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REVIEW ARTICLE

Biofilms: Architecture, Resistance, Quorum Sensing and Control Mechanisms

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Abstract Biofilm is a mode of living employed by many pathogenic and environmental microbes to proliferate as multicellular aggregates on inert inanimate or biological substrates. Several microbial diseases are associated with biofilms that pose challenges in treatment with antibiotics targeting individual cells. Bacteria in biofilms secrete exopolymeric substances that contribute to architectural stability and provide a secure niche to inhabiting cells. Quorum sensing (QS) plays essential roles in biofilm development. Pathogenic bacteria in biofilms utilize QS mechanisms to activate virulence and develop antibiotic resistance. This review is a brief overview of biofilm research and provides updates on recent understandings on biofilm development, antibiotic resistance and transmission, and importance of QS mechanisms. Strategies to combat biofilm associated diseases including anti-biofilm substances, quorum quenching molecules, bio-surfactants and competitive inhibitors are briefly discussed. The review concludes with updates on recent approaches utilized for biofilm inhibition and provides perspectives for further research in the field.

Keywords Biofilms · eDNA · Antibiotic resistance · Quorum sensing · Quorum quenching

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Introduction

Biofilm research has gained immense importance in recent times due to the insurmountable effects on health and disease manifestations. Several microbial infections have been associated with biofilm formation and pose challenges in treatment regimens [1, 2]. Alarming incidence of antibiotic resistance, unavailability of newer antibiotics and recalcitrant and chronic properties of biofilm associated diseases demand new control strategies [3–6]. Microorganisms in biofilms inhabit a matrix that glues them onto inert or biological substrates and provides communal benefits such as increased antibiotic resistance, slow growth, differential gene expression, elevated levels of lateral gene transfer, stress resistance and subversion of host defence mechanisms [7–13]. Biofilm formation in microbes occurs when multicellularity is induced in otherwise unicellular organisms in response to sensing their population density to have reached a certain threshold level [14]. Process of biofilm development can be broadly grouped into four different phases; *attachment* (on surface, inanimate or tissue), *sessile growth phase* governed by intercellular interaction (quorum sensing (QS) factors), *biofilm maturation* (induced exopolymeric substances (EPS) matrix synthesis) and *detachment* (induction of disassembly factors) [2, 15, 16]. Several clinically important pathogenic bacteria such as cystic fibrosis associated *Pseudomonas aeruginosa*, urinary- and catheter-associated *Proteus mirabilis*, spinal implants-associated *Staphylococcus epidermidis*, lower respiratory tract and surgical sites associated *Staphylococcus aureus*, pneumonia causing *Haemophilus influenza*, pacemaker-associated *Staphylococci* and *Streptococci*, and endodontic *Streptococcus mutans*, *Streptococcus sanguinis*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Lactobacillus casei* and

