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A critical review on innovative targets for signal disruption in *Enterococcus faecalis* infection management

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ABSTRACT

Enterococcus faecalis is a Gram-positive gut-associated microbe that commonly inhabits the human intestine and poses an immense threat to individuals with a compromised immune system, particularly in healthcare settings. It is also delineated for its various virulent factors, such as strong biofilm formation and resistance to multiple medications, which in turn challenge the current treatment strategies. Quorum sensing systems, particularly the Fsr and LuxS systems, play an important role in its virulence by regulating the synthesis of enzymes like gelatinase and serine protease, which help maintain biofilm stability and host tissue encroachment.

E. faecalis biofilms are resistant to antimicrobial medications, making the bacteria challenging to eliminate. This review explores *E. faecalis* quorum sensing systems and their role in biofilm formation, emphasizing these pathways as prospective targets for therapeutic intervention. Emerging techniques, which include the application of phages, probiotics, nanoparticles, and phytochemicals, can inhibit quorum sensing, disrupt biofilm growth and reduce *E. faecalis* pathogenicity. Targeting these processes may provide beneficial substitutes or complements to standard antibiotics, especially for antibiotic-resistant bacteria. These strategies aid in improving infection control and therapeutic effectiveness toward *E. faecalis* in healthcare settings.

1. Introduction

Enterococcus faecalis is a Gram-positive bacterium that thrives in the presence and absence of oxygen which is frequently identified in the human gut microbiota. It is classified as a member of the *Firmicutes* phylum, specifically the *Enterococcaceae* family [1,2]. It is recognized for its persistence and ability to grow in severe settings (e.g., high amounts of salt, bile, temperature fluctuations), which makes it simultaneously a symbiotic organism and a pathogenic species [3]. In individuals with good health, *E. faecalis* acts as an innocuous commensal, helping to maintain intestinal balance. In patients with immune-compromised situations or whenever it spreads to sterile places, it may trigger a variety of illnesses [4]. Virulence proteins facilitate its pathogenesis, like adhesins, cytolysin, and gelatinase (GelE) and the development of biofilm, which increases its potential for invasion into tissues and healthcare equipment [5]. On a clinical level, *E. faecalis* is a major contributor to nosocomial illnesses such as urinary tract infections (UTIs), infectious endocarditis, wound-related infections, and the presence of bacteria in the blood, as shown in Fig. 1 [6]. The *Enterococcus* species has been reported to induce ailments in dental roots, and the development of

Abbreviations: E. faecalis, *Enterococcus faecalis*; **GelE**, Gelatinase; **UTIs**, Urinary tract infections; **VRE**, Vancomycin-resistant strains; **QS**, Quorum sensing; **NPs**, Nanoparticles; **GBAP**, Gelatinase biosynthesis-activating pheromone; **SprE**, Serine protease; **CylM**, Lanthionine synthetase; **eDNA**, Extracellular DNA; **AI-2**, Autoinducer-2; **EPS**, Exopolysaccharides; **MIC**, Minimum inhibitory concentration; **NaOCl**, Sodium hypochlorite; **ENMs**, Engineered nanomaterials; **TC**, Trans-cinnamaldehyde; **TCS**, Signal transduction system; **ROS**, Reactive oxygen species; **AgNPs**, Silver nanoparticles; **GO**, Graphene oxide; **CHX**, Chlorhexidine; **CLSM**, Confocal laser scanning microscopy; **GSE**, Grape seed extract; **TTO**, Tea tree oil; **BBH**, Berberine hydrochloride; $Ca(OH)_2$, Calcium hydroxide; BEC_{50}, Biofilm eradication concentration; **RGO**, Reduced graphene oxide; **LTA**, Lipoteichoic acid.

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biofilm can exacerbate the condition [7].

In healthcare facilities, it is linked to catheter-related UTIs and reconstructive valve endocarditis, which contribute to significant mortality rates [8]. Various *Enterococcus* genera can be isolated from root canals with the ability to produce biofilms, which also brings up a concern about infections [9]. Endocarditis constitutes a big problem if infected by the *Enterococcus* genus, which might worsen the patient's condition. In addition, early bacterial infection induced by enterococci can eventually end up in biofilm-associated endocarditis [10].

Despite *E. faecalis* not being specifically identified on the World Health Organization's (WHO) 2017 Priority Pathogens List, the closely related *Enterococcus faecium* is classified as a high-priority pathogen due to its resistance to vancomycin-a characteristic frequently seen in *E. faecalis* strains. Global monitoring programs, such as WHO's GLASS and the CDC's Antibiotic Resistance Threats report, have highlighted the clinical significance of vancomycin-resistant enterococci (VRE), emphasizing the critical need to enhance antimicrobial management, prevention of infection, and biofilm-targeted treatments for combating *E. faecalis* in healthcare settings [11]. A critical challenge in treating *E. faecalis* infections is its intrinsic and acquired antibiotic resistance, particularly to aminoglycosides, cephalosporins, and the emergence of VRE.

This resistance, coupled with its biofilm-forming capacity, complicates treatment and infection control in healthcare settings [8]. The most significant characteristics of *E. faecalis* are their outstanding versatility in severe environmental circumstances and their potential for resistance to antibiotics. In order to combat this opportunistic infectious agent, different anti-virulence treatment methods, such as inhibiting quorum sensing (QS) systems, should be considered [12,13]. This article provides a comprehensive review of strategies and mechanisms targeting *E. faecalis* biofilms and QS inhibition.

Emerging techniques, such as the use of bacteriophages, probiotics, nanoparticles (NPs), and phytochemicals, have the potential to suppress QS, destabilize biofilm formation, and reduce the pathogenicity of *E. faecalis*. These techniques, which target important biological processes, could function as effective replacements or alternatives to existing antibiotic therapy, especially against antibiotic-resistant strains. Such approaches could considerably optimize preventative techniques and clinical outcomes for *E. faecalis* infections.

2. Fsr assisted quorum-sensing

The *fsr* locus in *E. faecalis* encodes a two-part regulatory mechanism that regulates virulence by detecting cell density. This 2.8-kb location contains four genes: *fsrA, fsrB, fsrD,* and *fsrC* [14]. The system controls genes including *gelE, sprE, ef1097,* and *ef1097b*. The *fsrA* gene codes FsrA, a LytTR-family response regulator with a DNA-adhering domain that attaches to regulating areas upstream of targeted genes such as *ef1097, fsrB,* and *gelE* [15]. Particularly, *fsrA* transcription is constitutively a functioning component of the QS mechanism. The *fsrB* gene encodes FsrB, a transmembrane protein that converts the propeptide FsrD into GBAP, a QS peptide. Ultimately, *fsrC* codes FsrC, a transmembrane histidine kinase that functions as a sensor transmitter for the *fsr* operon, as shown in Fig. 2 [16].

The FsrD propeptide (*fsrD*-coded) is transferred and converted to produce smaller lactone gelatinase biosynthesis-activated pheromone (GBAP) through FsrB. FsrC forms a portion of a two-component regulation system that reacts to extracellular GBAP by phosphorylating the intracellular response regulator, FsrA, which subsequently stimulates the transcription of *ef1097, ef1097b,* the *fsr* locus, *gelE* (which codes for gelatinase), and *sprE* (which encodes a serine protease) [18].

The *ef1097*-encoded pre-proprotein (170 amino acids) has been trimmed (the N-terminus 34 amino acids) and transferred via a Sec-assisted process, where GelE subsequently trims the precursor to generate enterocin O16 (68 C-terminus amino acids). ZBzl-YAA5911 (in a competitive manner) and NaCl (concentration-dependently) block the association of GBAP and FsrC. Ambuic acid suppresses FsrB function. Siamycin I, Sviceucin, and WS9326A all block FsrC phosphorylation [19]. In *E. faecalis*, the *fsr* system regulates factors associated with virulence and interaction between cells. As the overall population of *E. faecalis* rises, so does the level of GBAP in the extracellular setting, which is recognized by the sensor kinase FsrC. It phosphorylates the response regulator FsrA, activating it [20].

Phosphorylated FsrA then controls the transcription of the *fsrBCD, gelE-sprE* operons, and the *ef1097* gene, situated 800 kb upstream. Mutations or removal from one of the *fsr* genes (*fsrA, fsrB, or fsrC*) entirely inhibits the transcription of the downstream genes *gelE* and *sprE*, which are required for GelE and SprE synthesis [21]. These genes are strongly expressed in the wild-type varieties. *E.facecalis* strain OG1RF, with *ef1097* exhibiting a large increase through the late logarithmic and initial stationary phases of growth [22]. Ef1097 codes for enterocin O16 (EntV), which is secreted and processed via the Sec system and GelE. This bacteriocin-resembling peptide has antibacterial and antifungal properties, but *E. faecalis* is resistant, indicating a self-defensive strategy [23]. The *fsr* mechanism additionally regulates genes involved in surface adherence, self-destruction, and biofilm formation, demonstrating its importance not just in pathogenicity but also in metabolic and biofilm-related activities. More research must be conducted to understand better the framework and function of enterocin O16, as well as the mechanisms that protect *E. faecalis* from its potentially detrimental effects [24].

2.1. Virulence of fsr-assisted QS

The Fsr quorum-sensing system in *E. faecalis* modulates critical virulence components, such as GelE and SprE, which are coded by *gelE*

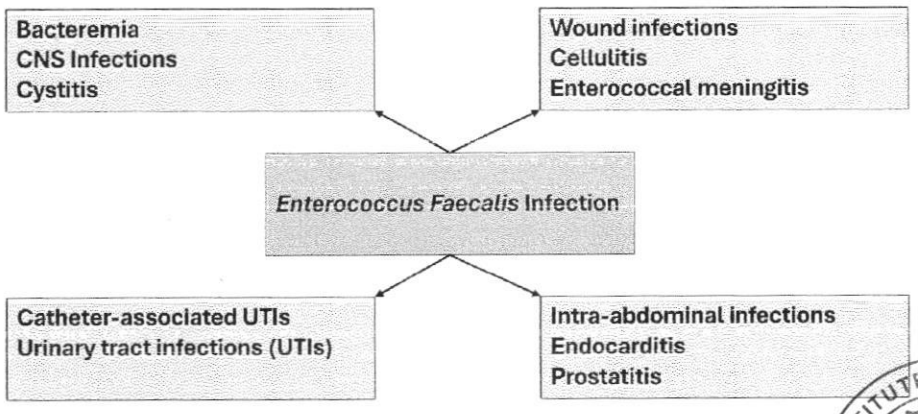

Fig. 1. Nosocomial infections contributed by *E. faecalis*.

