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REVIEW ARTICLE

The Impact of Antibiofilm Strategies in Controlling Microbial Colonization

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Abstract

Biofilms play a crucial role in persistent infections and antibiotic resistance owing to their structural complexity and protective matrices. This review focuses on current antibiofilm techniques to avoid or inhibit microbial colonisation. Key approaches involve the employing of antimicrobial peptides, quorum sensing inhibitors, quorum quenching agents, matrix-degrading enzymes, nanoparticles, and bacteriophages. These techniques inhibit biofilm formation, increase antimicrobial penetration, and impede biofilm integrity. The focus is on *Pseudomonas aeruginosa* and *Staphylococcus aureus*, two clinically relevant biofilm-forming bacteria. Quorum quenching, which interrupts microbial communication networks, has emerged as a viable technique for inhibiting pathogenesis and biofilm growth. Efforts have been made to identify various compounds used for inhibiting quorum Sensing. Various plant extracts and enzymes have been proven to affect quorum sensing profoundly. Another strategy is to inhibit the genes responsible for biofilm formation. Certain genes can be upregulated or downregulated to prevent biofilm formation, or component production can be minimised. A multidisciplinary strategy that incorporates these tactics can give effective approaches for treating biofilm-related illnesses in healthcare and industrial settings.

Introduction

A widespread misperception is that bacteria are mostly found as isolated, free-floating (planktonic) cells. Whereas, bacteria tend to live a sessile lifestyle, aggregating on both biotic and abiotic surfaces, which include medical implants, contact lenses, and industrial pipelines. These surface-associated microbial populations establish biofilms, which are complex multicellular aggregates encased inside a self-produced extracellular polymeric substance (EPS) matrix. [1]. Biofilms are made up of different bacterial and fungal communities that thrive in a variety of environments, from submerged surfaces to floating mats on liquid interfaces. This communal lifestyle enables critical survival activities, such as increased resilience to external stressors and the ability to eliminate hazardous metabolic wastes [2]. The EPS matrix performs both physical and biochemical activities, acting as a barrier that prevents antibiotic penetration and protects microbial cells from immune system clearance. The biofilm framework includes microenvironments and nutrition routes, which promote microbial diversity and resilience. Biofilm formation normally occurs in stages: initial reversible adherence of planktonic cells, irrevocable attachment helped by EPS production, maturation into organised communities, and cellular dispersion to colonise new surfaces [3].

Biofilms have significant significance in clinical settings as they are linked to nearly 80% of nosocomial (hospital-acquired) infections, of which *S. aureus* is the most notorious on the list, particularly those caused by indwelling medical equipment [4, 5]. The ESKAPE bacteria, which include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species, constitute some of the most challenging biofilm-forming pathogens. These organisms are well-known for their capacity to "escape" the effects of standard antibiotics and host immunological responses. Their ability to build persistent biofilms contributes greatly to multidrug resistance and pathogenicity, posing considerable hurdles in infection control [6]. Recognising and treating ESKAPE pathogens is crucial for developing effective

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antibiofilm medicines and infection control techniques [7]. Although biofilms have been known for over six decades, their architectural and functional complexity is only now beginning to be entirely understood [8]. Particularly, fossilised biofilms dating back 3.5-billion years indicate that biofilm development is an ancient and evolution-conserved surviving strategy [9]. Biofilm structure creates a defensive environment that allows bacteria to tolerate a variety of environmental challenges, such as antimicrobial exposure, nutrition constraints, and UV radiation [10]. This review aims to provide a thorough understanding of how microbial biofilms originate, function, and give rise to infections, particularly those produced by *S. aureus* and *P. aeruginosa*. It also investigates different current and new ways of preventing or breaking down biofilms, including the application of plant-based chemicals, enzymes, and tactics that disrupt bacterial interactions and gene expression. The purpose is to highlight effective techniques for controlling biofilm-related illnesses in medical and industrial settings.

Architectural organisation of biofilms

Biofilms are the aggregation of bacteria rooted in an extracellular matrix of exopolysaccharides or extracellular polymeric substances (EPSs), proteins, glycolipids, and some micro molecules like extracellular DNA. They can be found on both biotic and abiotic surfaces. They are complex and 3-dimensional structures. It can be defined as a microbially derived sessile community that exhibits a transformed phenotype for growth [11]. Even though biofilms arise from a single cell, different environmental conditions can potentiate the development of distinct subpopulations. Oxygen, nutrients, electron acceptors, etc., can cause diverse gene expression in a biofilm. The result of these characteristics leads to major problems in industries like biofilm formation by multi-species, which leads to high costs and the medical field, like the spread of infections, sepsis shock, and the risk of removal of surgical implants [3]. Beyond environmental impact, biofilms also play a critical role in medical contexts. Figure 1 illustrates the stepwise formation of biofilms, starting with the reversible adherence of planktonic bacterial cells to a surface. The stages of biofilm growth include the initial adhesion of planktonic bacteria and progressing to microcolony development, maturation into a structured community, and eventual dispersion. Understanding these stages is crucial for determining the intervention phases of biofilm formation. Analysing the EPS coating, it was found to behave technically as hydrogels exhibiting viscoelastic behaviour, which allows the biofilms to withstand mechanical stress. The bacteria will use nutrients and water trapped in the matrix of EPS. Another important biofilm function is QS (cell–cell communication) and horizontal gene transfer of genetic material [12].

Biochemical and structural components of biofilms

Biofilms are mainly composed of exopolysaccharides, extracellular proteins, and eDNA. These exopolysaccharides may be synthesised either intracellularly or extracellularly and