Actinomyces naeslundii, mediate infection through biofilm formation [7, 17]. Architecturally, bacterial mono-species biofilm is a three dimensional structure composed of genetically identical and functionally divergent cells that differentiate into distinct cell types and support biofilm establishment, maintenance and dispersal. Microbes in these communities become responsive to changing environment, nutrient deprivation and varying environmental insults by constantly evolving and spreading resistance to neighbouring cells. Community behaviour in otherwise independent cells is triggered upon sensing a certain threshold cell density through QS mechanisms [18, 19]. Cells in these assemblages are encased in a self-made extracellular polymeric matrix that comprises of polymers of sugars as major components along with polymers of proteins, lipids and nucleic acids that contribute to viscoelastic nature of the biofilm [20, 21]. Extra polymeric substance (EPS) also contains insoluble constituents such as amyloids, cellulose, surface adhesion molecules like pilli, fimbriae and flagella that provide rigidity and attachment property to biofilm [22]. Additionally, biofilms are known to express variety of structural proteins, aiding their diverse rheological properties [22]. Curli are functional amyloid fibres that are key protein components of EPS, produced by many species of *Enterobacteriaceae* including *Escherichia coli* and *Salmonella typhimurium*. These fibres are critical for biofilm development and provide resistance to desiccation, oxidative stress, protease and antimicrobial agents. Curli fibres act as scaffolding agent due to their extreme stability and display functional abilities in pathogenesis, host cell adhesion, invasion and immune activation [23]. Recently, the role of TasA, another structural protein from *Bacillus subtilis*, has been established in stabilizing biofilms by adopting a β-sheet-rich fibril conformation in vivo that provides resistance to proteases and thereby aids survival [24]. Among the four stages of biofilm formation, culmination stage of dispersal is quite weakly understood. Dispersal is a major event leading to spread of infectious bacterial cells that disperse and colonize other tissues or organs within a host [25]. Interestingly, regulation of biofilm dispersal is an emerging area in medicine that deals with combating the antibiotic-susceptible free dispersed bacteria and prevention of secondary biofilm infection [15, 26]. Biofilm disseminating agents targeting EPS are generally enzymes like deoxyribonucleases (deoxyribonuclease I) and glycoside hydrolases (dispersin B). Additionally, anti-biofilm peptides (cathelicidin), dispersal signals (nitric oxide and *cis*-2-decenoic acid), anti-matrix molecules (chitosan, rhamnolipids, urea) and sequestration molecules (EDTA and lectoferrin) may also be utilized for biofilm dissemination [27]. In order to synchronously function in a multicellular niche, bacteria necessarily adopt a reliable and efficient means of communication for intra-species and inter-species interactions. These interactions have been observed in biofilms of several food borne pathogens [28]. The food industry suffers a significant damage where 60% of the outbreaks are caused by biofilm associated infections leading to antimicrobial resistance, persistence, and virulence factor production [28, 29]. Bacteria in complex biofilms adopt QS as a means to communicate via signalling molecules to coordinate and cooperate for proliferation, sustenance and dissemination [14, 30, 31]. QS triggered in response to threshold population density regulates differential gene expression, and mediates virulence, acid tolerance and biofilm generation [32]. Three different modes of QS have been identified: first, comprising LuxI/LuxR-type QS specifically seen in Gram-negative bacteria, that use acyl-homoserine lactonases (AHL) as signal molecules; second, dealing with small peptides as signal molecules observed in Gram-positive bacteria; and third, adopted by both Gram-negative and Gram-positive bacteria utilizing LuxS-encoded autoinducer-2 (AI-2) [14, 30, 31]. AHL based QS is one of the most extensively studied mechanisms along with peptide based QS in Gram-positive bacteria.

In this review, we attempt to bring together the remarkable understanding available on structural components of EPS, mechanisms of antibiotic resistance and resistance transmission in biofilms, modes of biofilm maintenance and inhibition with an emphasis on QS mediated mechanisms, and strategies to control biofilm growth. Concepts discussed in the review are summarized in Fig. 1. We also present perspectives on development of new methods to combat biofilm infections.

Biofilm Stabilizing Components and their Roles

The progression of bacteria from a free-living state to a multicellular community is a complex and dynamic process involving multi-facet changes that lead to cellular reprogramming and alterations in expression profiles of cell surface molecules, nutrient utilization pathways, and virulence factors that facilitate growth in unfavourable conditions [15]. Broadly, biofilms comprise of two components, the microbial cells and secreted EPS that constitute 90% of the overall biomass. EPS contributes to overall establishment of biofilm structure and mainly constitutes of exopolysaccharides that provide sites for cohesion and adhesion interactions, proteins that act as carbon and energy source, and extracellular DNA (eDNA) for resistance gene transmission [21]. Recent reports suggest direct correlation between EPS production and growth of biofilm in nutrient rich environment [33]. Several studies have reported modification of EPS components in response to