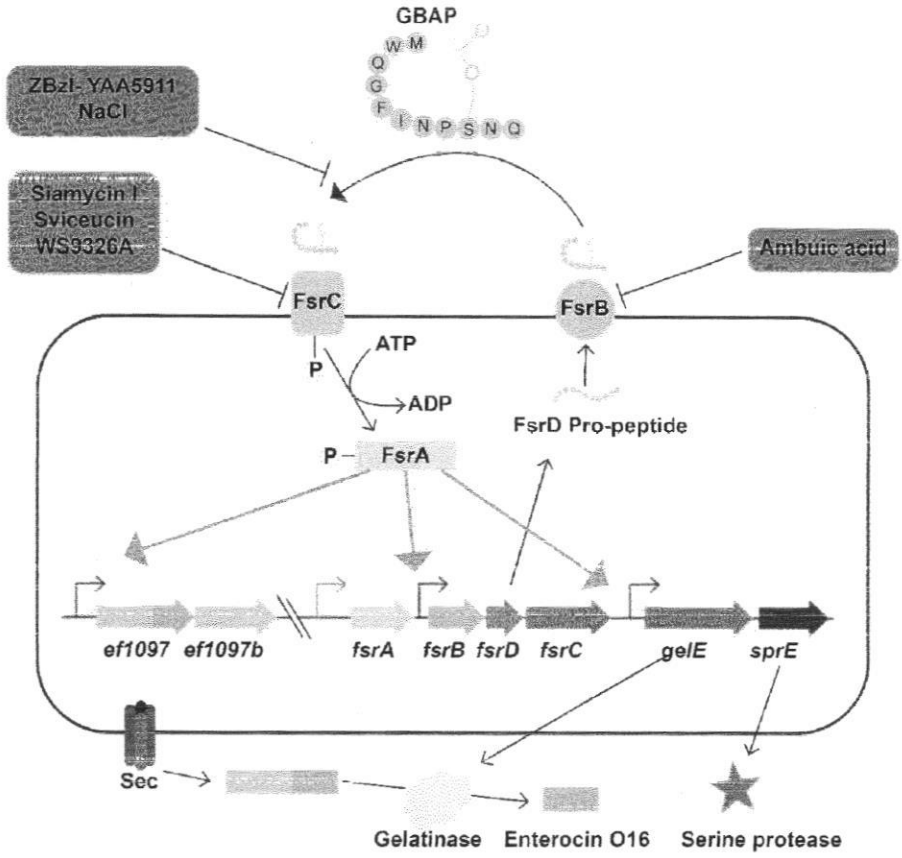

Fig. 2. The Fsr QS mechanism and its regulation in *E.faecalis* (Copyright © MDPI 2017. All rights reserved, reprinted with permission) [17].

and *sprE*, accordingly. These genes are situated next to the fsr genes and have a shared promoter, which contributes to tissue degradation, biofilm development, and pathogenicity [25]. GelE is a 34.5 kDa metalloprotease that disintegrates collagen, fibrin, hemoglobin, and complementary components (C3, C3a, C5a), as well as biofilm formation and modulating the immune response through protease-initiated receptor-2 [26]. This enzyme has been linked to long-term intestine inflammation and promotes *E. faecalis* migration into human cancer cells. GelE action is detected in 13–70 % of *E. faecalis* strains from the oral cavity and more than 78 % of clinical isolates resulting from infections [27].

SprE, a 25 kDa glutamyl endopeptidase, can also be an infectious factor in *E. faecalis*, although its mode for functioning in different hosts is uncertain [28]. The involvement of the Fsr system in pathogenesis has been investigated in a variety of animals, spanning rodents, rabbits, *Arabidopsis thaliana*, *Caenorhabditis elegans*, and *Drosophila melanogaster*. Genetic variation investigations of the *gelE*, *sprE*, and *fsr* genes revealed lower pathogenicity in these rodents, especially when GelE and SprE were interrupted [29,30]. Transcriptome research indicated that the Fsr system controls more than 75 genes, particularly those involved in surface proteins (e.g., EbpR), biofilm formation (BopD), and metabolic processes. GelE and SprE also have a role in controlling autolysin action, notably AtlA, which is responsible for cell death and extracellular DNA release in biofilm development [31]. GelE elimination promotes autolysis, whereas sprE deletion promotes fratricide, indicating a regulatory function in cell death. GelE also degrades collagen-adhering proteins, influencing bacterial attachment to collagen fibers, and the Fsr system encourages the buildup of biofilm via glycosyl transferases (GTFs), which may modify cell wall polysaccharides to impede antibiotic uptake [32].

In general, tackling the Fsr QS system may result in the development of innovative antivirulence medications for *E. faecalis* infections. Other regulatory mechanisms are probably involved as well, though they have not been fully discovered. The Fsr system is crucial to the pathogenesis of *E.faecalis*.

3. Regulation of cytolysin

E. faecalis Cytolysin, a pore formation toxin and Type-A lantibiotic, is classed as a two-component Class I lantibiotic (enterocin) that transforms after production. It is composed of precursor peptides coded by two genes, $cylL_L$ and $cylL_S$, situated in the *cytolysin* operon. When the level of $CylL_S$ exceeds a particular threshold, it activates the operon via a QS system [33]. Cytolysin can affect a variety of organisms, including prokaryote-eukaryote cells. The cytolysin operon, which can be found on the chromosome (located in a pathogenic island) or on a plasmid (pAD1), possesses eight genes [34]. The genes *cylR1* and *cylR2* encode regulatory proteins that are transcribed independently of the additional architectural and active genes: $cylL_L$, $cylL_S$, *cylM*, *cylB*, *cylA*, and *cylI*, as shown in Fig. 3. The genes $cylL_L$ and $cylL_S$ produce peptides with leading and core portions. Lanthionine synthetase (CylM) modifies these peptides by changing serine and threonine residues in the core peptides, resulting in uncommon amino acids such as methyllanthionine and lanthionine [35].

Cytolysin has a unique architecture, with one bridging in $CylL_S$ and two in $CylL_L$, forming an uncommon LL arrangement defined by the peptides instead of CylM. CylM removes water from serine and threonine residuals, transforming them into other molecules (Dha and Dhb), and generates structural crosslinks. CylB manufactures and releases peptides, removing the majority of the leading sections [36]. CylA, a SprE, stimulates peptides by eliminating six amino acids from their terminus and converting them into functional toxins. On the host cell surface, cytolysin monomers form a non-active complex. $CylL_L$ attaches to the cell membrane, whereas free $CylL_S$ functions as a messenger

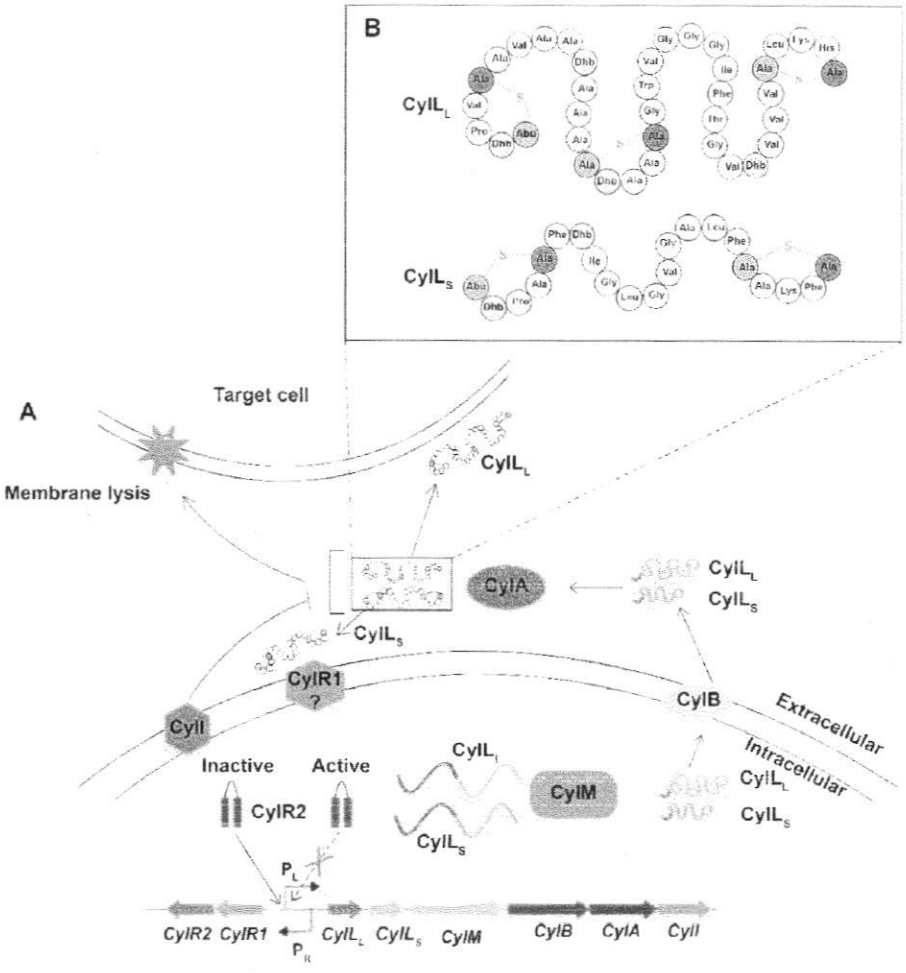

Fig. 3. The cytolysin QS system in *E. faecalis* (Copyright © MDPI 2017. All rights reserved, reprinted with permission)
(A) *The cylL$_L$* and *cylL$_S$* encode the toxin architectural components CylL$_L$ (bigger subunit) and CylL$_S$ (small subunit), respectively. CylM, by post-translation, modifies CylL$_L$ and CylL$_S$ central peptides before CylB processes and transports them beyond. CylA, an extracellular protease, eliminates six amino acid residuals (leading peptides) from CylL$_L$ and CylL$_S$, converting them into functional toxin monomers.
The signal transduction process that links extracellular CylL$_S$ adherence to CylR1 at the membrane and CylR2 detachment from the PL promoter is yet unclear. CylI produces self-immunity against cytolysin. **(B)** Morphology of CylLL and CylL$_S$ matured cytolysin monomers. Clinical proof shows that cytolysin-favorable microbes in bacteremia are as much as five more likely to cause death than non-cytolytic pathogens.

molecule [37].

When CylLS approaches a sufficient level, it adheres to the membrane protein CylR1, breaking its connection with the repressive CylR2 and inducing the replication of the cytolysin operon. The specific mechanism by which CylR1 conveys this signal remains uncertain [38]. The final gene in the operon, cylI, encodes the defense protein CylI, which defends the bacterium from its unique cytolysin. Despite CylI certainly adheres to zinc and has transmembrane regions, its specific involvement in safeguarding is still unclear.

3.1. Pathogenesis of cytolysin

Around 30 % of *E. faecalis* strains and 60 % of clinical strains synthesize cytolysin, a toxin with toxic effects that have been investigated *in vivo*, *In vitro*, and clinical contexts. Cytolysin has antibacterial properties towards a wide range of Gram-positive bacteria, including *Clostridia*, *Lactobacillales*, and *Staphylococcus* [39]. Whereas the particular mechanism of action is undetermined, cytolysin monomers CylL$_L$ and CylL$_S$ are structurally comparable to bacteriocin lacticin 3147 synthesized by *Lactococcus lactis*. Lacticin 3147 generates pores in membranes of bacteria in three steps, including lipid II attachment and complex formation; however, it is unclear whether cytolysin employs a similar method [40]. Cytolysin has been demonstrated to break down human erythrocytes, polymorphonuclear leukocytes, retinal cells, and intestine epithelial cells, indicating a function in hemolysis, endophthalmia, immunological suppression, and intestinal disorders [41]. In the mouse model, organisms carrying the cytolysin operon were more virulent than identical genes non-cytolysin microbes, and sterile rat fecal extract (SREF) was reported to worsen inflammation of the peritoneum [42].

Furthermore, bacteremia strains produce more cytolysin than endocarditis or healthier isolates [43]. Additional investigations revealed that the Fsr quorum-sensing system regulates cytolysin and *gelE* expression, which is controlled by host environmental parameters. While strains producing cytolysin or GelE boosted pathogenicity in infecting scenarios, the combined action of two of them failed to significantly enhance infectiousness, suggesting a potentially antagonistic association or a saturated effect [44]. GelE has been linked to the proteolytic breakdown of host substrates and sex pheromone-related peptides, and it may inhibit cytolysin function *In vitro*. However, simultaneous expression may aggravate disease *in vivo* [45]. Assessing these associations is crucial for innovating methods of therapy to combat cytolysin-mediated virulence, as cytolysin is involved in hemolysis, bacterial lysis, endophthalmia, inflammation of the heart valve, and other infectious characteristics following autoinduction in the vicinity of target cell membranes [46].

4. Luxs system

The LuxS gene is identified in various bacterial strains, including *E. faecalis*. It codes the LuxS enzyme, which stimulates the formation of 4,5-dihydroxy-2,3-pentanedione (DPD), a precursor to the messenger molecule autoinducer-2 (AI-2) [47]. AI-2 is considered to improve interspecies interaction in luxS-containing bacteria with a high density of cells.