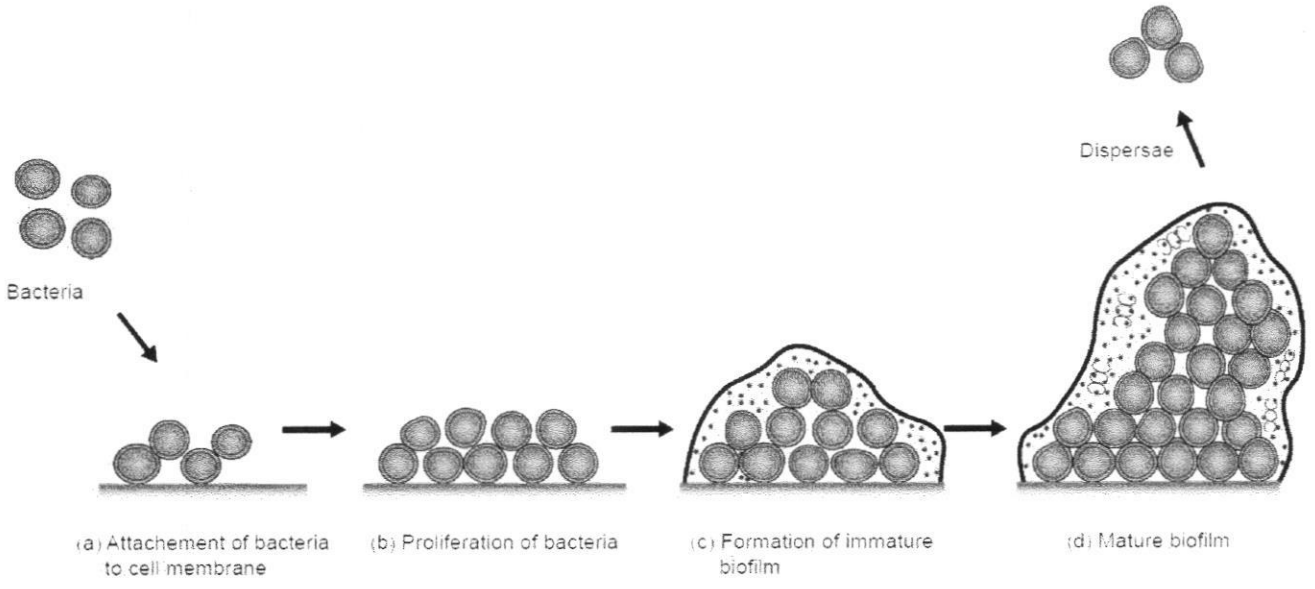

Fig. 1 *Stages of biofilm development.* The diagram depicts five key stages: **i** reversible attachment of planktonic cells, **ii** irreversible adhesion via EPS production, **iii** microcolony formation, **iv** maturation into a structured 3D biofilm, and **v** dispersion of cells to colonize new surfaces

later will be secreted to the outside in the form of linear or branched long strands forming large networks. They serve as the framework for other biomolecules for adherence [13]. EPS are critical components in the formation and maturation of bacterial biofilms and play various structural and functional roles within biofilms. Bacteria release these high-molecular-weight polysaccharides and play a crucial role in the adhesion of cells to both biotic and abiotic surfaces, thereby promoting the irreversible connexion required for biofilm formation. EPS generates a cohesive and protective extracellular matrix that helps the biofilm maintain its structural integrity, allowing complex, multilayered communities of microbes to develop [14]. This matrix also serves as a physical barrier, protecting the embedded bacteria from environmental pressures, such as antimicrobial chemicals, immunological reactions, and mechanical shear forces.

Furthermore, EPS play an important role in nutrient retention and diffusion, which promotes bacterial longevity and metabolic activity inside the biofilm. In addition, they strengthen intercellular communication through QS and horizontal gene transfer, both of which are necessary for biofilm maturation and the emergence of antimicrobial resistance. Because of their multifaceted activities, EPS are regarded as essential factors for biofilm resilience and potential targets for anti-biofilm treatment methods [15]. Different exopolysaccharides have different components and structural properties. Glucose, galactose, and mannose are the most abundant saccharides in the EPS matrix, followed by galacturonic acid, fucose, and arabinose [16]. Exopolysaccharides are not specific for biofilms and tend to increase due to stress, such as alginate synthesis in *P. aeruginosa,* which is composed of d-mannuronic acid residues interspersed with l-guluronic acid residues. Another example is colonic acid (M-antigen) in *Escherichia coli*, which is a repeated unit of l-fucose, d-galactose, d-glucuronate, and d-glucose, and decks with *O*-acetyl and pyruvate side chains [17].

Staphylococcus contains PIA or polysaccharide intercellular adhesin, composed of poly *N*-acetyl glucosamine, a linear polymer consisting of β-1,6-linked glucosamine residues. The exopolysaccharide in *Bacillus subtilis* is poly δ-glutamate, which also depends on the strain and conditions. Figure 2 depicts the structural components of the mature biofilms. The essential structural components of a biofilm, particularly EPS, provide mechanical stability, antibiotic resistance, and immunological responses. Extracellular proteins are an important component of biofilms. Some of these were attached to the cell surface. At the same time, others may be connected to polysaccharides to aid in biofilm formation and stabilisation, for example, glucan-binding proteins (Gbps) [18]. *S. mutans* biofilms play an important role in maintaining the biofilm architecture. Another example is amyloids, which are fibrous proteins produced by Pseudomonas spp. that lead to cell aggregation and increased biofilm formation. An important example of extracellular proteins is the BaP-biofilm-associated protein family, which includes proteins from *S. aureus* and Esp proteins from *Enterococcus faecalis,* which are involved in biofilm formation and infection [19].

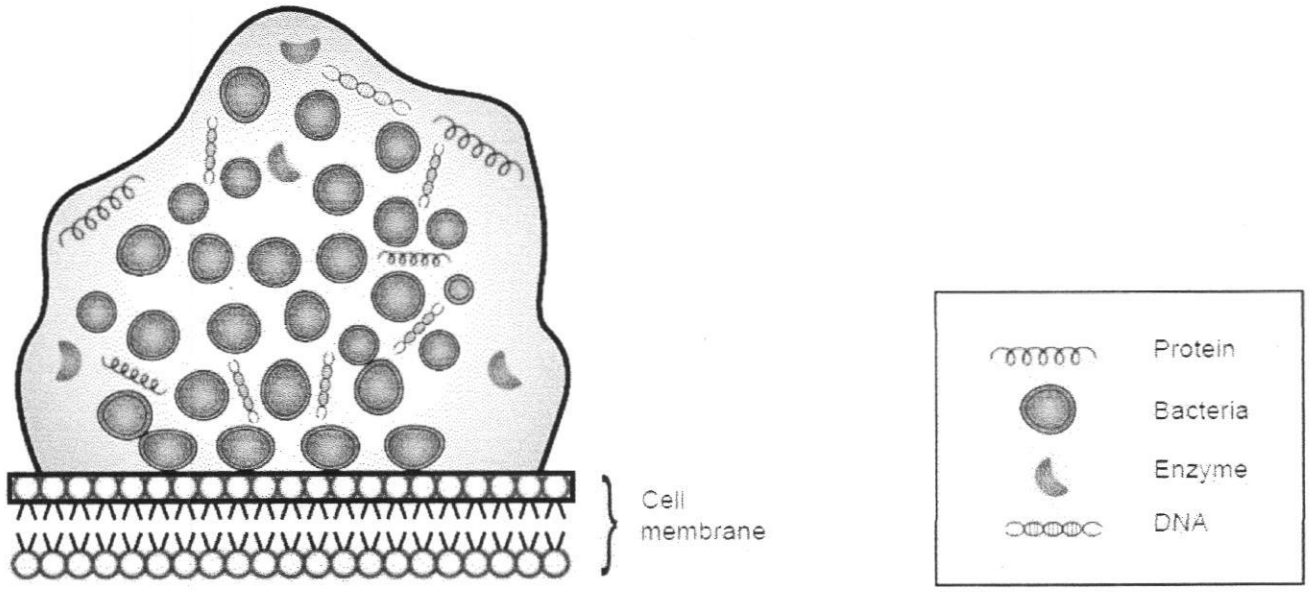

Fig. 2 *Structural components of a bacterial biofilm.* This illustration highlights key elements of the biofilm matrix, including exopolysaccharides (EPS), extracellular DNA (eDNA), and extracellular proteins