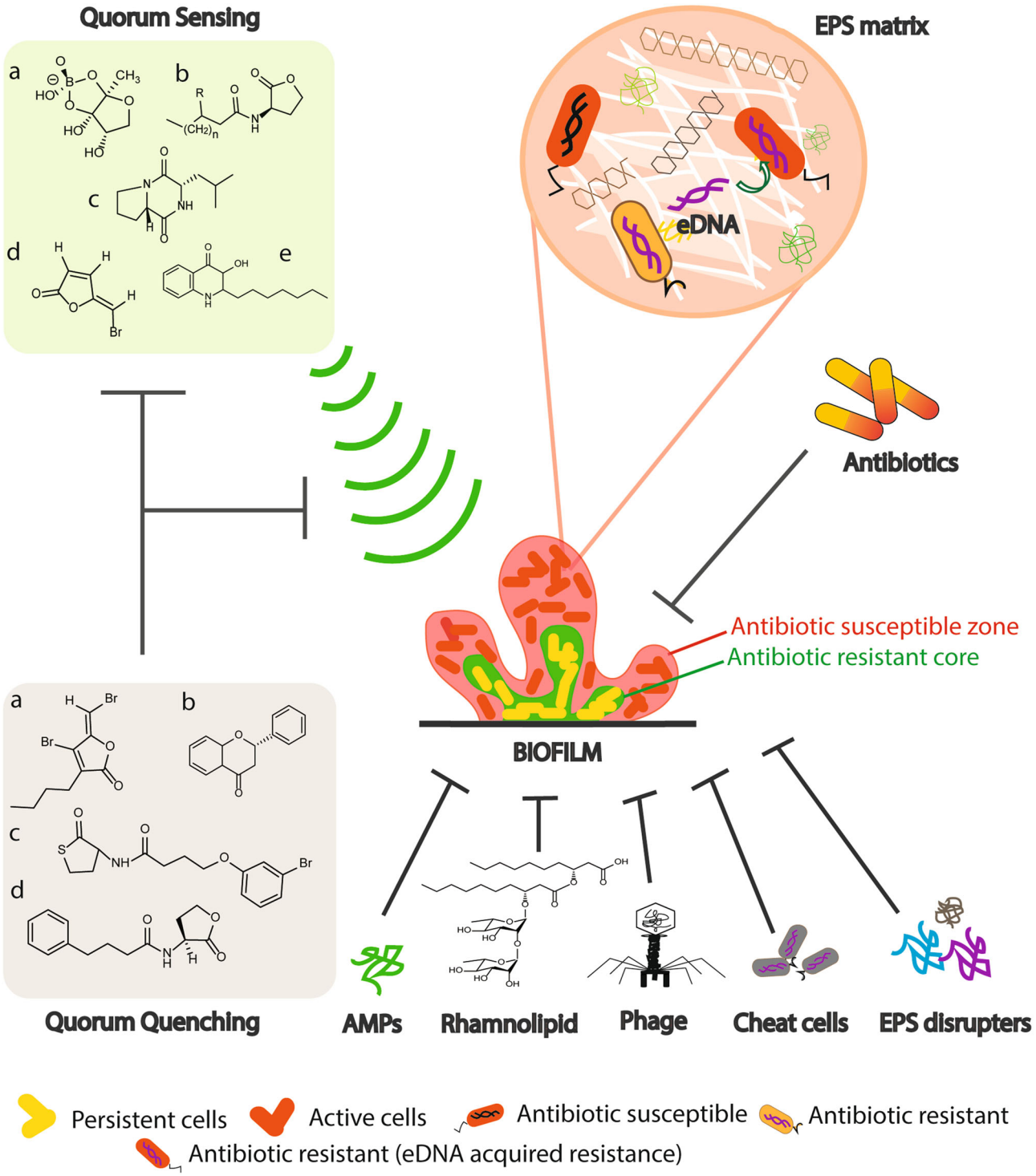

Fig. 1 The schematic depicts synergistic effect of quorum sensing (QS) molecules, where **a** AI-2, **b** AHL, R = H or OH, n = 0–18, **c** cyclo (L-Pro-L-Leu), **d** halogenated furanone, **e** 2-heptyl-3-hydroxyl-4-quinolone) aid in development of biofilm. Transmission of antibiotic resistance through extracellular DNA (eDNA) in the EPS matrix of biofilm is highlighted. The schematic of biofilm shows effect of antibiotics on different layers within the biofilm. The outer red layer represents antibiotic susceptible bacterial population (shown in red color) while the inner green layer represents antibiotic resistant subpopulation known as persister cells (shown in yellow color). Quorum quenching (QQ) molecules include **a** furanone C30, **b** flavanone, **c** meta-bromothiolactone analogue of LasR and RhlR, **d** 4-phenylbutanoyl HSL that function in inhibiting QS pathways either indirectly or through modification of QS molecules. Other strategies employed for treatment of biofilms including antimicrobial peptides (AMP), bio-surfactants (rhamnolipid), phage particles, cheat cells and different EPS disrupting enzymes are shown with block arrows