The LuxP receptor recognizes AI-2 in *Vibrio harveyi*, a Gram-negative bacteria; nevertheless, other bacteria that lack LuxP continue to react to AI-2, implying the existence of additional receptors such as LsrB or RbsB [48]. Nonetheless, no AI-2 receptors have been detected in Gram-positive bacteria such as *E. faecalis*.In *E. faecalis*, LuxS regulates genes associated with energy synthesis, cell wall formation, and metabolic processes. In spite of this, the process behind LuxS-mediated gene regulation remained unknown. Furthermore, LuxS has been connected to biofilm production; luxS elimination promotes biofilm formation and cell-surface hydrophobic properties [49]. Exogenous AI-2 can be employed to determine if this action is LuxS-dependent or solely metabolic, as resetting the methionine cycle without producing DPD could shed light on its involvement [50]. AI-2 signaling has also been linked to biofilm growth and secretion of protein toxins in Streptococcus pneumoniae, a closely related strain of *E. faecalis*, implying a more general role for AI-2 [51]. However, in *E. faecalis*, the precise involvement of AI-2 in communication between cells and biofilm development is unclear. Additional research is required to identify whether the LuxS system in *E. faecalis* is mainly metabolic or associated with QS [52].

5. *Enterococcus faecalis* biofilm formation

E. faecalis biofilms vary in physico-chemical attributes based on ecological and nutritional circumstances. In a nutritious environment, they formed conventional biofilm architectures characterized by bacterial surface cell aggregation and water passages. On the contrary, in nutrient-limited circumstances, adhering cell clumping develops irregularly [53]. During impoverishment, cells growing in biofilms demonstrated enhanced production of proteins and reduced nucleic acid levels.

E. faecalis biofilms are formed through complex interactions of released infectious factors, surface proteins, QS molecules, and extracellular DNA (eDNA) releasing regulators. They are responsible for the attachment of bacteria, biofilm development, resistance to mortality, and tissue injury [54]. Biofilms have various advantages for *E. faecalis*. They form a protective barrier that safeguards against external risks like drugs and immune system attacks. Furthermore, biofilms stimulate bacterial development and proliferation, increase the survival of bacteria by enabling the exchange of nutrients and messenger molecules, aid in bacterial adherence and localization in particular conditions, and enhance bacterial consumption of resources and adaptability [55]. Biofilms can considerably raise the required dosages of specific antibiotics by as much as 1000 times to battle illnesses caused by this pathogen [56].

Biofilm formation involves four major stages, as shown in Fig. 4. 1) Planktonic bacteria stick to an appropriate substrate; 2) Because adherent cells are permanently attached to the surface, they construct micro-colonies and release an extracellular polymeric substance (EPS) matrix. This irrevocable adhesion is critical because it signals the change from a changeable to a stable state, tightly attaching the cells to the surface [57]; 3) The biofilm grows via the formation of microcolonies and the building of water passageways framework. It endures a significant rise in layering, and when achieving full development, it obtains the optimum density of cells, eventually changing into 3-D communities partially controlled by QS; 4) Established biofilms liberate miniature colonies of cells from the main group, enabling them to migrate without restriction to new substrates and spread the illness to other sites [58–60].

Several genes are active in biofilm development, especially those responsible for surface adherence, accumulation, extracellular polymorphism, and the generation of toxins [62]. As a result, those gene products promote biofilm formation and invasion, leading to increased adherence to host tissues. Biofilm production is mainly regulated genetically by the following processes: QS, cyclic dinucleotide signaling, and smaller non-coding RNAs (sRNAs) [59].

GelE, a critical pathogenic component produced by the *gelE* gene, degrades collagen and additional proteins, besides enabling eDNA release by stimulating the main autolysin AtlA. Cytolysin, a substantial further element, aids in biofilm development by breaking down other bacterial cells and producing eDNA, which promotes bacterial agglomeration and stabilization in the biofilm matrix. Surface proteins or adhesins, which include pili and surface components of microbes that recognize adhesion matrix molecules, like Ace, play crucial functions in bacterial attachment to their host tissues and abiotic surfaces [63].

E. faecalis encodes adhesins like aggregation substances and biofilm-associated pili, both of which aid in biofilm formation on nutrient-rich surfaces and in endocarditis [64]. The QS mechanism in *E. faecalis*,

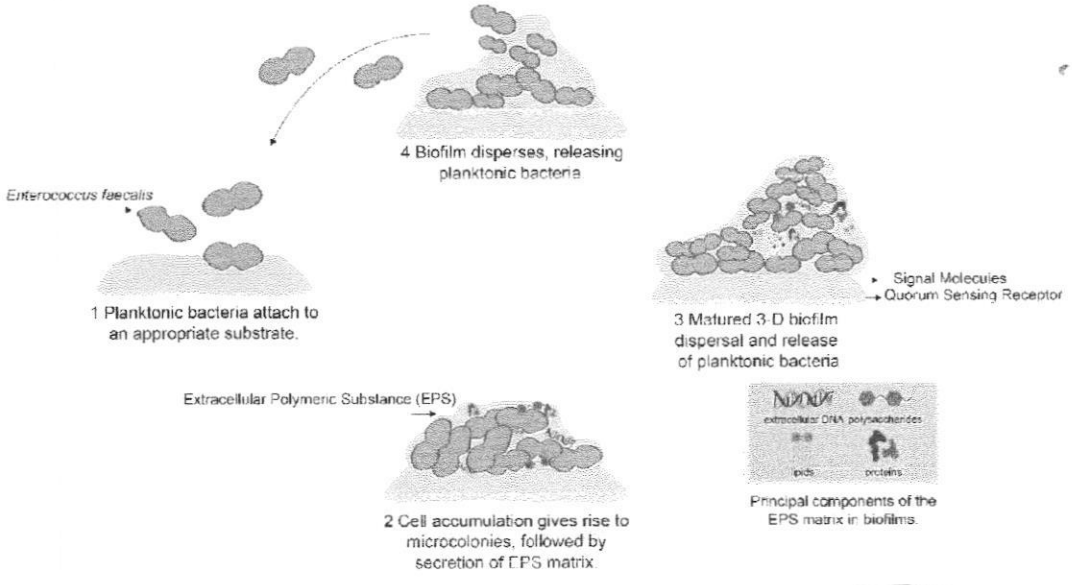

Fig. 4. Various stages of *E. faecalis* biofilm development (Copyright © Frontiers 2024. All rights reserved, reprinted with permission) [61].

specifically the fsr operon, mediates the transcriptional controlling of GelE and SprE, therefore exhibiting considerable impact on biofilm development [20]. Understanding these pathways paves the way for the development of specific anti-biofilm medications. *E. faecalis* interacts with a variety of bacteria to form and maintain biofilms. As an illustration, *E. faecalis* GelE increases the cross-feeding of heme derived from *Staphylococcus aureus* hemoproteins, increasing the formation of binary species biofilms [65]. In the early phases of biofilm development, AI-2 generated by *E. faecalis*, which functions as a chemoattractant, supports accelerated auto-accumulation and proliferation of *Escherichia coli*, hence increasing the robustness of combined-species biofilms towards antagonistic stressors [66]. *E. faecalis* enhances the formation of exopolysaccharides (EPS) in *Pseudomonas aeruginosa*, which promotes biofilm development and biomass via stimulating the expression of *Pel* and *Psl* [67].

The development of *E. faecalis* biofilms increases bacterial resistance to drugs and evades human immunological responses [68,69]. Antibiotics like ampicillin and vancomycin applied at highly concentrated levels (1000 × MIC (minimum inhibitory concentration)) were unable to eliminate *E. faecalis* biofilms, regardless of the amount of biofilms produced [70]. However, the synergistic therapy using antibiotics was determined to be ineffectual towards *E. faecalis* biofilms [71]. *E. faecalis* biofilms exhibit antimicrobial resistance due to their biofilm matrix, which can limit antibacterial dispersion and efficiency [72]. Moreover, *E. faecalis* biofilms with lower eDNA levels are more vulnerable to the lethal adverse effects of antimicrobial peptides and traditional disinfecting agents, which include sodium hypochlorite (NaOCl) [73]. The likelihood of propagating antibiotic resistance is increased in biofilms owing to a closer association of cells and a greater rate of horizontal transmission of genes.

Furthermore, the vancomycin-treated group had higher levels of the penicillin-adhering proteins that cause resistance to β-lactam antibiotics [74].

5.1. Various mechanisms targeted towards E. faecalis biofilms

Biofilms can be effectively eliminated by either hindering their development or disrupting and destroying established biofilm formation. These techniques can generally be categorized into the following categories: targeting enzymes related to biofilm development, regulating QS and transmitting signals systems, and destroying architectural components associated with biofilm, as illustrated in Fig. 5 [75].

5.1.1. Targeting enzymes involved in biofilm development

Biofilm development requires multiple synthetic enzymes, notably c-di-AMP synthase and ClpP protease. C-di-AMP synthase synthesizes c-di-AMP, a key messenger chemical in cells. This molecule has a major impact on numerous key cell processes, which include bacterial spread, pathogenicity, biofilm development, regulation of cellular arrangement, the production of fatty acids, and regulation of the host immunological response [76]. Chen et al. [77] used sequence alignment and molecular docking approaches to evaluate the effect of ST056083 on c-di-AMP synthase in *E. faecalis*. They also performed a variety of phenotypic studies to verify ST056083's inhibiting effect on *E. faecalis* growth and the development of biofilm.

The results of the experiment reveal that ST056083 preferentially targets the function of c-di-AMP synthase, thus efficiently reducing *E. faecalis* biofilm growth. The lack of ClpP protease alters the *E. faecalis* development pattern and lessens the polysaccharide matrix, limiting its capability to produce biofilms. It proposes a possible therapeutical pathway targeting ClpP protease for the therapy of *E. faecalis* infections [78].

Mabanglo et al. [79] discovered acyldepsipeptides, a new category of antibiotics with antibacterial capabilities toward ClpP targets. Therefore, the utilization of acyldepsipeptides offers the potential for efficiently treating antibiotic-resistant infections that result from *E. faecalis* biofilms.

Sortase A plays a crucial role in the longevity of *E. faecalis*, thus rendering it an attractive treatment option for infections. This enzyme catalyzes the interaction between surface proteins and cell wall peptidoglycan, which facilitates biofilm formation [80]. Sortase A is closely associated with the earliest phases of biofilm formation in *E. faecalis*, and its absence has significant effects on the attaching phase, resulting in insubstantial biofilm development [81]. Berberine hydrochloride has been demonstrated to efficiently prevent *E. faecalis* biofilm development by reducing mRNA expression related to Sortase A and EPS [82].