Biofilm dynamics: advantages and adverse impacts

Biofilm-forming microbes participate in the production and degradation of organic matter and the bioremediation of recalcitrants. Biofilms are involved in sewage purification. Biofilms are important for the treatment and nitrification of petroleum-contaminated groundwater [20]. Biofilms in rhizospheric soil help increase soil fertility and promote plant growth [21]. Another study showed that polymer degradation was efficiently promoted by microbial biofilms in the polymer, and a higher fitness was achieved in terms of reproductive competency. Biofilms are important for sulphur cycling in acid mine drainages [22]. EPS is an important component that helps detoxify heavy metals by precipitating after transformation under the extracellular activities of enzymes. The microbes present in the biofilm have higher metabolic diversity in terms of their function [23]. This is the reason for phenotypic plasticity and enhanced metabolic diversity in improving polymer degradation. Marine snow, an unusual structure, contains a biofilm of organic and inorganic materials capable of transforming particulate organic carbon into marine ecosystems [24].

In addition to their beneficial effects, biofilms have unadorned pathogenic effects. These bacterial communities often exhibit antibiotic resistance, virulence factor production, and slower growth rates [25]. Biofilms play an important role in the evolution of virulent and antibiotic-resistant strains by allowing horizontal gene transfer between the microbial communities. This genetic exchange promotes the spread of resistance amongst species. In addition, the EPS matrix generated by biofilms acts as a physical barrier, limiting antibiotic penetration and protecting embedded bacteria from the host immune response. These elements work together to increase the persistence and durability against biofilm-associated infections. Biofilms also resist substances other than antibiotics such as amoebae, bacteriophages, and biocides.. Another manifestation of biofilm that harms the body is the secretion of cytokines, which may cause wounds to the surrounding tissues rather than destroy the biofilm [26].

Structural and ecological classification of biofilms

Some biofilms may be bilayers, whilst others may be monolayers based on the interaction between cells, and it is observed that in monolayers, the interaction between the cells is low compared to the interaction between the cell and the surface. For monolayer attachment, the main adhesive structure observed is the flagellum or pilus, which helps increase attachment and accelerate biofilm formation. The other category is adhesin synthesis and synchronised transition to aid permanent attachment. Microbes develop multilayers when they adhere to each other and the surface; in addition, repulsion is observed depending on the surface characteristics [27]. For example, the cell wall properties of Gram-negative bacteria are determined by the negative charge of the O-antigen. Thus, a multilayered biofilm is possible only if the repulsive force is neutralised. To neutralise the repulsive force, O antigen-synthesising genes must be silenced, downregulated or mutated [28].

Mechanisms of biofilm formation

Cells that attach to the surfaces begin their cell division, forming microcolonies that produce extracellular polymers or EPS, which provide structure to the biofilm, which is highly hydrated and later forms water channels that allow the transport of nutrients and oxygen to the cells [29]. Biofilms progress via a five-stage process: (i) migration of planktonic cells and adherence to a surface. These cells will start biofilm production slowly, provided the conditions are apt; (ii) cell aggregation and matrix formation take place soon after the formation of EPS and attach to the surface firmly; (iii) development of microcolonies by the maturation of biofilms and layer formation takes place; (iv) a 3-D community is formed after the biofilm reaches a maximum cell density; and v) once maturation is attained, microcolonies are released which migrate to other surfaces and spread to other places [30]. Organisms associated with biofilms grow slowly because the cells have limited amounts of nutrients and oxygen. Certain cells detach either due to cell growth or division, and these cells then cause a systemic infection, reliant on various factors, including the host response [31].

Impact of biofilm formation on host infection dynamics

Biofilms have both positive and negative effects on human health. A possible positive impact is that of *S. epidermidis*, which obstructs the colonisation of other pathogenic bacteria by stimulating host cell immune defences and preventing adhesion. However, biofilms are also associated with both plant and human diseases [32]. An example is cystic fibrosis of CF caused by *P. aeruginosa*. After entering the lungs, this organism forms a CF-adapted pathogen from a virulent pathogen to stay in the lungs for decades. This adaptation

is caused by the overproduction of alginate, which forms a biofilm that can resist phagocytosis and antibiotics.

As a result, a distinct antibody response was developed, which stimulated granulocyte-mediated chronic inflammation, leading to severe damage to the lung tissues, as illustrated in Fig. 3. Figure 3 shows the physiological distinctions between a healthy lung and one infected with bacteria [33]. Biofilms play an important role in the persistence of chronic lung infections, because they protect bacteria from both drugs and the host immune system. They are frequently linked to respiratory disorders such as cystic fibrosis, chronic obstructive pulmonary disease (COPD), and bronchiectasis [34]. Biofilm formation causes excess mucus production, airway obstruction, and decreased clearance of the mucociliary system, all of which lead to respiratory dysfunction. The presence of biofilms also causes ongoing inflammation, which causes epithelial tissue damage and a gradual reduction in lung functionality. This disturbance to normal gas exchange worsens respiratory symptoms and increases the risk of consequences. To enhance clinical results, effective treatment regimens must target and eradicate biofilms [35].

Antibiotic resistance mechanisms in biofilms

Resistance to antibiotics and the host immune system are important bacterial characteristics. Biofilms are approximately 10,000 times more resistant to antibiotics than their counterparts. However, the cause of antibiotic resistance has not yet been completely studied. This may be because of the activity of multiple factors that aid in the protection of biofilms. The possible mechanisms of antibiotic resistance may be due to any or all of these factors [36]. Limited penetration of antibiotics, horizontal gene transfer, protection of the EPS matrix, reduced growth rate, persister cells, efflux pumps, and reduced growth rate. The limited penetration of antibiotics may be due to the strong resistance of the EPS matrix, which contains charged polysaccharides and eDNA that prevents the entry of antibiotics. Studies have shown that the penetration of ampicillin is lower than ciprofloxacin [37].