changes in nutrient availability and temperature [15]. These biofilm components, together with the constituent cells, cause serious environmental issues like biofouling in industrial bioreactors [34]. Abundance of polymeric proteins, cellulose, rhamnolipids, cell debris, gel-forming polysaccharides, amyloids and eDNA provide elasticity, robustness and high tensile strength to biofilms, and increase microbial proximity thus facilitating communication via QS [15, 22]. Extracellular matrix also modulates biochemical properties thereby regulating diffusion, adhesion and cohesion to create a suitable acidic environment for biofilm formation. Additionally, large cell biomass in pathogenic biofilms aids in decreasing diffusion and achieves a serial resistance model of diffusion that adversely affects permeability of antibiotics used for treatment [35–37]. Usage of weak acid drugs in such cases is known to enhance permeability and effectiveness of antibiotics [35]. Based on functional divergence EPS can be categorized into three major classes (Table 1). Class I constitutes the architectural EPS that are involved in signal and structural regulation, class II comprises of protective EPS that provide protection against host immune system and physiological stresses and class III that contains aggregative EPS involved in adhesion and biofilm development.

Antibiotic Resistance Mechanisms in Biofilms

Biofilm mode of living endows microbes with various survival benefits, of which, transmission of antibiotic resistance is the most advantageous. Interestingly, microbes in biofilms are several-fold more resistant to antibiotics as compared to their free living planktonic counterparts. Microbes in biofilms prefer a sessile way of living and constantly evolve with environmental fluctuations. Upon availability of conditions for rapid growth, these cells revert to the planktonic lifestyle [38]. Biofilms of *P. aeruginosa, S. pneumonia* and other pathogenic microbes cause severe and chronic diseases like cystic fibrosis, pneumonia, meningitis, and osteomyelitis [19]. Biofilm associated diseases contracted from contaminated surgical and bio-medical equipment including catheters, contact lenses, implants etc. are resilient to treatment with conventional antibiotics due to poor penetration and occurrence of deeply rooted persister population [39]. Nutritional stress in biofilms leads to generation of a heterogeneous population of slow-growing and starved persister cells. In contrast to upper layers of antibiotic-susceptible cells, persister cells are extremely tolerant to antibiotics and occupy the internal core of biofilms [19]. Extensive and unregulated usage of antibiotics has led to development of several antimicrobial resistant strains including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus*, carbapenem-resistant *Enterobacteriaceae* and multidrug-resistant *Acinetobacter*. While exact molecular mechanisms underlying antibiotic resistance need further understandings, pathways involved in conferring protection against oxidative stress, expression of efflux pumps, protective barrier provided by components of EPS, heterologous mode of growth of sub-population, and diffusion reaction inhibition used to volatilize, precipitate, chelate and chemically modify the anti-microbial drugs, have been implicated [39, 40]. Additionally, certain microbes utilize conversion of mucoid exopolysaccharide into alginate exopolysaccharide to acquire antibiotic resistance [41]. Biofilms of *P. aeruginosa* produce rhamnolipid, elastases, proteases and pyocyanin molecules that cause lung tissue damage and provide resistance against antibiotics [42].

Cryptic eDNA: Mediator of Resistance Transmission in Biofilms

Several biofilm bacteria are known to release extracellular DNA (eDNA) that facilitates remodelling of extracellular matrix and helps in tethering microbial cells in clusters by promoting acid base interactions [43, 44]. While eDNA is known to commonly occur in most biofilms, mechanisms of externalisation of this DNA need further understanding. Recent reports suggest an autolysis mediated and active secretion of eDNA into exopolymeric matrix under control of QS [43]. A calcium ion-regulated autolysin, AtlA, mediated mechanism has been linked with release of eDNA in case of *S. mutans* both in vivo and in in vitro models [45]. In another recent study, phosphodiesterase, gdpP gene was implicated in eDNA release. The gene product facilitates degradation of secondary messenger cyclic-di-AMP and xdrA which affects expression of several downstream genes including genes involved in cell wall homeostasis. A gdpP mutant showed remarkable decrease in release of eDNA, suggesting importance of cell wall instability in eDNA release [46]. Notably, in some cases release of eDNA has been linked with enhanced competency in microbial cells suggesting possible involvement of intrinsic mechanisms in lateral gene transfer. eDNA serves wide range of purposes in biofilm generation. It acts as key adhesion molecule in early stages of biofilm formation, and as structural component of mature bacterial aggregates, and in twitching motility structures [44, 47]. eDNA chelates and inactivates cationic antibiotics because of inherent negative charge on the surface and contributes to antibiotic resistance [48]. In certain cases eDNA has been observed to bind to Pel, the protein facilitating cell–cell interactions within biofilm and

