelatinase, and gene expression in *E. faecalis*. In addition, we looked at the influence of cinnamaldehyde on RAW264.7 macrophages' interaction with biofilm and planktonic *E. faecalis* in terms of intracellular bacterial clearance, NO generation, and macrophage migration in vitro. According to our research, cinnamaldehyde attenuated the biofilm formation potential of planktonic *E. faecalis* and gelatinase activity of the biofilm at non-lethal concentrations. The expression of the quorum sensing fsr locus and its downstream gene *gelE* in biofilms were also found to be significantly downregulated by cinnamaldehyde. Results also demonstrated that cinnamaldehyde treatment increased NO production, intracellular bacterial clearance, and migration of RAW264.7 macrophages in presence of both biofilm and planktonic *E. faecalis*. Overall these results suggest that cinnamaldehyde has the ability to inhibit *E. faecalis* biofilm formation and modulate host innate immune response for better clearance of bacterial colonization.

Introduction

Enterococcus faecalis inhabits the oral cavity and gastrointestinal flora as an opportunistic pathogen but also causing severe urinary tract infections, surgical wound infections, bacteremia, and bacterial endocarditis in humans and animals [[1], [2], [3]]. *Enterococcus faecalis* play a dual role as commensal organisms in gastrointestinal tract and nosocomial pathogen in hospital-acquired infection [4]. Either mutation or insertion of external genetic material in *E. faecalis* is responsible for their innate resistance against most of the presently available antibiotics [5]. In addition, they have also been reported for potential biofilm formation on biotic/abiotic surfaces that were subsequently covered with a hydrated matrix containing various biomolecules including exopolymeric substances, proteins, polysaccharides and nucleic acids [6]. Bacteria are protected from immune system and antimicrobial chemicals in the biofilm state [7]. *E. faecalis* is proven to grow as biofilm

characterized with enhanced antibiotic tolerance, which is challenging in treatments of biofilm-related infections [8]. In some chronic infections, Biofilm even induces tolerance in bacteria that are susceptible to antibiotics [9].

In bacterial infections, the importance of biofilms in drug tolerance leads to increasing interest in the characterization of the factor responsible for biofilm formation. Enterococcal surface protein (ESP) is a high molecular weight surface protein (1873 amino acids) and an ortholog of *Staphylococcus aureus* biofilm associate protein (Bap). Tendolkar et al. (2004) demonstrated that Esp significantly enhanced *E. faecalis* biofilms formation in a glucose-dependent manner. Bap is a biofilm-associated surface protein of *S. aureus*, previously reported to be involved in biofilm formation [2]. Recent study on screening of natural biomolecules targeting Bap revealed polyphenols could prevent amyloid-like aggregation and suggested that they can be used to treat *S. aureus* biofilm and other bioflm-related infections [11].

Due to their antibacterial and antibiofilm capabilities, phytochemicals have shown a substantial potential to combat microbial infections [12,13]. The effectiveness of plant-derived chemicals against biofilms of pathogens that cause common and/or deadly illnesses, like *Escherichia coli*, *Pseudomonas aeruginosa*, and *S. aureus*, has been thoroughly evaluated [14,15]**.** Many natural compounds, including Epigallocatechin-3-gallate, Proanthocyanidins, Quercetin, Licoricidin, and Curcumin, have been reported to have an antibiofilm action against a variety of pathogenic bacteria, including *E. faecalis* [[16], [17], [18], [19], [20]]. Cinnamaldehyde is predominately exists in cinnamon essential oils and widely reported to have antimicrobial and antibiofilm potential against a wide range of bacterial pathogens including *E. faecalis* and *S. aureus* [21,22]. Thus the present study was planned to study ESP binding potential of cinnamaldehyde using computational approach and to confirm the *E. faecalis* biofilm inhibitory potential using invitro models.

Section snippets

Computational analysis of ESP and cinnamaldehyde interaction

The docking analysis was carried out using Auto dock tools (ADT) v1.5.4 and Autodock v4.2 programs [23]. Chemical structure ligand cinnamaldehyde was downloaded from Pubchem database. Three dimensional structures of target protein; *E. faecalis* ESP (PDB ID: 6ORI ↗), (https://www.rcsb.org/structure/6ORI ↗) Ligand was docked to target protein complexes with the molecule considered as a rigid body and the ligand being flexible. The search was extended over the whole receptor protein used as blind…

Computational docking of ESP and cinnamaldehyde interaction

This study was conducted with a view to identifying new natural biofilm inhibitory agents targeting the ESP. We herein report the binding potential of cinnamaldehyde to *E. faecalis* ESP using molecular docking computer software. Table 2 includes a list of all ligand-receptor residue interaction, hydrogen bonds, distance of hydrogen bonds and molecular docking binding energies (kcal/mol) involved in cinnamaldehyde:ESP interaction. The above interaction exhibited the binding energy value of −5.55…

Discussion

Enterococci can develop biofilms on abiotic surfaces, like other gram-positive microbes can [4]. This increases enterococci's high level of innate tolerance to antibiotics, but the exact mechanisms governing enterococcal biofilm formation and maintenance are yet unknown. Bacterial adhesion to the biomaterial is the first stage in the colonization of catheters and the creation of biofilms. Enterococcal Surface Protein (Esp) is the bacterial protein in Enterococci that is important in biofilm…

Conclusion

The findings of this study showed that non-lethal concentrations of cinnamaldehyde decreased the development of *E. faecalis* biofilms, altered the structure of biofilms, and inhibited gelatinase activity. Cinnamaldehyde inhibits biofilm formation by downregulating genes involved in *E. faecalis* Fsr/luxS quorum sensing system. In addition, cinnamaldehyde plays an important role in the clearance of intracellular pathogens by macrophage. More specifically, cinnamaldehyde enhances the NO production,…

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Ethics approval

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CRediT authorship contribution statement

Balasubramanian Sennammal Akshaya: Writing – original draft, Methodology, Formal analysis. **Kumar Premraj:** Writing – original draft, Software, Investigation, Formal analysis. **Christian Iswarya:** Software, Formal analysis. **Suganthi Muthusamy:** Software, Resources, Formal analysis. **Hairul-Islam Mohamed Ibrahim:** Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Hany Ezzat Khalil:** Visualization, Validation, Software. **Vaishnavi Ashokkumar:** Methodology, Formal…

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

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