Biofilms produced by *S. aureus* exhibit plasmid-borne antibiotic resistance genes by conjugation/mobilisation. Plasmids bearing antibiotic-resistant genes can be easily transferred via horizontal gene transfer at higher transfer frequencies. Aggregated biofilm cells are protected physically by EPS, such as alginate of *P. aeruginosa* biofilms, which protects them from the attack of human leukocytes. There is a certain subpopulation of cells known as persister cells whose growth rate is zero. These cells are later converted to virulent cells because the persisting antibiotics only target cell division or growth [38]. Efflux pumps help to pump out toxins, antibiotics, etc., whilst some pump genes are upregulated, contributing to antibiotic resistance. Certain antibiotics target only dividing cells, but since oxygen availability and nutrients are lower, the metabolic growth and division rate are very slow, thus

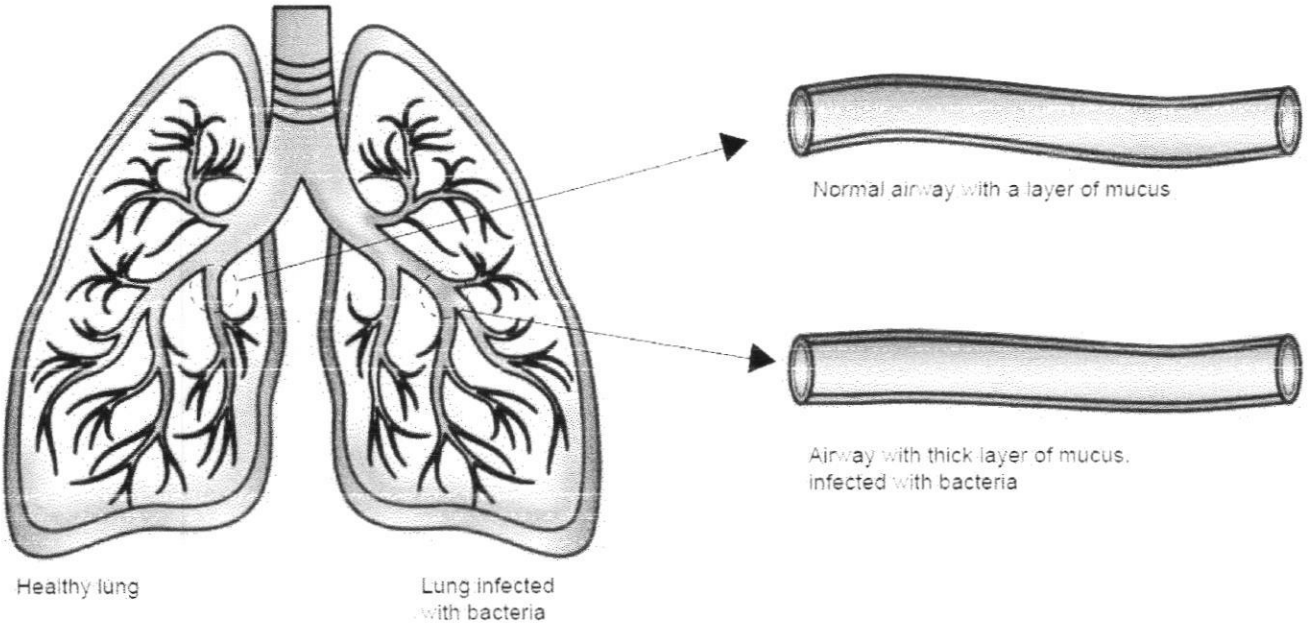

Fig. 3 *Comparison of a healthy lung and a lung infected with biofilm-forming bacteria.* The image contrasts normal lung architecture with one affected by chronic infection, illustrating how biofilm-producing pathogens lead to mucus overproduction, airway obstruction, persistent inflammation, and progressive tissue damage

becoming antibiotic-resistant. A good example is the sensitivity of *E. coli* to β-lactam drugs [39].

Biofilm production by *S. aureus*

S. aureus is a Gram-positive, ubiquitous, opportunistic pathogen considered the most common agent of skin and soft tissue infections and can be found in the nasopharynx, skin, eye, intestine, and urogenital tract as normal flora. It can crack skin barriers through the wound or surgical incision, causing infection. When infections caused by *S. aureus* become chronic, it will result in biofilm formation occurs. *S. aureus* at first adheres to a solid substrate, after which a cell–cell adhesion occurs further, resulting in the multiplication of the bacteria to form a multi-layered biofilm encased in EPS. Numerous surface proteins are involved in the biofilm formation process, together with biofilm-associated proteins, *S. aureus* surface protein G, fibronectin-binding proteins, and staphylococcal protein A [40].

S. aureus has been the major organism forming biofilms in the food industry, especially in the dairy process, and they are resistant to sanitizers. It is also the most frequent biofilm former since they are commensals on human skin and mucosa, and thus are an important reservoir of dissemination to other sites of the body. In addition, Methicillin-Resistant S. aureus (MRSA) strains form biofilms that require intensive treatment and medical device replacement. The structural components of EPS in biofilms depend on the type of microorganism present, and variation can be observed even amongst the same species. Different biofilms produce different quantities of EPS [41].

Role of quorum sensing in biofilm formation in *S. aureus*

Biofilms can control their population by synchronising gene expression and Quorum sensing (QS). Quorum Sensing is the ability of an organism to perceive and respond to cell density by secreting specialised signal molecules called autoinducers that are specific to each species. Almost all bacteria, including *S. aureus,*' speak of using chemicals for words. When the cell density was low, the concentration of autoinducers was very low; as the density increased, the concentration also increased. A critical threshold for autoinducers is reached at a specific cell density, leading to the activation or repression of target genes. This variation helps bacteria in a combined response, benefitting the entire community by maintaining the biofilm size [42, 43]. The molecule responsible for QS in *S. aureus* is a peptide derived from the *agr D* gene, which regulates transcription and codes proteases involved in biofilm dispersal. *S. aureus* has two QS systems: (i) The presence of '*agr*'— an accessory gene regulator capable of controlling the expression of toxins and virulence factors and (ii) the presence of the *luxS* gene that induces cell division in the initial stages of infection [44]. Figure 4 shows the QS mechanism of QS in bacterial interactions. The QS mechanism is a bacterial communication system controlled by autoinducers that coordinate collective activities, such as pathogenicity and biofilm maturation. Quorum quenching disrupts this signalling pathway and offers a viable therapeutic strategy.