Table 1 Types of exopolymeric substances (EPS)

Sub-class	Organism	Genes involved	Role	Regulation
Class I architectural EPS (regulation of biofilm formation and structure, secondary messenger signaling)				
Calonic acid (UDP-glucose, galactose, glucuronic acid)	*Enterobacteriaceae Salmonella sp*	*wca* cluster	Abiotic surface binding, pathogenesis, biofilm biodiversity, and phenotype	Rcs phosphor relay system (Rcs D/B/C and LuxR)
Cellulose (β-1,4 linked D-glucose)	*G. xylinus S. enterica*	bcs (bacterial cellulose synthesis) cel (cellulose)	Protection from mutagenic UV radiation	c-di-GMP level, AdrA
Class II protective EPS (protection from Opsonic and non-opsonic phagocytosis, maintenance of hydrated biofilm environment)				
Alginate (polymer of acetylated 1, 4-linked β-D-mannuronic acid and α-L- glucuronic acid)	*P. aeruginosa, A.vinelandii* other *Pseudomonads*	algD-A	Protection against anti-microbial drugs, calcium chelation, scavenge reactive oxygen in chronic lung infection (cystic fibrosis)	Positive regulator σ-AlgT (AlgUAσE/o22/RpoE)
				Negative regulator Anti-sigma MucA
Capsular PS	*E. coli S. pneumonia* other gram-positive and gram-negative bacteria	wyz dependent, synthase dependent, and ATP-binding cassette transporter dependent	Protection from desiccation, opsonophagocytosis. Maintenance and dispersal of biofilm. UTI infection	Environmental Conditions- Low CPS phase and High CPS phase
Levan (β-fructans, Levansucrase)	*P. syringae E. amylovora B. subtilis*	sacB-sacC	Physico-chemical properties of the biofilm	Activator:- Sucrose 2 component sensor kinase LadS (*P. syringae)* SacX/SacY (*B. subtilis*)
Class III aggregative EPS (Surface adhesion, complex structure formation)				
PIA (polysaccharide intracellular adhesion)–β-1-6 linked 2-amino-2-deoxy-D-glucopyranosyl residues	*S. aureus S. epidermidis*	*icaADBC* locus	Cell-Cell Interaction, Bacterial Aggregation Diseases-Endocarditis, Osteomyelitis	Negative regulation-icaR and LuxS system (quorum sensing)
				Positive regulation- σ^B, RsbU and Spx protein (*S. aureus*)
				Induction by high NaCl, Glucose, temperature, anaerobiosis
Pel (thick Pellicle)	*P. aeruginosa*	*Pel* operon	Pellicle formation, Adherence to surface, Aggregate formation in broth culture	c-di-GMP, FleQ
Psl [D-mannose, D-glucose, and D-rhamnose (3:1:1)]	*P. aeruginosa*	*Psl* operon (*pslA-O*) 15 genes	Attachment, resistance and biofilm formation, systemic infection evasion of phagocytosis	c-di-GMP, FleQ

aid antibiotic resistance [43]. Efficiency of gene transfer in biofilm inhabiting cells is extensively studied in *Neisseria gonorrhoea* biofilm where the acquisition of double-drug resistance is mediated through gene transfer between single-drug resistant gonococci. The study revealed importance of biofilm age in determining transfer efficiency. Early stage biofilm exhibited higher gene transfer efficiency that decreased with increasing biofilm age. While biofilm architecture is not known to affect gene transfer efficiency, loose biofilms under selective conditions facilitate spread of double-drug resistant bacteria [11].