5.1.2. Inhibiting the growth of E. faecalis biofilm by modifying bacterial interaction and signaling routes

QS is a bacterial interaction system that generates and detects signaling molecules called autoinducers (AI). When approaching a given threshold, these chemicals cause changes in bacterial behavior,

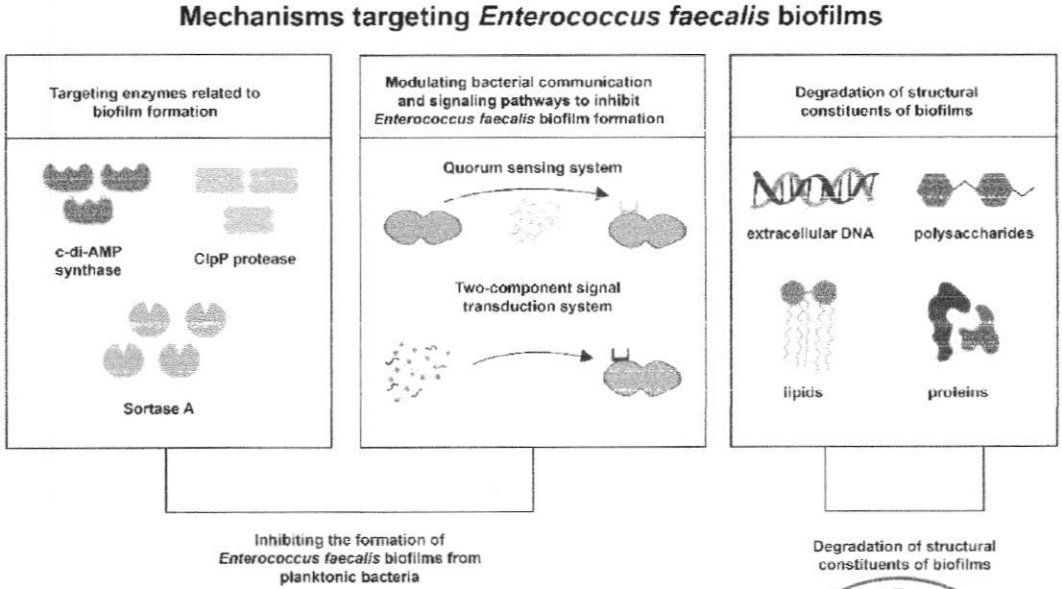

Fig. 5. Various mechanisms targeted towards *E. faecalis* biofilms (Copyright © Frontiers 2024. All rights reserved, reprinted with permission) [50].

involving modification of EPS generation, bacterial surface adherence, expressing virulence proteins, and eventual biofilm development [83, 84]. Targeting bacterial QS is a promising approach to reduce *E. faecalis* biofilm development and persistence. QS inhibitors could target QS at many phases, including signal production, degradation of signaling molecules, competition for signal adhering to cognate receptors, and inhibition of QS-assisted gene transcription [85].

Trans-cinnamaldehyde (TC) suppresses QS systems and alters gene expression related to biofilm development. Inhibiting QS has been shown to limit the invasion of bacteria and breakdown of host tissue constituents by restricting the generation of hydrolytic enzymes, which include proteases and GelE [86,87]. The LuxS gene synthesizes Autoinducer-2 (AI-2), a messenger molecule that enables inter-bacterial interaction. When bacterial community density approaches a threshold, the messenger molecule AI-2 becomes released into the surroundings, causing bacteria to exhibit QS behavior [88]. Knocking down the LuxS gene alters AI-2 expression, which affects the *E. faecalis* buildup of biofilm. Particularly, loss of LuxS gene expression limits biofilm size and distinctness while also creating inadequacies in secretions and fibrous interactions, hence drastically limiting *E. faecali's* potential to produce biofilm [50]. One possible method is to prevent *E. faecalis* from forming biofilms by interfering with the production or transfer of AI-2 signaling molecules.

The Fsr QS system plays a vital role in forming biofilm by producing GelE, which forms one of the primary stages in biofilm formation [19]. Cinnamaldehyde decreases the growth of biofilm by altering gene expression inside the *E. faecalis* Fsr QS system [89]. QS antagonists reduce GelE synthesis, which is regarded as vital in averting the breakdown of host tissue constituents resulting from the colonization of bacteria [90]. Cinnamaldehyde, in particular, inhibits the transcription of the *fsrB* and *fsrC* genes in *E. faecalis* biofilms. This downregulation lowers response regulatory phosphorylation, which inhibits gelE-sprE operon transcription. As a result, this inhibition additionally decreases gelE action, eventually inhibiting biofilm formation [91]. Tea tree oil contains α-pinene, which may interfere with QS mechanisms and so inhibit the development of *E. faecalis* biofilms [92]. Furthermore, probiotics may exhibit an anti-biofilm effect by interrupting QS through gene control [93].

Bacteria's signal transduction system (TCS) works in conjunction with the QS system to detect and react to surrounding signals. It regulates bacterial physiological activities such as biofilm growth and maturity by controlling gene expression and enzyme activity [94]. *E. faecalis* has 17 two-component, with walRK recognized as the single important TCS that is critical to assessing the strain's survival [16]. WalRK in *E. faecalis* exerts regulatory dominance over a variety of metabolic activities, including synthesizing cell walls, survival from extreme osmotic stress, and biofilm development. In addition, WalRK has been established as a critical target in fighting *E. faecalis* biofilm. By intervening with the auto-phosphorylation mechanism of WalK, it efficiently inhibits the functioning of the WalRK system, reducing *E. faecalis* biofilm formation.

5.1.3. Degrading structural components of matured biofilm

Biofilms, composed of EPS, can be targeted on disintegration by breaking down specified structural constituents such as proteins, eDNA, and polysaccharides. The eDNA structure within EPS is deemed significant [95]. It has been identified that eDNA performs an important role in the development, stability of structure, and development of *E. faecalis* biofilm [96]. Yu et al. [88] employed deoxyribonuclease (DNase) as an enzymatic agent to successfully disintegrate eDNA molecules, reducing its function in both *E. faecalis* cells and biofilms. The action drastically lowered the biofilm's stability. In addition, they discovered that suppressing eDNA increased the vulnerability of *E. faecalis* biofilms to NaOCl treatment.

Earlier studies have shown that silver nanoparticles (AgNPs) and graphene oxide (GO) can produce oxidative stress and generate reactive oxygen species (ROS) [97]. These ROS molecules can efficiently associate with proteins and eDNA in the EPS, destroying the biofilm architecture [98,99]. Furthermore, the phage-based polysaccharide depolymerase shows remarkable promise in disrupting biofilm construction by targeting bacterium polysaccharides in capsular form and destroying the EPS matrix [100,101].

6. Emerging strategies to Disrupt E. faecalis Biofilm and quorum sensing systems

In recent years, novel tactics have been employed to attack *E. faecalis* biofilms, which pose a considerable issue in healthcare facilities due to their persistence. NPs, phytochemicals, phages, and probiotics are used in the current approaches, as illustrated in Fig. 6. NPs, displayed as poriferous frameworks, are used because they can break through biofilms and provide antimicrobial drugs directly to bacterial cells. Plant-derived treatments, such as phytochemicals, include biologically active substances such as alkaloids, vital oils, and polyphenols that can inhibit biofilm growth, attachment and QS system [102].

Bacteriophages, which appear as virus-resembling fragments, are viruses that attack and lyse *E. faecalis* by attacking biofilm-associated cells [103]. Finally, probiotics, also known as good bacteria, are employed to compete with or suppress infectious bacteria by competitive exclusion, antibacterial agent synthesis, or biofilm destruction [104].

6.1. Phytochemicals

The application of biological compounds, especially phytochemicals, as supplements or substitutes for conventional antimicrobial medicines has gained popularity in recent times. Bioactive substances called phytochemicals originate from plants, and several of them have shown antibacterial, anti-inflammatory, and antibiofilm capabilities. These substances, which include alkaloids, flavonoids, polyphenols and terpenoids, have demonstrated potential in preventing the emergence of biofilms, lowering bacterial attachment, and increasing the sensitivity of bacteria enclosed within biofilms to antimicrobial agents.

The antibiofilm actions of phytochemicals on *E. faecalis* are especially important in periodontal therapies and nosocomial illness [105, 106].

Research has demonstrated that phytochemicals can alter biofilm growth at several phases, including the initial attachment, development, and dispersal, as shown in Fig. 7. Phytochemicals disrupt QS signaling by reducing the synthesis of autoinducing peptides (AIPs), preventing signal receptor binding, or degrading signal molecules. This disruption interrupts the coordinated expression of biofilm-promoting genes, which leads to attenuated biofilm development that is more susceptible to medications. Furthermore, certain phytochemicals alter bacterial membrane permeability and efflux pump activity, hence increasing antibiotic intake and retention [108].

Phytochemicals lessen the risk of chronic infections and recurrence by inhibiting QS-mediated resistance mechanisms and boosting bacterial susceptibility. The capability to target numerous bacterial activities at the same time makes them beneficial alternatives to conventional medications for treating recurring *E. faecalis* infections [109].

6.1.1. Trans-cinnamaldehyde

Trans-cinnamaldehyde (TC) is derived from the bark of the cinnamon tree, containing a substantial amount of TC (65 %–80 %). Significant research has concentrated on TC and its related compounds, demonstrating their considerable antibacterial activity against many infectious yeasts and molds [110,111]. Ali et al. [112] found that TC was as effective as 1 % NaOCl and 2 % CHX (chlorhexidine) in removing *E. faecalis* biofilms from dental discs within 15 min. In particular, TC had long-lasting antimicrobial activity on *E. faecalis* biofilms, limiting regeneration in spite of ideal growth circumstances. In contrast, live

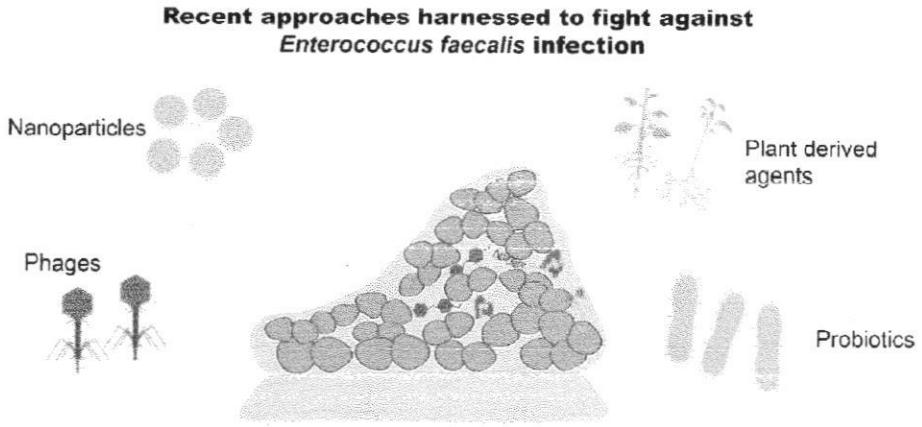

Fig. 6. Various novel approaches for targeting *E. faecalis* (Copyright © Frontiers 2024. All rights reserved, reprinted with permission) [91].

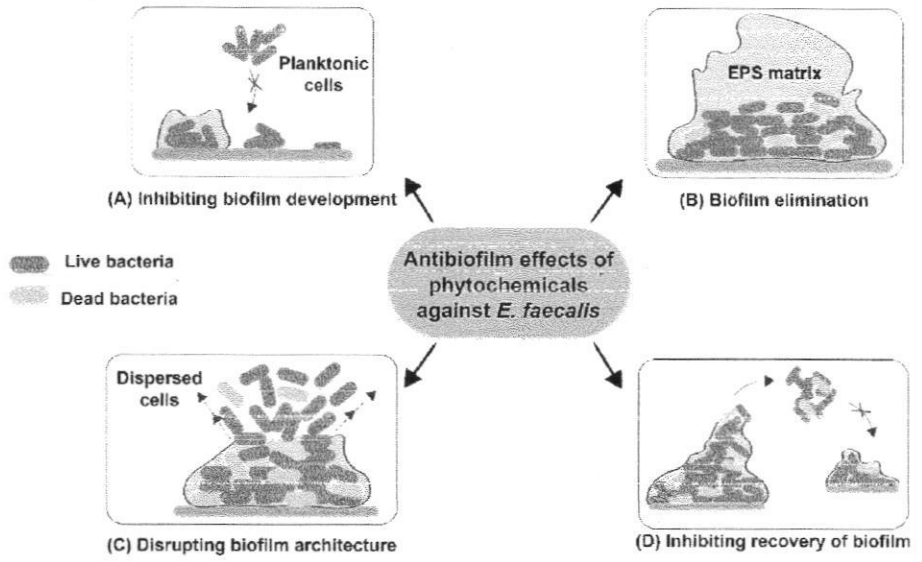

Fig. 7. Antibiofilm beneficial effects of phytochemicals towards *E. faecalis* (Copyright © Wiley 2022. All rights reserved, reprinted with permission) [107].