Research has proved that the Agr system-induced peptides (AIP) sense population and, thus QS system can be triggered by the depletion of glucose and the addition of AIP. This system has two promoters: P2, which codes for the autoinducing ligand AIP, and P3, which codes for RNA III. In the biofilm formation process, the Agr- QS system is repressed so that the expression of *S. aureus* colonisation factors is halted, and further activated during dispersion. Agr QS is necessary for communicating inside a mature biofilm to establish 3D, which requires PSMs- phenol-soluble modules [45].

PIA-dependent and independent biofilm formation

Polysaccharide intercellular antigen or PIA is produced from UDP- N- acetylglucosamine through intercellular adhesion (*ica*) locus invitro *icaR* and *icaADBC*, the two products of the ica locus are essential for biofilm formation. SrrAB, a staphylococcal respiratory response regulator, induces PIA in anaerobic environments. Various environmental factors, such as temperature, ethanol, and glucose, also control Ica. Strong negative regulation was provided by *icaR* by binding *ica* [46]. Biofilms can be developed in an ica-independent manner. However, biofilm formation was unaffected, even when *the ica ADBC* operon was deleted. Research has shown that biofilm formation in MRSA strains is unaffected even if the *ica* locus is deleted. FnBP-fibronectin-binding proteins were also found to mediate the formation of biofilms by the regulation of Atl-autolysin and sigB. Bap biofilm-associated proteins help develop biofilms independently without PIA production under cell–cell aggregation [47]. Autolysin is an enzyme capable of hydrolysing Staphylococcus cells and phage genes when there is a shift from lysogenic to lytic and is globally regulated [48].

Approaches for inhibiting microbial biofilm development

Studies exploring the physiology of biofilms are important, and the search for new approaches to control biofilms is enormously challenging. Healthcare-associated infections face great challenges owing to biofilm infections. *S. aureus*

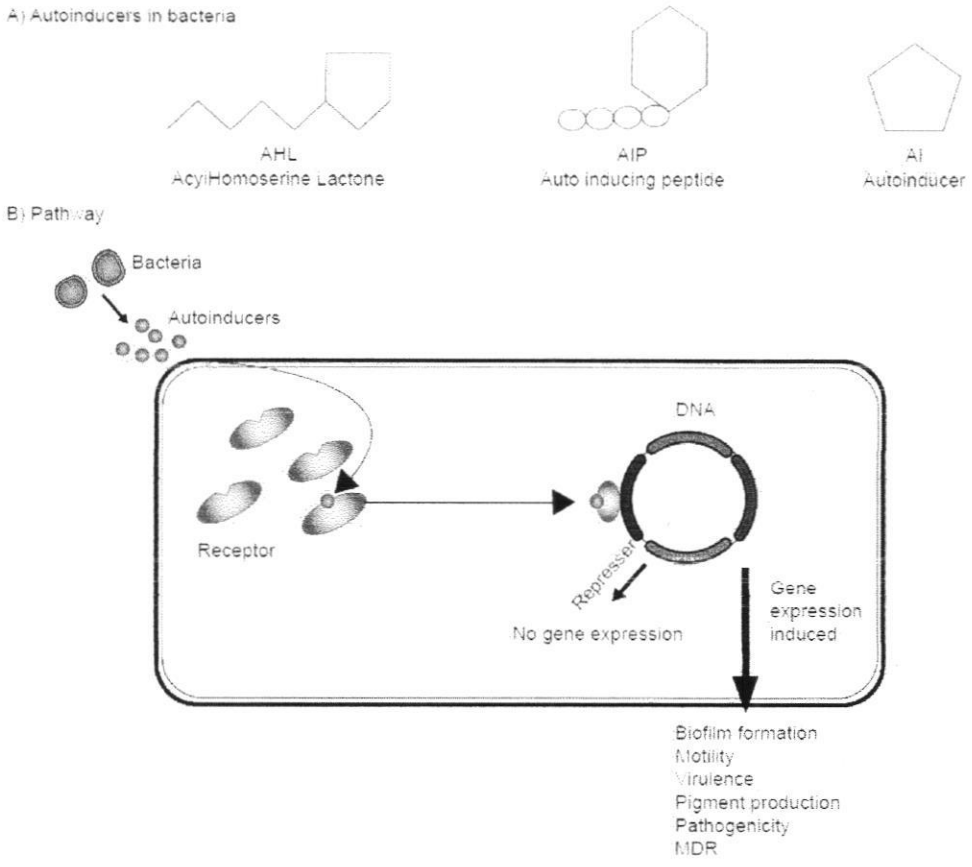

Fig. 4 *Quorum sensing mechanism in bacteria.* This diagram illustrates the bacterial communication system regulated by autoinducers. As cell density increases, signal molecules accumulate and activate quorum sensing pathways, leading to the coordinated expression of genes involved in biofilm formation, virulence, and dispersal. Disruption of this system, known as quorum quenching, represents a promising antibiofilm strategy

is susceptible to nearly all antibiotics. Unfortunately, it has the exclusive ability to develop antibiotic resistance, especially beta-lactams such as methicillin, and transmission of antibiotic resistance genes makes this organism difficult to treat. The discovery of new drugs for the treatment of S. aureus infections is of prime importance. One of the reasons for the difficulty in predicting and treating infections is the ability to form biofilms, which contributes to therapeutic failure [49]. Biofilms are a clinical challenge because of their antimicrobial resistance and the persistence of these organisms in inaccessible sites. Owing to this great antimicrobial resistance, the development of novel alternatives is necessary. Developing effective tools to eradicate biofilms will provide insights into improving the treatment of biofilm-related infections and slowing the spread of antibiotic resistance. Some strategies that can be adopted to prevent biofilms are preventing antiadhesive or anti-communication molecules, weakening or disrupting biofilms, and killing them [50]. Figure 5 outlines the various approaches employed to eliminate or disrupt the biofilm development. Antibiofilm techniques include enzymatic EPS breakdown and the use of antimicrobial peptides, nanoparticles, bacteriophages, and QS inhibitors, all of which target different aspects of biofilm integrity and resistance.