Biofilm Development and Imperative Roles of Quorum Sensing

Biofilm formation involves several stages including initiation, establishment, maintenance and dispersal. Free-living bacterial cells upon reaching a certain threshold density acquire the potential, through QS mechanisms, to live as multicellular entities where cell-to-cell communication is established by secretion of small signalling molecules. These signals lead to a wide range of effects on bacterial genetics and physiology [14]. The effects are manifested in

the form of alterations in gene expression profiles and lead to generation of phenotypes that are known to mediate biofilm development, competence, nodulation, antibiotic production, motility, and virulence in pathogens [49, 50]. Several environmental factors such as pH, nutrition and signal flow rates, control QS mediated functioning in biofilms [51]. QS functions through a feed forward mechanism where signalling molecules bind their receptors on recipient microbial cells and activate expression of several genes including genes that are responsible for synthesis of these molecules [52]. Wide range of QS signalling molecules are known in both Gram-positive and Gram-negative bacteria [14, 30, 31]. These signals mainly include N-acyl homoserine lactones (AHLs), oligopeptides (cyclic thiolactone), furanosyl borate (AI-2), hydroxyl-palmitic acid methylester, and methyl dodecanoic acid [14]. Gram-negative bacteria utilize diffusible AHLs that cross cell membranes and bind to regulatory proteins in recipient cells. Gram-positive bacteria, on the contrary, rely upon peptide-based sensing mechanisms that require membrane bound receptor histidine kinases. Oligopeptides like thiolactone, furanone signals (LuxS system) in *Vibrio harveyi* induced by AI-2, hydroxyl–palmitic acid in *Ralstonia solanacearum,* diketopiperazines in *P. aeruginosa,* methyl dodecanoic acid and quinolone are other examples of QS signalling molecules [14, 53]. Gram-positive *B. subtilis* and *S. aureus* utilize eukaryotic-like Ser/Thr kinase mediated regulation of biofilm formation. PrkC in *B. subtilis* is a dimeric Ser/Thr kinase that phosphorylates an external myelin basic protein (MBP) and undergoes autophosporylation utilizing a conserved K40 residue. Deletion mutant of *prkC* and a K40R mutation in PrkC resulted in decreased sporulation efficiency and reduction in biofilm formation suggesting crucial roles of this protein in biofilm growth [54]. PrkC in *Bacillus anthracis* regulates phosphorylation of GroEL chaperone that plays important roles in biofilm formation in pathogenic *Mycobacteria* and *Streptococci*. PrkC phosphorylates six threonine residues in GroEL and facilitates binding of GroES co-chaperone. *prkC* deletion in *B. anthracis* resulted in defective biofilm formation that could be rescued with GroEL over-expression. Over-expression of phosphorylation-site mutants of GroEL could not rescue growth in biofilms suggesting essential roles of PrkC-mediated phosphorylation in functioning of GroEL in biofilms [55]. Stk1, a PrkC homolog in *S. aureus*, deactivates LuxS protein through phosphorylation and eliminates production of AI-2 thereby affecting biofilm formation. Several pathogenic microbes harbour genes for eukaryotic-like Ser/Thr kinases, suggesting evolutionary importance of these enzyme systems in regulating biosynthesis of signalling molecules and controlling biofilm mode of living [56].

Not limited to bacteria, QS has been identified in eukaryotic microbes (*Candida* and *Histoplasma*) and recently also in viruses [31]. QS mutants in bacteria have been implicated in reduced virulence making QS systems potential anti-microbial targets [31]. QS mechanisms are extensively studied in pathogenic bacteria including, *S. aureus*, *Bacillus cereus*, *P. aeruginosa*, and *Vibrio cholerae.* LuxI/LuxR is a prototype of QS in Gram-negative bacteria. LuxI is an AHL synthase producing AHL that at high concentrations binds to and stabilizes cognate LuxR receptor. AHL-LuxR receptor protein complex triggers expression of several genes while autoinducing LuxI expression in a feedforward reaction to produce more AHL and cause signal amplification. Several pathogenic microbes utilize LuxI/LuxR-type QS circuit to induce virulence [51]. An AI-2 autoinducer based QS pathway discovered in *V. Harveyi* is involved in interspecies communication and occurs in both Gram-negative and Gram-positive bacteria [14].