E. faecalis was found ten days after treatment with CHX. These outcomes emphasize the possibility of it as a successful therapy for treating *E. faecalis* infection throughout root canal procedures. The phytochemical TC's biological function is constrained by its lipophilicity, which reduces its ability to dissolve in watery settings, including the hydrate EPS matrix. This drawback could decrease TC's efficacy in facilitating the development of biofilm [113,114].

TC disintegrates bacterial cell membranes, enhances permeability, and leaks essential contents, resulting in cell death. It also inhibits biofilm development by preventing initial attachment, degrading the shielding EPS matrix, and inhibiting quorum sensing, which is required for bacterial interaction and pathogenicity. Furthermore, TC causes oxidative stress and affects essential metabolic pathways, reducing bacteria's survival.

Despite its efficiency, TC's lipophilicity limits its ability to dissolve in aqueous conditions, lowering its bioavailability in biofilm-rich locations. Thus, novel delivery techniques are required to improve clinical effectiveness [115].

Hu et al. [116] used acidic sophorolipids (ASL) as a type of surfactant to encompass TC molecules and build a complex that enhanced the antibacterial and anti-biofilm characteristics of hydrophobic trans-cinnamaldehyde. Their investigations revealed that the TC-ASL complex substantially enhanced antibacterial activity, resulting in a dramatic reduction in both the production and quantity of *E. faecalis* biofilms when compared to employing TC alone. When exposed to air or blood vessels, TC degrades and converts into cinnamic acid, reducing its antibacterial activity. This breakdown raises questions regarding its clinical importance, prompting additional research into its *in vivo* activities and therapeutic properties. Subsequent investigations should concentrate on identifying or manufacturing stable TC variants, owing to the compound's intrinsic volatility and poor biological action.

The TC-ASL combination improves the antibacterial and anti-biofilm characteristics of TC by increasing its solubility, stability, and transport. ASL, a biosurfactant, encases hydrophobic TC molecules, improving dispersion in aqueous conditions and preventing TC breakdown into cinnamic acid, which decreases its action [117]. This compound facilitates synergistic membrane disruption, enabling TC to penetrate and harm bacterial cells efficiently. It also increases biofilm entry and destroys the extracellular matrix, resulting in a substantial decrease in *E. faecalis* biofilm production and bulk. Furthermore, the TC-ASL complex increases TC's QS inhibition, which reduces bacterial interaction and pathogenicity. In general, the TC-ASL complex addresses TC's intrinsic shortcomings and offers a promising path to better clinical performance.

Furthermore, investigating the application of additives, which include surfactants, nanomaterials, and additional substitutes, may be critical to enhancing TC's dissolution and permeation, possibly boosting its therapeutic efficacy [86].

6.1.2. Quercetin

Quercetin, a flavonoid that occurs in fruits and vegetables, is known for its anti-inflammation, antioxidant, and anti-aging capabilities [118]. Quercetin's mechanism of action against *E. faecalis* biofilms involves inhibiting QS, which is required for bacterial interaction and biofilm formation [119]. The fsr QS system in *E. faecalis* regulates GBAP synthesis, which initiates the FsrC-FsrA signaling cascade, resulting in the development of crucial virulence proteins such as *gelE* and *sprE*. Quercetin disrupts this process by downregulating the fsr operon, resulting in reduced GBAP synthesis and QS system initiation. This suppression inhibits the expression of QS-regulated genes such as *gelE* and *sprE*, which are required for biofilm formation and virulence. It also interferes with glycolysis and protein folding, which further destabilizes the biofilm architecture.

It further impairs signal transduction by disrupting the membrane-bound sensor protein FsrC, as well as suppressing QS-regulated gene expression, which limits the synthesis of enzymes required for biofilm formation [120]. In addition, quercetin may function as a QS inhibitor, inhibiting signal receptors and preventing bacterial transmission. These combined actions hinder biofilm formation and diminish virulence in *E. faecalis*. Particularly, quercetin was discovered to suppress biofilm generation by up to 95 % at sub-MIC levels by interference with critical metabolic processes such as glycolysis and folding of protein [121].

This disturbance destabilizes the biofilm framework, rendering *E. faecalis* more vulnerable to antimicrobial treatments. Qayyum et al. [122] reported that quercetin has substantial inhibitory activity against *E. faecalis*, with MIC recorded at 512 mg/L. The compound's efficiency was demonstrated using modern techniques such as scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM), which provided visual evidence of biofilm decline. Furthermore, proteomic and real-time PCR investigations demonstrated that quercetin interfered with key bacterial proteins, limiting their activity and hence suppressing biofilm development [123].

6.1.3. Grape seed extract (GSE)

Grape seed extract (GSE) has cancer-fighting, antioxidant, anti-inflammation, and antibacterial properties, making it useful for regulating and eliminating oral microbes [124].GSE, produced from *Vitis vinifera*, has shown remarkable potential for suppressing *E. faecalis* biofilms and inhibiting QS. Studies have shown that the polyphenols in GSE have strong anti-biofilm action. They limit biofilm adherence and development by targeting surface proteins and releasing virulence indicators such as GelE, which is essential for *E. faecalis* biofilm stability. GSE also affects *E. faecalis*' QS system, namely the fsr operon, which controls the expression of genes involved in biofilm development and persistence. By inhibiting QS, GSE reduces the generation of messenger molecules required for bacterial interaction and biofilm growth, lowering biofilm strength and pathogenicity [125]. Soetanto et al. [126] used the CLSM technique to investigate the antibacterial impact of 6.5 % of GSE on the *E. faecalis* biofilm [126]. Investigations suggested that GSE demonstrates greater antibacterial efficiency towards *E. faecalis* biofilms than 2 % CHX and saline solution.GSE can improve tissue stabilization towards collagenase breakdown in the course of therapy of demineralized dentin while also displaying minimal harmful effects on human cells [127].

These findings make GSE a promising candidate for more investigation as an additional therapy for targeting *E. faecalis* biofilms in dental infections, probably boosting the effectiveness of standard antimicrobial therapies.

6.1.4. Tea tree oil

Tea tree oil (TTO) is sourced from *Melaleuca alternifolia* leaves. TTO is widely utilized as a secure, organic, and effective antioxidant with powerful antibacterial and antifungal characteristics, which encompass a wide spectrum of biological activity and safety [128,129]. In the scenario of battling *E. faecalis* biofilms, Qi et al. [128] evaluated the effect of different TTO dosages on biofilm development. The results revealed that TTO levels beyond 0.25 % significantly inhibited biofilm growth and eliminated existing *E. faecalis* biofilm. TTO has been found to prevent biofilm development and QS in *E. faecalis* [130]. TTO's crucial phytochemicals, including terpinen-4-ol and α-terpineol, disrupt biofilm construction by lowering bacterial adhesion and EPS synthesis [131]. TTO also disrupts QS pathways, specifically the fsr system and luxS-assisted signaling, reducing the synthesis of virulent factors such as GelE and cytolysin. These acts weaken the biofilm and reduce the bacteria's capacity to trigger illnesses [132].

6.1.5. Berberine hydrochloride

Berberine hydrochloride (BBH), an alkaloid produced from *Rhizoma Coptidis*, has antibacterial and anticancer activities [133,134]. It impedes biofilm stability by lowering the adherence of bacteria and degrading the extracellular matrix, which is essential for biofilm longevity. Furthermore, BBH interacts with the QS system, notably the fsr pathway, which modulates virulence factors like gelE [135]. BBH inhibits these QS signals, reducing biofilm development and the bacteria's capacity to cause illnesses.

BBH's combined effect makes it an intriguing treatment for reducing antibiotic-resistant illnesses caused by biofilms. Chen et al. [82] discovered that BBH reduces the production of *E. faecalis* biofilms and facilitates their dissemination by decreasing the mRNA expression of Sortase A and EPS.

6.1.6. Propolis

Propolis, a resin that is naturally made by bees, comprises bioactive components, including esters, flavonoids and phenolic acids that have been investigated for their antibacterial effects, specifically against *E. faecalis* [136]. Propolis possesses key therapeutically active components such as flavonoids, which may interact on bacteria microbial membranes or cell walls, causing operative and structural impairment [137]. The bioactive components in propolis, particularly flavonoids such as caffeic acid phenethyl ester (CAPE),galangin and pinocembrin, disrupt the early stages of the formation of biofilm. Propolis lowers bacterial attachment to surfaces, weakens the EPS matrix, and impairs the structural strength of formed biofilms [138]. Propolis additionally attacks *E. faecalis* QS processes that control virulent factors, including GelE and cytolysin, which are required for biofilm development and pathogenesis. The functional components in propolis may suppress the expression of QS-related genes, culminating in reduced synthesis of these virulent factors [139].

6.1.7. Aloe vera

Aloe vera originated from the *Liliaceae* family and has a cactus-like appearance. Aloe vera has considerable therapeutic, antibacterial, antiviral, and anti-fungal capabilities, in addition to favorable hypoglycemia effects [140]. *In vitro* investigations have demonstrated that Aloe vera has antibacterial powers towards *E. faecalis* that are greater or equivalent to the effects of saline and calcium hydroxide ($Ca(OH)_2$) compared to CHX, NaOCl, and propolis.

Moreover, Ghasemi et al. [141] discovered *In vitro* that Aloe vera has more considerable antibacterial effects on *E. faecalis* biofilms than Ca $(OH)_2$. Aloe vera gel possesses phenolic chemicals, glycoproteins, and anthraquinones, which can help to prevent *E. faecalis* biofilm development. Aloe vera extract dramatically reduced *E. faecalis* biofilm development. It disturbed the biofilm matrix, reducing bacterial adherence to surfaces. Aloe-emodin, a constituent of Aloe vera, was recognized for its antibacterial activity against biofilm-forming bacteria. Aloe vera can enter the biofilm matrix, interfere with QS signals, and decrease EPS formation, all of which are necessary for biofilm persistence [142,143]. Saponins found in aloe vera are identified for their detergent-like characteristics, which aid in disrupting biofilm stability.

Aloe vera has anti-QS action, which is most probably attributed to the existence of phenolic chemicals and flavonoids that impede

signaling molecules like AI-2 in Gram-positive bacteria such as *E. faecalis* [144]. Aloe vera gel hindered *E. faecalis* cellular communication by decreasing the synthesis of QS-controlled virulence determinants. Aloe vera can prevent the formation of signaling molecules required for QS, reducing biofilm development and pathogenicity without damaging the bacteria and lowering selective pressure for resistance [145].

6.1.8. Triphala

Triphala, an ancestral Ayurvedic compound made of three therapeutic fruits (*Emblica officinalis*, *Terminalia bellirica* and *Terminalia chebula*), has demonstrated great potential for antibacterial action, especially toward *E. faecalis* biofilms and QS. It possesses tannins, polyphenols, and vitamin C, which provide considerable antioxidant, anti-inflammatory, and antibacterial characteristics [146].

Triphala mouthwash substantially decreased *E. faecalis* biofilm production in clinical conditions, indicating its potential application in endodontic treatments. Triphala's active constituents disrupt the EPS matrix, inhibiting bacteria from establishing stable biofilms and increasing the effectiveness of other antimicrobial therapies [147].

Triphala's substantial polyphenol content inhibits QS by interfering with signaling molecules like AIs. Gallic acid is present in Triphala and recognized for its antibacterial and antioxidant characteristics, it has been linked to biofilm suppression and QS disruption [148]. Chebulagic Acid and Chebulinic Acid found in *Terminalia chebula* are excellent at interrupting QS pathways while inhibiting bacterial biofilm formation. The ellagic Acid present in *Emblica officinalis* has demonstrated promising action toward biofilms by reducing bacterial attachment and QS signals [149].