Inhibition or prevention is considered to be the best approach for combating biofilm formation. This technique can be used as a prophylactic mode during implantation to prevent bacterial adhesion, which should be developed during manufacturing. Bacterial adhesion can be prevented by targeting the molecules responsible for the initial attachment. Another inhibition technique may be enzymes that degrade biofilm matrix components, like DNase. If the prevention of biofilm misses the mark, other approaches like weakening, i.e. avoiding biofilm, can be chosen. Virulence-causing factors or communication molecules can be targeted [51]. Another example is the inhibition of microdomains using zaragozic acid [52]. Triggering the disruption is another important strategy for biofilm disruption. Molecules such as cis-2- decenoic acid, D- D-amino acids have been shown to disperse biofilms produced by *S. aureus*. The last approach is to eliminate the biofilms. AMPs or animal antimicrobial peptides have been shown to have anti-inflammatory effects, and thus destroy bacteria at low concentrations. These substances can function synergistically [53].

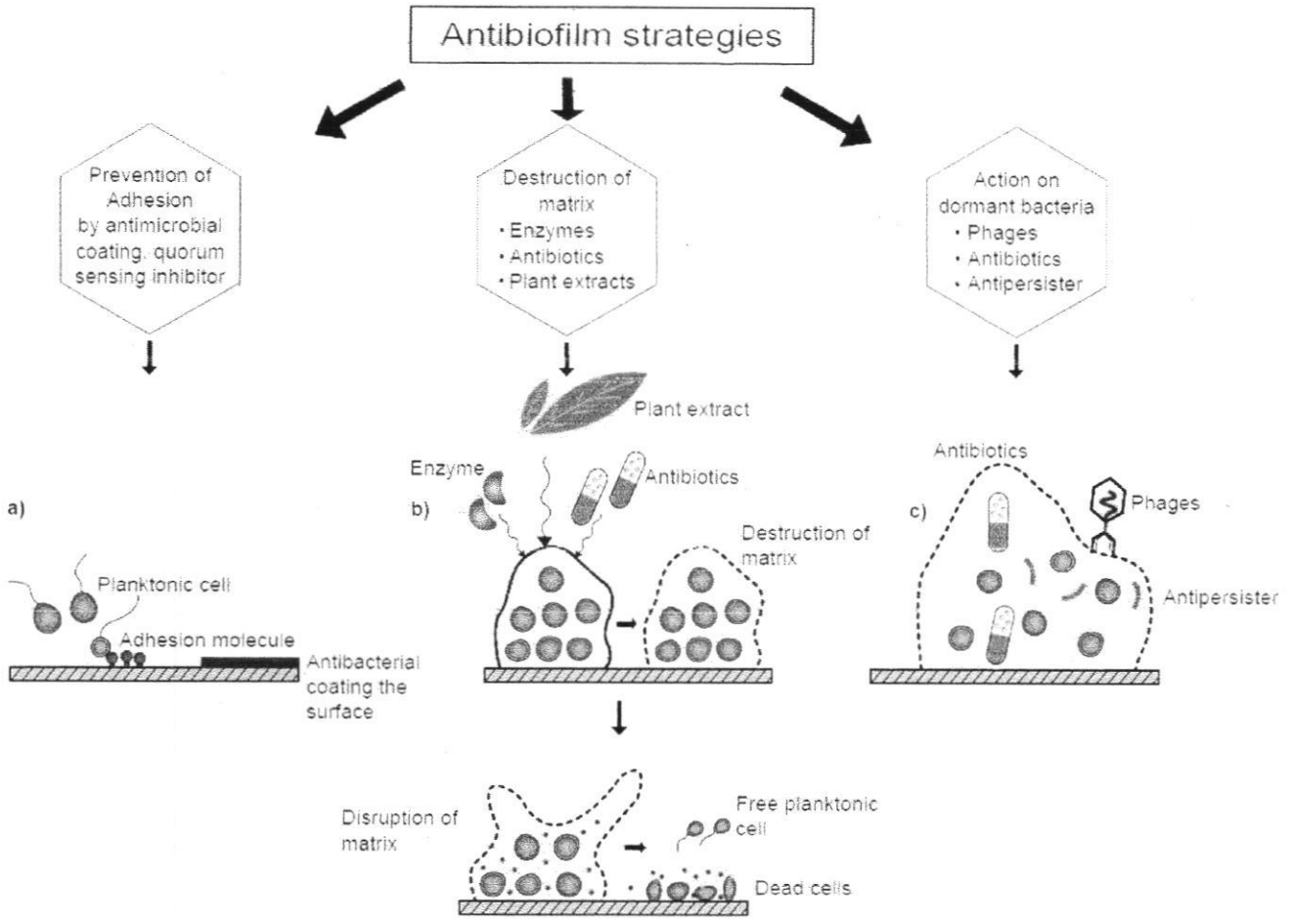

Fig. 5 *Approaches for biofilm disruption.* The figure summarizes various antibiofilm strategies, including enzymatic degradation of the EPS matrix, use of antimicrobial peptides, quorum sensing inhibitors, nanoparticles, and bacteriophages. These approaches target different stages of biofilm development and integrity to enhance treatment efficacy against biofilm-associated infections

Phytochemical approaches to combat *S. aureus* Biofilm formation

A substance can act by diverse mechanisms, such as interfering with the key enzymes that help form biofilms, enzymes that target the matrix, and the biosynthesis of proteins important for forming biofilms and their maturation. Another challenge is that the traditional antibiotics developed to kill planktonic bacteria do not significantly affect biofilm-encased bacteria because they are sessile [54]. Therefore, it is important to develop alternative treatment strategies. Medicinal plants are considered one of the principal sources of bioactive molecules that can prevent biofilm formation.

The World Health Organization (WHO) also distinguishes plants as the backbone of primary health for over half of the world. Bioactive plant-based products can benefit health when included as feed and food components [55]. Several plant extracts have been found to synthesise biomolecules such as phenolic compounds, with great antimicrobial and antioxidant effects. Myrtenol, a phytoconstituent of *Myrtus communis* L. (Myrtaceae) essential oil is used in folk medicine [56]. Myrtenol has been identified as a potential antibiofilm agent that acts at suboptimal concentrations. *Piper regnellii* (Miq.), an herbaceous plant found in tropical and subtropical areas, is crucial for reducing biofilm formation. Leaves, roots, and infusions are used to treat wounds and skin irritation. Leaf extracts of *P. regnellii* have been proven to have good antibacterial activity against *S. aureus*, and neolignan eupomatenoid-5 is effective against methicillin-resistant *S. aureus Eucalyptus globulus*, and *Juglans regia* was efficient against all *S. aureus* strains tested.