Strategies to Control Biofilm Growth

Emergence of antibiotic resistance in biofilm associated diseases has led to identification of several anti-biofilm compounds of prokaryotic and eukaryotic origin. QS regulation, being imperative to development and virulence in biofilms, has been a target for identification and generation of anti-biofilm compounds [14, 30, 31]. These molecules deactivate QS system by a phenomenon termed as quorum quenching (QQ) [14]. Interference with QS signals, and disruption of eDNA, proteins, lipopolysaccharides, EPS and secondary messengers, constitute mechanisms used to inhibit biofilm development [41]. Architectural stability and robustness of biofilms in addition to increased rate of resistance transmission and low antibiotic permeability pose tremendous challenges to antibiotic treatment. Disruption of biofilms in certain cases may require mechanical, physical and chemical strategies [19]. QQ molecules, adhesion inhibitors, EPS disruptors and competitive microorganisms are some of the routinely employed means of eradicating microbial biofilms [57]. Natural/synthetic chemicals aimed against the QS system are known as quorum sensing inhibitors (QSI) that work through the QQ process [58–61]. QSIs are broadly divided into three distinct categories. First category includes enzymes that degrade autoinducers by chemically modifying them, for example, lactonases, amidases, reductases and cytochrome oxidases found in bacteria, archaea, fungi, marine species and several other microorganisms. Comparative genome analysis has revealed the presence of several copies of genes encoding AHL acylases and AHL lactonases in *Pseudomonas* spp. and *Bacillus* spp., respectively [62]. Studies have also revealed remarkable biodiversity and polymorphism in genes of AHL lactonases in *Bacillus* spp.

[63]. Additionally, mammalian paraoxonases PON1, PON2 and PON3 that target AHLs and display broad substrate specificity against organophosphates, arylesters, gamma-lactones and delta-lactones are also included in this category [64]. The second category comprises of natural compounds such as phenolic derivatives (tanins, flavonoids, phenylpromene etc.), indole derivatives, alkaloids, furanones, lactones, organosulphur compounds and acetaldehydes [64, 65]. Third category of synthetic analogues of QS molecules includes QSIs such as AHL analogues (macrolides, azithromycin, furanyl hydrazide, and cyclohexanone) and lactone analogues (N-(heptyl-sulfanyl acetyl)-L-HSL, HepS-AHL and 4-phenylbutanoyl HSL) [14]. Anti-biofilm substances derived from natural sources along with synthetic analogues, chelating agents, lantibiotics and antibiotics are also used to inhibit biofilm growth. Combination therapy comprising of more than one antibiotic is used to treat bacterial infections arising from multispecies heterogeneous biofilms [1]. Additionally, antimicrobial peptides occurring naturally or generated through genetic engineering (broad-spectrum bactericidal peptide R-FV-I16) are effective against biofilm dispersal. Several specifically targeted antimicrobials (STAMPs) are designed to selectively work on pathogens while non-pathogenic bacteria remain unaffected. Biofilm degrading enzymes could also be employed to disrupt the EPS matrix, affecting biofilm stability and allowing dispersal of microbes. Dispersing agents together with antimicrobials can be beneficial in dealing with rigid biofilms. Disrupters such as deoxyribonuclease I derivative (DNAse 1L2) from human stratum corneum and α- amylase from *B. subtilis* are known to disrupt *P. aeruginosa* and *S. aureus* clinical MRSA strain biofilms, and *P. Aeruginosa* ATCC 10145 and *Staphylococcal* biofilms, respectively. Dispersin B (DspB) from *Actinobacillus actinomycetemcomitans* is known to hydrolyze EPS of *S. aureus* biofilms and promote antibiotic penetration [1].These compounds degrade eDNA and matrix adhesion molecule, poly (1, 6)-N-acetyl glucosamine (PNAG) in many Gram-negative and Gram-positive bacteria including *Staphylococcus dermis* and *S. aureus* [27]. Agents reducing surface adhesion property of biofilm are important in controlling biofilm formation and used as anti-fouling agents [66, 67]. Disinfection with surfactants is a common practice for biofilm removal from steel and glass surfaces in food and dairy industries. While most effective surfactants include anionic, ionic and alkali compounds, other acidic compounds, phenolics, iodine, chlorine, caustic products and peracetic acid are also used to inhibit biofilm formation by altering the physio-chemical properties of surfaces [68]. Surfactants also retard growth of flagella which results in poor microbial adhesion to substratum and hindrance in biofilm formation [69]. Biosurfactants such as rhamnolipids produced by *Pseudomonas* spp. can inhibit biofilms of several pathogens like *S. aureus, Salmonella enteritidis* and *Listeria monocytogenes*, and hold potential to replace synthetic detergents, foaming agents, emulsifiers, solubilizers, and wetting agents in food industry [70]. Biological agents such as enzyme-based detergents, phage particles and cheat cells are gaining popularity due to their anti-biofilm potential. Pre-conditioning with proteolytic and other enzymes has been effective in preventing biofilm production by *Lactobacillus bulgaricus* and *Lactobacillus lactis* in dairy industry. Phage particles are also employed to kill antibiotic sensitive and resistant microbes in wound biofilms, and implant- and catheter-related infections [3]. Cheat cells, in a social community of bacteria, comprise of mutated intraspecies/competitive strains incapable of biosynthesizing QS-dependent secreted essential products (public goods). Such cells depend for these goods on cooperative producer cells and exploit them by utilizing the exo-products without contributing towards the metabolic cost of synthesis. When introduced in pathogenic biofilms, less virulent/medically beneficial cheat cells can compete for resources, hinder biofilm development, and out-number and restrict population of the deprived pathogen [31]. Additionally, in multispecies competitive environment, production of antagonist metabolites by certain species hampers growth of competing organisms. *B. subtilis* for example, produces surfactin that prevents biofilm production by *Salmonella enterica*, *E. coli* and *P. mirabilis* [71]. A classic example of exploitive competition is seen in co-culture of *P. aeruginosa* and *Agrobacterium tumefaciens* where *P. aeruginosa* utilizes a surface blanketing phenomenon to spread and occupy the ecological niche via swarming and twitching motility [72]. Invader microorganisms down regulate the secretion of adhesive molecules, inhibit cell-to-cell communication and may degrade components of EPS [72, 73].