Triphala has antibacterial efficiency towards *E. faecalis* biofilm, with decreased toxic effects, ease of accessibility, cost-efficiency, and other health advantages such as antioxidants and anti-inflammation characteristics [150]. Table 1 summarizes the potential of several phytochemicals to inhibit *E. faecalis* biofilm.

6.2. Nanoparticles

Biofilms are well-structured bacterial communities that encapsulate themselves within a self-secreted matrix of EPS, mainly composed of different polysaccharides, proteins, lipids, and nucleic acids [165]. Heterogeneity in biofilm properties of *E. faecalis* serves as an extraordinary protective shield with enhanced bacterial resistance to antibacterial agents and antibiotics and also shields them from immune responses [166].

Such biofilms are mostly associated with infections, and it is challenging to eradicate using conventional antibacterial treatments, often leading to chronic diseases and augmented bacterial resistance [167]. NPs, measuring 1–100 nm, have distinctive physico-chemical features owing to their small dimensions, substantial surface area-to-mass ratio, and increased chemical response, offer a promising solution to combat these resilient biofilms, making them particularly effective in biomedical applications [168,169]. NPs lower than 5 nm tend to be more biocompatible and beneficial to biological processes, causing minimal disruption of biological and physiological processes [170,171].

Additionally, they have a distinctive ability to enter tissues and penetrate through bacterial cell walls and biofilm matrices via electrostatic interactions [172]. NPs, especially metal-based silver (Ag), zinc oxide (ZnO), gold (Au), copper oxide (CuO), titanium dioxide (TiO_2) and carbon-based (graphene oxide (GO)) and their nanocomposites, are being fabricated to improve interaction with biological systems at cellular and molecular levels, allowing them to perform specialized tasks like targeted drug delivery and antimicrobial action with minimal disturbance to biological processes [173].

NPs have unique qualities such as high surface area-to-volume ratios and improved reactivity, enabling them to penetrate and break *E. faecalis* biofilms and are readily known for their potent antimicrobial properties [174,175]. NPs could break into the EPS layer and have direct antibacterial effects on the biofilm's bacterial cells [176].

Table 1
Phytochemicals towards *E.faecalis* biofilm inhibition.

Bioactive Compound	Class	Performance	References
Proanthocyanidins	Flavanol	50 % inhibition in biofilm growth at 62.5 μg/ml	[151,152]
Quercetin	Flavanol	Reduced biofilm formation by 95 % at 256 μg/ml	[153]
γ-Terpinene	Hydrocarbon monoterpene	BEC_{50} (Biofil eradication concentration) – 416 μg/ml	[154]
Asiatic acid	Pentacyclic triterpene	Suppression of biofilm development and bacterial cell count at 0.75 × MIC.	[154]
Betulinic acid	Pentacyclic triterpene	BA-succinyl ester (25 μM) and BA-ketone derivates (100 μM) significantly reduce biofilm development	[155]
Oleanolic acid	Pentacyclic triterpene	Reduced buildup of biofilm by over 50 % at 62.5 μg/ml	[156]
Ursolic acid	Pentacyclic triterpene	Suppression of biofilm formation at 100 μM	[157]
Cinnamaldehyde	Cinnamaldehydes	Reduced biofilm survivability (72-h old) at 0.5, 0.75, and 1 % wt/vol	[112,158]
Myricitrin (myricetin-3-o-rhamnoside)	Flavonol	Biofilm generation is reduced by more than 60 % at 250 μg/ml.	[159]
Glabridin	Isoflavonoid	Reduced biofilm metabolic action by 11 % at 25 μg/ml.	[160]
Licoricidin	Isoflavonoid	Reduced biofilm development by 31.6 % at MIC – 1.56 μg/ml.	[161]
Curcumin	Curcuminoid	Hindrance of biofilm formation by 23 % at 5 mg/ml. The percentage of damaged cells at 20 μmol/L is 47.5 %.	[162,163]
Urushiol	Alk(en)yl catechol	Destruction of biofilm cells (1 week old) at 10 wt%	[164]

6.2.1. Metallic NPs

Metal NPs can penetrate biological barriers and biofilm structures, bypassing the EPS matrix that protects bacterial cells. Especially their nanoscale size and high reactivity enable them to interact and disrupt biofilm integrity producing antimicrobial effects to the bacterial cells within the biofilms. Scientists have proposed various surface-functionalized nanocomposite materials to increase the specificity and efficacy of engineered nanomaterials against biofilm [177].

Functionalization involves modifying the NP surfaces with chemical or biological ligands that enhance targeting to bacterial cells, reduce cytotoxicity to human cells, and enable controlled release of antimicrobial agents [178]. Among the most extensively studied metal-based NPs, AgNPs are well-known for their intrinsic antibacterial effects from ancient times [179]. AgNPs release Ag^+ ions that interact with bacterial cells, producing ROS that induce oxidative stress, damaging cell membranes, DNA, proteins, and metabolic processes [180].

This multifaceted mechanism is particularly effective against antibiotic-resistant biofilms like those formed by *E. faecalis*, a common pathogen in dental infections [181]. Additionally, AgNPs can interfere with bacterial QS, a cell-to-cell communication system that regulates

gene expression and biofilm stability in *E. faecalis* [182]. AgNPs interrupt the fsr regulatory-mechanism in *E. faecalis*, which reduces factors associated with virulence and inhibits biofilm formation. AgNPs reduce GBAP formation, degrade or adhere to signal molecules, disrupt signal reception by destroying sensor proteins, and suppress QS-regulated genes [182]. Furthermore, AgNPs cause oxidative stress, which further disrupts signaling within cells. AgNPs significantly inhibit the QS-mediated control, lowering biofilm stability and virulence in *E. faecalis*. Au@Ag core-shell NPs have been proposed for controlling *E. faecalis* infection with reduced cytotoxicity and prolonging antibacterial effects [183].

The ability of these metal oxides to generate ROS and release antimicrobial ions makes them promising for biofilm-targeted applications. Ag-chitosan NPs, for example, are AgNPs coated with chitosan, an antibacterial polymer that improves adhesion to biofilms. This synergistic effect enhances the NP's binding to biofilms while contributing to its antimicrobial effects, proving particularly effective against *E. faecalis* biofilms [184]. Studies have also demonstrated the synergistic effects of combining NPs with other antimicrobial agents. For instance, Balto et al. [185] showed that combining AgNPs with calcium hydroxide ($Ca(OH)_2$) led to a biofilm prevention rate of over 90 %. Doxycycline-loaded polymeric NPs have proven effective against *E. faecalis* biofilms, especially in dental applications, as these particles can penetrate biofilms on dentin, eradicating existing biofilms, inhibiting bacterial colonization, and disrupting biofilm architecture [186]. AgNPs have been shown to interfere with QS-regulated gene expression, reducing the synthesis of enzymes required for biofilm stabilization.

6.2.2. Carbon-based NPs

GO, a carbon-based nanoparticle, has unique structural and electronic properties that allow it to interfere with QS in bacteria. GO adsorbs QS molecules, preventing their accumulation in biofilms and disrupting the QS signaling essential for biofilm stability. It is combined with GO's inherent antibiofilm properties, making it an effective tool against *E. faecalis* [187] and chronic wound pathogens [188]. Reduced graphene oxide (RGO), a derivative of GO, has also shown excellent antibiofilm effects with reduced cytotoxicity, making it safer for biomedical applications [189]. Lee et al. [190] demonstrated that combining electric currents with GO improved the effectiveness of sodium hypochlorite (NaOCl), a commonly used disinfectant in root canal treatments. Similarly, GO-NPs can adsorb QS molecules, avoiding buildup and inhibiting QS action. This combined action of biofilm breakdown and QS suppression makes NPs a strong tool for targeting *E. faecalis* biofilms [191].

6.2.3. Hybrid and functionalized NPs

Functionalization can increase NP targeting to biofilms or bacterial cells, minimize toxic effects on human cells, and allow for monitored distribution of antimicrobial chemicals. For instance, ZnO-NPs have shown antibacterial efficacy against *E. faecalis* and other bacterial pathogens, *Serratia marcescens* [192]. ZnO-NPs effectively combat biofilms by creating ROS and Zn^{2+} ions that disrupt bacterial cell wall stability and metabolism. These particles have exhibited excellent antibiofilm action towards *E. faecalis*, even at low doses, suggesting a potential treatment for antibiotic-resistant biofilm-associated infections [193]. Similarly, CuO-NPs also release Cu^{2+} ions that disrupt bacterial metabolic pathways and cell membrane integrity, destabilizing biofilms [194]. Engineered nanomaterials like QDs are gaining popularity for their photocatalytic and redox-active properties, which generate ROS and offer unique mechanisms for antimicrobial action [195].

Cerium oxide (CeO_2) also emerged as promising as it can switch between Ce^{3+} and Ce^{4+} oxidation states, allowing them to scavenge or generate ROS as needed, adapting to various biological environments [196,197]. Whereas, TiO_2 NPs, when exposed to UV light, also generate ROS, which is valuable for non-invasive biofilm disruption on medical devices and other surfaces prone to biofilm formation [198]. In addition, these NPs were found to inhibit bacterial colonization of obstructed dentinal tubules by combating bacteria for that particular site. Furthermore, it has been proven that these materials can attach to the exterior EPS within *E. faecalis* biofilms, severely affecting their architecture organization and overall stability.

Parolia A et al. [199] examined the antibacterial impact of chitosan-propolis NPs as an intracanal medication toward *E. faecalis* biofilm in root canals and determined that NPs were more efficient in lowering *E. faecalis* colony formation.

The potential of novel engineered nanomaterials (ENMs) extends beyond dental applications, showing promise in treating a range of biofilm-associated infections. Despite the promise of innovative ENMs-enabled biofilm disruption approaches, significant limitations remain in improving disinfection outcomes and controlling biofilm formation [200].

Metal-based NPs, drug-loaded NPs, and naturally derived NPs can target infections that resist conventional therapies. However, realizing the full therapeutic potential of nanotechnology requires extensive preclinical and clinical testing to ensure safety, efficacy, and compatibility with human biology [201]. Key considerations include understanding ENM cytotoxicity, optimizing dosing protocols to avoid adverse effects, and evaluating the long-term impact of exposure [202]. Additionally, given the increased use of NPs, researchers are investigating whether bacteria might develop resistance mechanisms to NPs, as they have with traditional antibiotics [203]. However, in order to demonstrate how effective they are in treating biofilm illnesses, they must undergo extensive testing and assessment in clinical settings [204].

6.3. Phages (bacteriophages)

Phages are viruses that can infect and destroy bacteria. They have a distinctive capability to penetrate bacterial biofilms and cause destruction. Phage treatment has been shown to be more effective than traditional antibiotics, especially when infections are triggered by multi-drug resistant (MDR) biofilms [205]. Phages have specificity to certain bacterial species or even distinct strains, rendering them an excellent treatment alternative for specifically targeting and eradicating infections [206].

Phages usually have a highly positive, safe profile and therapy using phages is linked with a minimal risk for negative responses and adverse effects [207]. Phages destroy bacterial biofilms using a variety of complicated methods, as shown in Fig. 8. The phages reproduce within bacterial cells, causing cell lysis followed by the expulsion of offspring phages that eventually break down biofilms. Certain phages attack inactive cells, stay dormant until they reactivate, and then lyse them [208]. Endolysins and holins are enzymes encoded by phages that disintegrate bacterial cell walls, allowing infectious particles to be released and biofilm formations to break down. Furthermore, phage-produced depolymerases break down the EPS matrix in biofilms, weakening them and enabling a deeper entry and more efficient elimination. Phages impair bacterial communication systems, such as QS, and interfere with the control mechanisms necessary for biofilm sustaining and stabilization [209].