Studies have shown that hexane extracts of *Salvinia auriculata* Aubl and *Hydrocleys nymphoides* and ethanol extract of *Buchenau* could inhibit biofilm formation by *S. aureus* by approximately 50% [55]. Researchers have shown that the inhibitory effect of ethanolic extracts of Senna macranthera, Baccharis dracunculifolia, and dichloromethane extract of Artemisia absinthium was 80% [57]. Polyphenols that are effective against bacterial infections can inhibit biofilm formation or dispersal of biofilms that have already

formed. *Syzygium gerrardii* and essential oil from *Syzygium aromaticum* showed good activity against biofilm formation by *S. aureus*. In addition, *S. gerrardii* can be used to develop anti-staphylococcal products that can be used as sanitisers to clean surfaces [57]. *Krameria, Aesculus hippocastanum* extracts and *Chelidonium majus* yielded four compounds that inhibited biofilm formation in *S. aureus Vaccinium macrocarpon*-American cranberry extracts contain active constituent proanthocyanins (PAC) that were reported to inhibit the growth and biofilm production of Gram-positive bacteria, including *Staphylococcus* sp [58].

Researchers have shown that tannic acid inhibits biofilm formation by *S. aureus* without inhibiting bacterial growth in multiple biofilm models [59]. Tea tree oil extracted from the tea tree *Melaleuca alternifolia* can eradicate biofilm formation in *S. aureus,* including MRSA variants, by causing damage to the extracellular matrix (ECM), which in turn removes biofilm from a surface, and is also thought to disrupt the factors promoting adherence of the biofilm to the substratum [60]. Cinnamaldehyde, found in cinnamon, has proven to be efficient in preventing biofilm formation in a dose-dependent manner. Derivatives from *Rubus ulmifolius*, such as ellagic acid, significantly limit film formation and enhance susceptibility to antibiotics, such as oxacillin and daptomycin [61].

Enzymatic disruption of *Staphylococcus aureus* biofilms

Biofilm formation can also be controlled by preventing EPS matrix secretion by bacteria or by treating the established biofilm with other products, such as enzymes [62]. Enzymes are biological catalysts, mainly proteins, that accelerate biochemical reactions without being consumed or altered or will increase the rate of reaction without changing the chemical equilibrium of the reactants and products. All enzymatic functions depend on different environmental factors, such as the activity or specificity of the enzymes [63]. Gram-positive bacteria contain cytoplasm, cytoplasmic membrane, and cell wall, whereas Gram-negative bacteria contain an outer membrane also in addition to this. Cell walls contain a peptidoglycan layer, with a specific characteristic of alternating L and D amino acids, playing an important role in protecting the cell from osmotic disruption [64].

Gram-negative bacteria possess an additional feature of the presence of a layer of lipoprotein that is covalently bound to the peptidoglycan layer. This layer is the major barrier that prevents the entry of bile salts and aids in protection against digestive enzymes. The EPS provides additional safety by acting as a barrier. The heterogeneous nature of EPS indicates the need for a heterogeneous combination of enzymes [65]. Researchers have already proven the activity of enzyme mixtures for the degradation of EPS. The matrix of a biofilm is composed mainly of PIA or polysaccharide intercellular adhesin, which is also known as PNAG or poly-N-acetyl-D-glucosamine, eDNA, and extracellular proteins, and can be degraded by the required concentration of polysaccharide hydrolase, proteolytic hydrolase, or DNase [66].

Polysaccharides hydrolyse polysaccharides into mono- or oligosaccharides by targeting α-1, 4-, β-1,4-, or β-1,3-glycosidic linkages. Dispersion B, another polysaccharide hydrolyzer, can hydrolyse PNAG, an important component of the biofilm matrix. The peptide bonds of proteins, including EPS and the cell wall, can be cleaved with the help of lysostaphin. Finally, DNase can denature eDNA and prevent conjugation and horizontal gene transfer by the bacteria. Adhesions can also be prevented [67].

Enzymes play an important role in EPS degradation in biofilms. Applying enzymes for the degradation of EPS is an effective option for industries requiring complete biofilm removal [68]. EPS has a different array of macromolecules, and because the enzymatic functions are different, combining enzymes with assorted substrates or other physicochemical treatment methods is essential for the complete removal of EPS. Another strategy for eliminating biofilms includes excluding QS molecules by QQ or quenching the Quorum Sensing system, thereby reducing pathogenic expression [69]. Various techniques inhibit the QS mechanism, prevent signal synthesis and degradation of QS molecules, and do not prevent the receptors or the cascade of signal transduction from binding to QS molecules. These factors highlight the importance of the QQ enzymes. α-Amylase is an important potent biofilm inhibitor involved in the hydrolysis of α-1,4 glycosidic linkage. The marine bacterium *Bacillus cereus* produces amylase in the presence of a 96.1% calcium chloride enzyme capable of inhibiting *S. aureus* [70].

This enzyme also disrupts the EPS of mature biofilms. *B. subtilis* can also inhibit and reduce biofilm formation by 90% and disrupt the EPS matrix by 82% in a temperature range of 37-60^{0}C [69]. Another marine bacterium, *B. subtilis* S8-18, produces extracellular α-amylase, which exhibits notable activity against MRSA biofilm formation (approximately 73.07%). Other Bacillus spp., such as *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, and *Bacillus stearothermophilus*, produce amylase enzymes at high temperatures. Studies have shown that the amylase enzyme also shows excellent activity against *S. aureus* of marine origin. Proteins are another important factor in an EPS matrix [71]. The biofilm of *S. aureus* contains an enormous amount of surface-associated and extracellular proteins. Of all the proteins, adhesion initiation and biofilm formation were promoted by BaP biofilm-associated surface proteins. Biofilm formation can be controlled by breaking the protein barrier by degrading protein enzymes, also known as proteolytic enzymes [72].

This group of enzymes hydrolyses the peptide bonds that connect to amino acids. An innovative sulfhydryl plant protease, ficin, has been efficaciously used to prevent *S. aureus* biofilms. Proteases are hydrolytic products of bacterial metabolism that affect host cell proteins, thereby facilitating bacterial invasion and growth [73]. Subtilisin is a broad-spectrum serine protease obtained from many *Bacillus sp.* strains that breaks the peptide bonds in the protein structure. Biofilms attach to surfaces through proteinaceous adhesins.

Subtilisin breaks adhesin, resulting in the separation and dispersion of biofilms. Researchers have shown that immobilised enzymes possess greater competency against biofilms [74].