Conclusion and Future Perspectives

Biofilm associated microbial infections are subject of major concern in health, food, agriculture and industrial sectors. Attributes like biofilm dispersal and spread of infection, high efficiency of genetic exchange via eDNA, reduced susceptibility to antimicrobial agents, enhanced ability to form endotoxins (in case of Gram-negative bacteria), and inherent resistance against host immune system make inhibition of biofilm infections a tough ordeal. Efforts are being concentrated to identify biofilm specific transcriptome, estimated to be ~ 10% of genome, to identify genes involved in different stages of biofilm development. This strategy would enable generation of stage-specific control mechanisms targeting specific molecular processes. In parallel, there is an increase in

search for natural and more effective inhibitors of QS molecules by taking cautious note of QSI resistance developing in the background. EPS acts as a major contributor of emergent detrimental attributes observed in biofilm associated infections. Nanoparticle based antibiotics are an emerging area of research with promising results. Phosphatidylcholine-coated Au nanoparticles filled with gentamicin (GPA NPs) have been found to be more effective in killing Gram-positive and Gram-negative pathogens in biofilms, as compared to the planktonic counterparts [74]. Efforts are also on to create nanotechnology-based biomaterial with anti-fouling, bactericidal and anti-biofilm properties. These materials being resistant to biofilm formation and being bio-compatible, non-toxic, and cost-effective serve as advantageous option for making biomedical devices [75]. Natural detergents with biofilm corroding properties could be developed for clearing biofilms through sanitization in health, food and dairy industries. Further studies to unravel molecular mechanisms underlying microbial community existence should provide insights on the intrinsic programming that universally governs biofilm development. Last two decades of path-breaking research in this field has uncovered various aspects of this complex social lifestyle of bacteria. Community living is a natural phenomenon involving genetically similar cells to differentiate and acquire phenotypically distinct characteristics. Bacterial differentiation commonly arises from asymmetric cell division leading to progeny with diverse features and roles. Distribution of specific roles is induced through concentration dependent cellular factors and in response to changing population density. During this phase, a small population of functionally senescent persister cells accumulate in intricate regions within biofilms. A deeper understanding on triggers and molecular events mediating senescence, responsive differential gene expression resulting in phenotypic variation, inducers of division-of-labour behaviour, and chemical environment and gradients within biofilms should provide novel clues to the dynamic multispecies consortium with a single aim of better living.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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