Phage-assisted breakdown of bacterial biofilms and QS suppression need a complex interaction between bacteriophages and their bacterial hosts. Phages can successfully destroy preexisting biofilms by many mechanisms: they produce lytic enzymes, such as tail-associated depolymerases and peptidoglycan hydrolases, that degrade the EPS required for biofilm integrity [210]. Furthermore, some phages activate host bacterial enzymes, which leads to biofilm destruction. Certain phages use hydrophobic channels inside biofilms to penetrate and lyse bacteria from within, whereas other types disrupt QS-regulated interaction by releasing inhibitors such as lactonases, which impair biofilm development and promote structural collapse [211]. These activities can be considerably improved when phages are coupled with antimicrobials such as antibiotics, xylitol, or natural substances like honey, resulting in

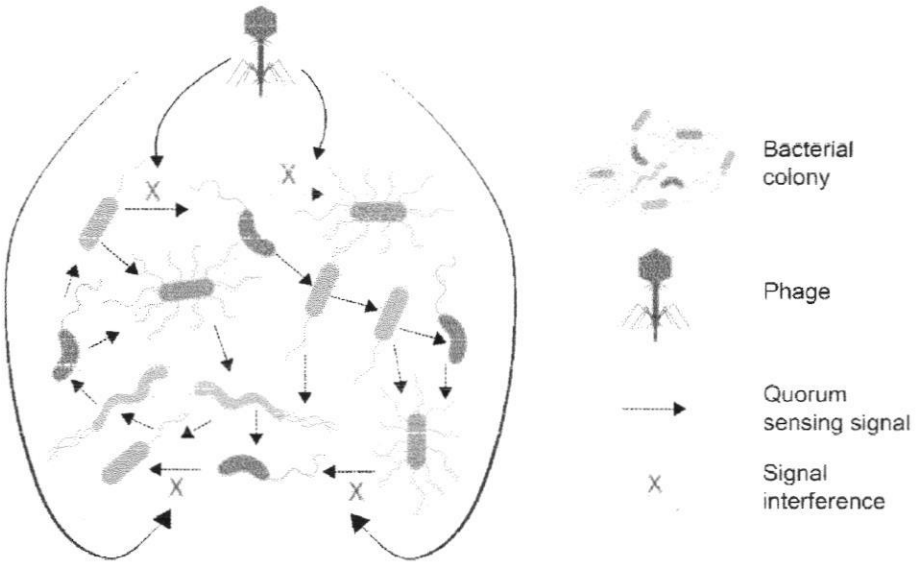

Fig. 8. Phage-assisted biofilm disruption and QS inhibition (Copyright © Frontiers 2024. All rights reserved, reprinted with permission) [61].

synergistic biofilm elimination [212]. Phages also affect bacterial QS systems to aid in infection and proliferation. QS systems control the development of surface appendages (e.g., flagella, pili) and membrane proteins that act as phage receptors; phages can change these QS pathways to promote or prevent adsorption [213]. Furthermore, phages like VP882 encode QS-responsive proteins, which identify bacterial community density and induce rupture rather than lysogeny under high-density conditions [214].

Phage treatment is frequently used in conjunction with antibiotics to improve activity towards biofilms. This strategy combines phages' ability to destroy biofilms with antibiotics' ability to eradicate bacteria. Phages and antibiotics work better together to destroy *Enterococcus* biofilms, according to research. The combinatorial effect of phage and gentamicin resulted in a considerable reduction in *E. faecalis* biofilm biomass when compared to either treatment alone [215]. Integrating phages with QS inhibitors is a potential method. QS inhibitors that block the fsr QS system in *E. faecalis* hinder bacteria from forming biofilms or producing virulence proteins and make the bacterium more susceptible to phage attacks.

The synergistic effect of QS inhibitors with phages towards targeting biofilm-forming *Enterococcus* resulted in a considerable reduction in biofilm stability and increased phage effectiveness [216]. Shlezinger et al. [217] discovered that treatment with EFLK1 phage at concentration 1.2×10^8 PFU/well significantly disrupts and decreases 72 h biofilm development in the *E. faecalis* V583 strain, resulting in a considerable reduction of around 81 %.

E. faecalis phage SHEF2, derived from the oral cavity of a patient with an infected root canal, was able to eliminate the biofilm produced on the surface of polystyrene *In vitro* and treat an *E. faecalis* infection in a zebrafish model. It can cure antibiotic-resistant *E. faecalis* infections [218]. Investigations in the model of mice have shown that combining phage treatment with ampicillin significantly improves effectiveness towards Vancomycin-resistant *E. faecalis* [219]. The combined action of vancomycin with phages reduced *E. faecalis* biofilm biomass by 87 %, demonstrating their efficiency in disrupting and suppressing biofilm formation [217].

Furthermore, Song et al. [220] investigated the bacteriophage vB_EfaM_LG1 (LG1), derived from medical facility sewage, and identified that when combined with an antibiotic, it greatly suppressed and disintegrated the *E. faecalis* biofilm. Phages play a key role in horizontal gene exchange amongst communities of bacteria, which may result in the propagation of antibiotic-resistant genes [221]. This process allows genetic material to migrate between bacteria through methods other than usual reproduction, frequently via a technique known as transduction, in which phages mistakenly bundle bacterial DNA and transport it to new host cells. One of the most significant implications is the propagation of antibiotic-resistant genes [222]. When phages spread these genes across bacteria, they can lead to the creation of multidrug-resistant species, also known as superbugs.

This not only increases bacterial adaptation but also creates significant challenges for infection treatment, making phage-driven transfer of genes a major concern in both microbiology and medicine [223]. Phage-derived defensive systems allow them to keep up with other phages, which eventually benefits the host bacteria [224]. A variety of tactics are used to improve phage treatment and combat bacterial resistance. These include boosting phage dose and employing highly lytic phages to eradicate microbes quickly, in addition to adopting wide-spectrum or highly effective phages, sometimes known as phage cocktails, for targeting varied strains while minimizing resistance [225]. Most bacteriophages have a limited host range, so phage cocktails must be precisely customized to specific bacterial strains. This precision targeting enables phages to target pathogens despite disturbing beneficial microbiota; however, mismatched phages may result in inadequate biofilm elimination or therapy failure, particularly in polymicrobial or strain-diverse diseases [226]. Resistance to therapeutic phages in *E. faecalis* can emerge through processes such as receptor alteration or CRISPR-Cas systems; however, the use of varied phage cocktails and phage-antibiotic combinations can help limit this risk [227].

Integrating phages and antibiotics can improve antimicrobial effectiveness while lowering the risk of resistance. Furthermore, exploratory phage development by laboratory-assisted mutual evolution with bacteria can result in "trained" phages that are more efficient toward resistance strains [228]. Phage treatment holds great potential as a substitute for antibiotics for the treatment of *E. faecalis* infections. However, its clinical implementation involves overcoming significant difficulties, including regulatory vagueness, the requirement for tailored and quickly adaptive phage formulations, scalability manufacturing challenges, potential host immune system neutralization, and the continual evolution of bacterial resistance [229]. These concerns emphasize the significance of ongoing research and infrastructure development to facilitate the incorporation of phages into mainstream medical practice.

Prospective studies should concentrate on expanding our knowledge of phage-bacteria associations, enhancing phage development and

distribution systems, and creating regulatory systems to ensure both safety and effectiveness [230]. Furthermore, integrating phage therapies with antibiotics or new antimicrobials may aid in resistance reduction. By addressing these limitations, phage therapy has the potential to change the approach to MDR and biofilm-dependent illnesses [231].

6.4. Probiotics

Probiotics are live bacteria that, when consumed in sufficient quantities, can offer health advantages. Probiotic compositions often contain species such as *Lactobacillus* and *Bifidobacteria* taken from healthier people's gut microbiota [232]. Research has shown that they have medicinal promise, especially in terms of gut health and digestive diseases. Furthermore, probiotics have shown potential in treating chronic inflammation-related conditions, resulting in a fascinating area of research in microbiology and therapeutic approaches [233].

Probiotics have shown promise in fighting *E. faecalis* infections, notably by dealing with biofilm development and QS, which are crucial components in the bacteria's pathogenesis and capacity to resist therapy. Probiotics fight *E. faecalis* biofilms by specific mechanisms that impair biofilm integrity and diminish bacterial pathogenicity. One major technique is to degrade the EPS matrix, which is required for biofilm stability. Probiotic-derived enzymes, such as proteases and DNases, degrade essential EPS components, particularly eDNA and surface-associated proteins like biofilm-associated protein (Bap), degrading the biofilm framework [234].

Furthermore, probiotics block QS, specifically the *fsr* signaling system in *E. faecalis*—which regulates the development of virulent factors such as GelE and SprE. Probiotics interfere with these signaling pathways, preventing the synchronized behavior essential for successful biofilm development [235]. Probiotics modulate host immune responses by increasing anti-inflammatory cytokine production (e.g., IL-10) and suppressing pro-inflammatory mediators (e.g., IL-6, TNF-α) by association with TLRs on epithelial and immune cells. Due to their dual function of disrupting biofilms and modulating immunity, probiotics are promising adjuncts in controlling persisting *E. faecalis* infections, particularly in the setting of antibiotic resistance [236].

Probiotics such as *Lactobacillus* species may be competing with *E. faecalis* for surface adherence areas in the gastrointestinal or mouth mucosa, therefore reducing biofilm development in its early stages [237]. Some probiotics synthesize bactericides (e.g., *Lactobacillus* and *Bifidobacterium* species), organic acids, and hydrogen peroxide, all of which may readily hinder *E. faecalis* development and disrupt the biofilm framework [238]. Certain probiotics release enzymes such as proteases and DNases, which destroy the EPS in the biofilm matrix, affecting the biofilm's structural integrity.

Probiotics can improve the host's immune response by increasing the development of antimicrobial peptides and immune cell types that fight biofilm-forming bacteria like *E. faecalis* [239]. Some probiotics can inhibit the AIs employed in *E. faecalis* QS systems. *Lactobacillus reuteri* has been demonstrated to synthesize components that inhibit infectious bacteria's QS, preventing the coordination necessary for biofilm development and pathogenicity [240].

Several probiotic strains may deteriorate or sequester messenger molecules utilized in QS, such as AHLs (acyl-homoserine lactones), limiting the bacterial interaction essential for biofilm formation and stability. Probiotics may reduce the expression of critical genes connected with QS processes, restricting bacteria from initiating biofilm-related processes or producing virulent factors [241]. *E. faecalis* uses a peptide-based QS system, specifically the *fsr* system, to regulate important virulence genes, such as *gelE* and *sprE*, which are required for biofilm formation and tissue invasion. Probiotics may interfere with this interaction by degrading signal peptides or decreasing the production of QS-related genes [242]. *Lactobacillus plantarum*, for example, has been demonstrated to severely impede *E. faecalis* biofilm formation by inhibiting the *fsr* system and reducing gelatinase activity, resulting in inadequate biofilm architecture and pathogenicity. Furthermore, probiotics may release antimicrobial compounds, such as bacteriocins and organic acids, which inhibit *E. faecalis* proliferation and QS activity. Probiotics show promise in preventing or controlling infections triggered by biofilm-forming, antibiotic-resistant bacteria such as *E. faecalis* [243].

Lactobacillus plantarum is known for its ability to prevent biofilms and have antimicrobial action against a variety of diseases [244]. *Lactobacillus rhamnosus* GG has been shown to inhibit biofilm development and modify host immune responses for combating *E. faecalis* [245]. *Bifidobacterium* strains can prevent *E. faecalis* biofilm development and synthesize metabolites that impair QS signals [246]. Jung et al. [247] discovered that lipoteichoic acid (LTA) synthesized by *Lactobacillus* can suppress *E. faecalis* biofilm development, with a persistent inhibiting impact at the earliest stages. Furthermore, LTA produced by various *Lactobacillus* species, including *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus rhamnosus*, showed a comparable capacity to suppress the formation of *E. faecalis* biofilms.