Earlier studies have also shown that the entrapment of subtilisin in ceramics reduced the biofilm content of *S. aureus*, *S. typhimurium,* etc. Under optimised conditions, immobilised subtilisin reduced biofilm formation by 3–4 times in several bacteria, and the highest of eightfold was found in *S. aureus* [75]. Metalloprotease, specifically serratiopeptidase (Spep), produced by *Serratia marcescens,* inhibited the adhesion and invasion of different species of *Staphylococcus*. Spep eliminated *S. aureus* more effectively than the other proteases. Another metalloprotease, Serratia simulans, is lysostaphin (Lst), which is zinc dependent [76]. Lst hydrolyses the pentaglycine interpeptide bonds of the peptidoglycan layer, which forms the cell wall and is a primary defence mechanism of a cell against antimicrobial agents 0.4 µg/mL of Lst was found to cause cell death and detaching of biofilms in *S. aureus*. Recombinant Lst was effective against MRSA and was 2–eightfold sensitive and effective against biofilms. Immobilised Lst was also useful for eliminating biofilms formed by *S. aureus* on coated surfaces [77]. Cysteine proteases, especially papain and bromelain, have been found to eliminate biofilms. Papain is known to control *S. aureus* both in the free and immobilised forms and was found to decrease both the protein and carbohydrate content of biofilms [78].

Gene inhibition strategies against *S. aureus* biofilm development

Biofilm formation is an intricate process that is divided into various phases, such as attachment, accumulation, maturation, and dispersal. Of the various phases, primary emphasis was given to attachment and accumulation, arbitrated specifically by a group of proteins known as microbial surface components that recognise adhesive matrix molecules (MSCRAMMs) [79]. PIA or polysaccharide intercellular adhesin depends on genes within the *icaADBC* operon, and studies have shown that it consists of PNAG- polymeric *N*-acetylglucosamine. Mechanisms for controlling biofilm formation in *S. aureus* include QS, pH changes, and proteases [80]. Researchers have proved that if *agr* QS is activated and protease treatment is done, *S. aureus* will inhibit its biofilm and disperse the already formed biofilms 1% v/v culture supernatant of *P. aeruginosa* could inhibit biofilm formation of *S. aureus* to about 90%.

The supernatant induced the expression of *aur, clp, aspA, splA*, and *sspA*, which are endogenous protease genes, and *saeS, hla,* and *agrA*, which are regulatory genes. In addition to reducing the hydrophobicity of the cell surface and cell charges, the *icaAB* gene is downregulated, thus leading to a low amount of adhesin as a function of the *ica* locus, leading to reduced intracellular adhesion between cells [81]. Vitexin significantly affects S-mediated movement and protease secretion via agrAC downregulating [82]. A two-component signal transduction system encoded by *the agr* locus, an accessory gene regulator of *S. aureus,* results in the upregulation of secreted proteins and downregulation of surface proteins.

The inhibitory activity of agr groups—agrB (secretion of autoinducing pheromones), agrD (binding and activation of histidine kinase receptor), and agrC (activation of response regulator agrA) will ultimately affect virulence gene expression. Quercetin and tannic acid are two of the chief components found in *Alnus japonica,* which repress the intercellular adhesion genes *icaA* and *icaD*, inhibiting biofilm formation by over 70% [83]. Crystal violet staining indicated biofilm reduction in the presence of shikimic acid by breakdown of the biofilm structure. Shikimic acid downregulates the transcription of sarA and upregulates agrA, thereby inhibiting biofilm formation by *S. aureus* by interfering with the initial steps of biofilm formation. In response to nisin, a bacteriocin, 601 genes of *S. aureus* were regulated, 327 were upregulated, and 274 were downregulated. Different transporter genes showed high antimicrobial activity against *S. aureus*.

The sliding movement of *S. aureus* results in its rapid translocation from one place to another, and adherence to surfaces promotes colonisation, resulting in the formation of a biofilm network. Research on *S. aureus* has established that the regulation *of ica* expression and biofilm formation involves regulatory elements other than σ^B and IcaR [84]. The additional regulatory locus is agr, the accessory gene regulator, which encodes a two-component QS system that controls the regulatory RNA molecule (RNA III) that encodes toxins and their expression, leading to reduced biofilm formation. Another regulatory locus is sar sarA-Staphylococcal accessory regulator A, a DNA-binding protein that enhances biofilm production. Mutations in this locus reduce biofilm formation. Sar A is a global virulence regulator even in MRSA. This proves that both agr and sarA act as molecular switches to regulate biofilm formation [85]. Researchers have shown that thymol reduces the expression of sarA by downregulating *ica A, icaD, fnbB*, and *hla*, without altering

agrA and agrC. Thymol inhibits the synthesis of virulence factors such as PIA and haemolysin [86].

Recent anti-biofilm approaches in controlling microbial colonization

Incorporating advanced post-harvest strategies is critical for maintaining microbial safety and the quality of fresh produce. Recent studies have emphasised the efficacy of edible coatings and natural antimicrobials for sustainable postharvest management. For instance, the application of edible coatings enriched with-calcium chloride has been shown to positively influence physiological and biochemical responses in mango fruit during cold storage, thereby prolonging shelf life and minimising spoilage [87]. Similarly, nanoemulsion-based edible coatings provide promising results for protecting the freshness of fruits and vegetables by forming protective barriers that limit bacterial growth and water loss [88]. Antimicrobial strategies, such as the use of beeswax combined with lemongrass oil, have shown substantial inhibitory effects on *Alternaria alternata*, a key post-harvest pathogen of bitter gourd, demonstrating the potential of natural products to minimise microbial growth and maintain quality during storage [89]. These findings are consistent with broader sustainable food system aims, which highlight the use of environmentally friendly post-harvest measures to minimise waste and increase food safety. Collectively, these studies highlight the importance of anti-biofilm techniques, particularly when using natural biodegradable coatings to reduce microbial colonisation and preserve postharvest quality in fresh fruit.

Conclusion

This review examines microbial biofilms thoroughly and meticulously, concentrating on their structural complexity, resistance mechanisms, and clinical importance, particularly in infections caused by *S. aureus* and *P. aeruginosa*. It discusses the stages of biofilm development and the involvement of EPS, quorum sensing systems, and genetic regulation in biofilm resilience and pathogenesis. This study discusses a wide range of current antibiofilm methods, including the application of quorum quenching agents, antimicrobial peptides, enzymatic degradation, nanoparticles, and bioactive plant extracts. The focus is on molecular interventions and nature-derived compounds, which are intriguing alternatives to traditional antibiotics. This review emphasises the critical need for novel, multi-targeted, and long-term antibiofilm techniques, especially in the light of the development of antimicrobial resistance. Future research should focus on designing synergistic medications that use physical, chemical, and biological techniques to undermine biofilm integrity. Natural compounds and enzyme-based treatments should also be highlighted for their translational potential, assisted by enhanced drug delivery technologies and nanotechnology. Furthermore, consistent testing methodologies and interdisciplinary research are necessary to address the gap between laboratory findings and clinical or industrial application.

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Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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