Thus, LTA generated from *Lactobacillus* species serves as a potential anti-biofilm agent, providing prospective uses in the prevention or treatment of illnesses linked with *E. faecalis*. Bohora et al. [248] examined three probiotics: *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Bifidobacterium bifidum*. The study investigated how these probiotics inhibited bacterial growth in both planktonic and biofilm phases. All probiotics inhibited growth during the planktonic stage. In the biofilm phase, adding 30 % poloxamer 407 (Pluronic F-127) to the De Man, Rogosa, and Sharpe media with probiotics resulted in various degrees of suppression of *E. faecalis* biofilm development. The study also identifies poloxamer 407 as a possible technique for delivering probiotics into the root canal system. Shaaban et al. [249] investigated the influence of multi-strain probiotic supernatants combining *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus* on *E. faecalis* biofilms. After 24 h, multi-strain probiotics effectively lowered biofilm development compared to $Ca(OH)_2$. After 7 days, both probiotics and Ca $(OH)_2$ showed comparable antibacterial actions, drastically lowering biofilm populations. The data imply that an extended contact period may improve antimicrobial benefits, but further investigation is necessary to discover the most efficient concentration of multi-species probiotics.

Safadi et al. [250] studied the effectiveness of probiotics as a replacement irrigant for removing *E. faecalis* biofilms. The study investigated the effects of three probiotic strains (*Lactobacillus casei*, *Lactobacillus plantarum*, and *Bacillus coagulans*) on pre-existing *E. faecalis* biofilms. The data revealed that both *L. casei* and *Lactobacillus plantarum* effectively destroyed developed *E. faecalis* biofilms and reduced their regeneration, whereas *Bacillus coagulans* failed to demonstrate equal efficiency. The advantageous effects of probiotics on host health are well known.

Still, the investigation has aroused worries concerning potential hazards in immune-weakened or severely unwell individuals, such as malfunctioning various organs or shock. While the research continues to be in the beginning stages, further *in vivo* investigations are required to corroborate these conclusions [251]. These investigations must first begin with rigorous experimental designs and detailed biosafety evaluations to assure both effectiveness and safety. Furthermore, subsequent studies should look into novel administration mechanisms, such as biocompatible transporters and sustained-release compositions, to improve the potential for therapy as well as the safety of probiotics for widespread clinical use [252].

7. QS-based regulation and biofilm dispersion

The *fsr* QS system in *Enterococcus* modulates pathogenesis and gene expression, notably biofilm formation. The fsr operon regulates QS-related genes; however, its influence on biofilm development may be indirect by affecting other biofilm-determinant variables. The *fsrA*

promotes the production of *fsr* genes, whereas *fsrD* codes a pro-peptide that is converted into GBAP, the messenger molecule produced by FsrB. FsrC activates a phosphorylated sequence that additionally regulates the *fsr* system. Its major function is to induce GelE synthesis, which may influence biofilm growth [253].

The *fsr* QS system in *Enterococcus* may control the development of biofilm, but its role varies by variety and strain. Enhanced GelE action has been associated with the discharge of eDNA, which is an essential component of *Enterococcus* biofilms [254]. Some investigations have found that inhibiting the *fsr* system reduces biofilm development. Furthermore, the *fsr* system regulates the production of adhesive proteins and surface proteins, which are required for surface attachment, a fundamental stage in biofilm growth on both host tissues and non-living surfaces like healthcare equipment [255].

The major product of the *fsr* system, GelE, is involved in the breakdown of proteins, which aids in invading tissue and infection. Whereas the biofilm dispersal process in *Enterococcus* is not entirely comprehended, phage-assisted dispersal has been observed in *P. aeruginosa* [256].

Recent research has highlighted the capability of phage-antibiotic combined action to break biofilms. *E. faecalis* genome contains roughly 7 prophages, and AI-2 signaling can activate prophage expression, resulting in self-destruction. This forms voids in the biofilm, which aids in the dissemination of non-infected cells; however, the exact molecular pathways remain unknown [257].

Phage therapy has emerged as a promising approach. The phage EFDG1, isolated from sewage, effectively targets biofilms formed by clinical strains of *Enterococcus*, including vancomycin-resistant strains [214]. Phage therapy demonstrated efficacy in both *In vitro* and *in vivo* infection models. However, more research is needed to investigate the differential gene expression in dispersed cells from *Enterococcus* biofilms, as has been studied in other bacterial species [258]. Several drugs, namely synerazol, phenalinolactones A-D, and BU-4664LMe, have been found to be prospective AgrA and Fsr QS inhibitors. Moreover, acetylsalicylic acid and trifluoperazine were investigated for their association with StrA, showing significant amino acid residues and bonds at the site of action. The results indicate that the determined compounds could efficiently suppress QS, providing viable techniques to reduce the infectiousness of the *E. faecalis* bacterium [259,260].

8. Conclusion and future Perspectives

E. faecalis is a powerful invasive pathogen due to its adaptability in protecting biofilms, expressing virulent factors, and developing immunity to numerous antibiotic classes, including VRE. The QS processes, especially the Fsr and LuxS systems, are critical in controlling the expression of genes involved in biofilm formation and pathogenicity, such as GelE and SprE. These QS-regulated mechanisms are vital to *E. faecalis* pathogenesis because they allow the bacteria to establish colonies, survive, and hinder host immunological responses and antimicrobial treatments, particularly to healthcare equipment and wounded tissues. Prospective therapeutic techniques addressing *E. faecalis* QS systems offer a potential replacement to conventional antibiotics, particularly given biofilms' increased capacity to resist common antimicrobial drugs. Innovations in QS inhibitors, including TC, plant-based medicines, and biofabricated NPs, have the potential to reduce biofilm growth and pathogenicity [37].

When used with traditional antibiotics, these techniques may improve treatment success by disrupting biofilm stability and reducing bacterial resistance processes. Furthermore, phage therapy and modified bacteriophage-sourced enzymes suited to destroying the biofilm extracellular matrix provide an additional strategy for addressing *E. faecalis* biofilms. For the advancement of multi-target, combinatorial medicines, further investigations should target understanding the molecular processes driving QS-regulated biofilm propagation, stress control, and antibiotic resistance. Genomic and proteomic investigations may uncover innovative targets within QS mechanisms that can be used to limit the establishment and dissemination of *E. faecalis* biofilms.

Expanding their knowledge of these regulatory networks can help researchers design more accurate antivirulence and antibiofilm therapies, resulting in better healthcare administration and lowering the incidence of *E. faecalis*-related illnesses in hospital settings.

CRediT authorship contribution statement

Kayeen Vadakkan: Writing – original draft, Writing – review & editing, Conceptualization. **Gajanan Sampatrao Ghodake:** Supervision, Validation, Writing – review & editing, Conceptualization. **Chin Wei Lai:** Validation, Supervision, Data curation. **Selvaraj Vijayanand:** Supervision, Investigation, Conceptualization. **Janarthanam Hemapriya:** Supervision, Validation, Data curation.

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.

References

[1] N. Li, G. Tan, Z. Xie, W. Chen, Z. Yang, Z. Wang, S. Liu, M. He, Distinct enterotypes and dysbiosis: unraveling gut microbiota in pulmonary and critical care medicine inpatients, Respir. Res. 25 (2024) 304, https://doi.org/10.1186/s12931-024-02943-7.

[2] H.M.S. Goh, M.H.A. Yong, K.K.L. Chong, K.A. Kline, Model systems for the study of enterococcal colonization and infection, Virulence 8 (2017) 1525–1562, https://doi.org/10.1080/21505594.2017.1279766.

[3] B. Krawczyk, P. Wityk, M. Gałęcka, M. Michalik, The many faces of enterococcus spp.—Commensal, probiotic and opportunistic pathogen, Microorganisms 9 (2021) 1900, https://doi.org/10.3390/microorganisms9091900.

[4] A. Daca, T. Jarzembowski, From the friend to the foe—enterococcus faecalis diverse impact on the human immune system, Int. J. Mol. Sci. 25 (2024) 2422, https://doi.org/10.3390/ijms25042422.

[5] G. Sangiorgio, M. Calvo, G. Migliorisi, F. Campanile, S. Stefani, The impact of enterococcus spp. in the immunocompromised host: a comprehensive review, Pathogens 13 (2024) 409, https://doi.org/10.3390/pathogens13050409.

[6] F. Nappi, Current knowledge of enterococcal endocarditis: a disease lurking in plain sight of health providers, Pathogens 13 (2024) 235, https://doi.org/10.3390/pathogens13030235.

[7] H. Liu, S. Nio, Y. Shen, Sodium hypochlorite against Enterococcus faecalis biofilm in dentinal tubules: effect of concentration, temperature, and exposure time, Odontology 112 (2024) 390–398, https://doi.org/10.1007/s10266-023-00850-9.

[8] M. García-Solache, L.B. Rice, The enterococcus: a model of adaptability to its environment, Clin. Microbiol. Rev. 32 (2019), https://doi.org/10.1128/CMR.00058-18.

[9] A. Alziyadi, M. Al Adwani, H. Abdulaziz, R. Alloufi, A. Althagafi, A. Alsulaimani, A. Alotaibi, Enterococcus faecalis infective endocarditis associated with colorectal cancer, Cureus (2024), https://doi.org/10.7759/cureus.55096.

[10] C. Nye, A. Maxwell, H. Hughes, J. Underwood, Enterococcus faecalis bacteraemia and infective endocarditis - what are we missing? Clin. Infect. Pract. 21 (2024) 100336 https://doi.org/10.1016/j.clinpr.2023.100336.

[11] G. Mancuso, A. Midiri, E. Gerace, C. Biondo, Bacterial antibiotic resistance: the Most critical pathogens, Pathogens 10 (2021) 1310, https://doi.org/10.3390/pathogens10101310.

[12] L. Guan, M. Beig, L. Wang, T. Navidifar, S. Moradi, F. Motallebi Tabaei, Z. Teymouri, M. Abedi Moghadam, M. Sedighi, Global status of antimicrobial resistance in clinical Enterococcus faecalis isolates: systematic review and meta-analysis, Ann. Clin. Microbiol. Antimicrob. 23 (2024) 80, https://doi.org/10.1186/s12941-024-00720-w.

[13] K. Vadakkan, K. Sathishkumar, S. Kuttiyachan Urumbil, S. Ponnenkunnathu Govindankutty, A. Kumar Ngangbam, B. Devi Nongmaithem, A review of chemical signaling mechanisms underlying quorum sensing and its inhibition in Staphylococcus aureus, Bioorg. Chem. 148 (2024) 107465, https://doi.org/10.1016/j.bioorg.2024.107465.

[14] D. Ashraf, M.I. Shaaban, R. Hassan, A.M.A. El-Aziz, Polidocanol inhibits Enterococcus faecalis virulence factors by targeting fsr quorum sensing system, BMC Microbiol. 24 (2024) 411, https://doi.org/10.1186/s12866-024-03548-x.

[15] M.F. Del Papa, M. Perego, Enterococcus faecalis virulence regulator FsrA binding to target promoters, J. Bacteriol. 193 (2011) 1527–1532, https://doi.org/10.1128/JB.01522-10.

[16] J. Zhang, R. Fang, Q. Peng, S. Wu, L. Lei, The regulations of essential WalRK two-component system on Enterococcus faecalis, J. Clin. Med. 12 (2023) 767, https://doi.org/10.3390/jcm12030767.

[17] L. Ali, M. Goraya, Y. Arafat, M. Ajmal, J.-L. Chen, D. Yu, Molecular mechanism of quorum-sensing in enterococcus faecalis: its role in virulence and therapeutic