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REVIEW

What makes another life possible in bacteria? Global regulators as architects of bacterial biofilms

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Abstract

Biofilm structures are the main mode of evolutionary reproductive adaptation of bacteria, and even these features alone, are sufficient to make them the focus of genetic and physiological studies. As this life form is a multicellular-like life form coordinated by genetic and physiological programming, it is quite different from the planktonic form. In bacterial biofilms, which are often composed of more than one species in nature, there is a clear division of labor, nutrient channels, and a language (signaling) established between the cells forming the biofilm. On the other hand, biofilms, especially formed by pathogens, cause important industrial and clinical problems due to their high resistance to environmental stress conditions. Obtaining new data on the molecular basis of bacterial evolution and understanding the intra- and inter-species ecosystem relations in this context, as well as finding permanent solutions to the serious problems they create, are directly related to a detailed understanding of the genetic regulation of bacterial biofilm structures. Today, it is becoming increasingly certain that environmental signals effective in the transition from planktonic form to biofilm form and their receptor/response molecules are generally managed by similar systems and global regulator molecules in bacteria. In this sense; Besides the quorum sensing (QS) systems, cyclic adenosine monophosphate-catabolite suppressor protein (cAMP-CRP) and bis-(3′–5′) cyclic dimeric guanosine monophosphate (c-di-GMP) signaling molecules are of critical importance. In this review article, current information on bacterial biofilms is summarized and interpreted based on this framework.

Keywords Biofilm · Genetic regulation · Quorum sensing · cAMP-CRP · c-diGMP · Bacteria

Introduction

It is possible to describe bacterial biofilms as multicellular-like life forms that are formed by one or more free-living species by adhering to biotic or abiotic surfaces and each other, surrounded by an extracellular polymeric matrix they produce (de la Fuente-Núñez et al. 2013; Flemming et al. 2016). The multicellular-like form definition essentially refers to the cooperation and task sharing of the cells that make up the biofilm in this new life form. In other words, the preferences of microorganisms between independent life forms and biofilm forms represent a process that requires a total genetic and physiological transformation. First of all, it is necessary to define what conditions force independently-living cells to undergo such a massive transformation. Thus, it will help us to develop a correct perspective on biofilm forms and to understand the evolutionary forces involved in selecting such a transformation as an alternative.

The microbial fossil studies based on the analysis of conical stromatolites indicate that bacteria biofilm forms have a history of 2.9 billion years on earth (Petroff et al. 2013). In this process, which corresponds to 500 million years after the first bacterial fossil record, it is thought that bacteria developed biofilm forms for their adaptation to adverse environmental conditions on earth. The formation of biofilm forms is one of the main ways for bacterial resistance to many environmental conditions that force bacterial growth, such as low nutrient supply, high degree of microbial antagonism, changes in salt and sugar concentrations, high pressure, presence of antimicrobials, low water activity and ultraviolet radiation. The resistance of biofilm structures to all these adverse environmental conditions, despite the contribution of other genetic and physiological rearrangements,

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is mainly due to the extracellular polymeric substance (EPS), which is a common characteristic of bacterial biofilms. This structure, which we can call the main material mixture used in the construction of the castle of biofilm community in question, consists of the main components of carbohydrates, lipids, proteins, and extracellular DNA prepared in the aqueous phase. The castle of bacterial biofilm communities is built directly by the members of these communities. The main materials of the mortar used in the construction of the said castle are carbohydrates, lipids, proteins, extracellular DNA, and water. The architects and workers of this castle are also members of the mentioned communities (Flemming et al. 2007, 2016). Biofilm castles not only act as a barrier to adverse environmental conditions but also serve as nutrient reservoirs to withstand the famine created by these conditions. Castle dwellers use the quorum sensing alphabet to perceive and respond to negative environmental signals, that is, to communicate and to get organized. The division of labor of the castle residents in war and peace is a process that is governed by much more complex regulations (genetic and biochemical).

Biofilm structures can be homogeneous bacterial communities consisting of a single species, or heterogeneous bacterial communities established by the cooperation of more than one species. Heterogeneous biofilms are mostly encountered in natural conditions. Interactions between species in these heterogeneous biofilm communities need to be largely elucidated. Biofilms formed by bacterial pathogens are the main source of persistent bacterial infections and are responsible for 85% of total bacterial infections. Bacterial biofilms, which cause major problems in the health and food industries due to the persistent infections they cause, are also used in the production of many organic compounds or the treatment of domestic and industrial wastes (Liu et al. 2022a, b). In addition to these versatile and critical effects of bacterial biofilms on life, the structural and functional multicellular organization of planktonic forms has become the focus of microbiological studies.

Biofilm formation

The basic stages of bacterial biofilm formation are defined as an attachment to organic or inorganic surfaces, colonization, extracellular polymeric matrix production, maturation, and dispersal (Fig. 1).

In the first phase of contact of bacteria with biotic or abiotic surfaces, along with Brownian motion, Wan der Waals forces, gravitational forces, hydrodynamic forces, flagellar motion, chemotaxis and electrostatic interactions, fragile adhesives produced by cells also show activity. Since these forces can be easily eliminated by different compelling environmental effects, the process in question is defined as reversible adhesion (Kreve et al. 2021; Luo et al. 2022).

As a result of the prolongation of this process directed by the weak forces mentioned above; in addition to bacterial fimbrial adhesins, genetic regulation of non-fimbrial bacterial surface proteins such as biofilm extracellular polymeric substance (EPS) matrix exopolysaccharides, autotransporter proteins, and porins is promoted, and all these adhesive elements produced to play a role in the realization of irreversible adhesion (Lee et al. 2018). In this irreversible attachment stage, the transition from individual movement (swimming) to community movement (swarming) occurs with negative regulation of flagella synthesis in bacteria moving with flagella. Following the irreversible attachment, the process of microcolony formation and coating of the said microcolonies with the extracellular polymeric matrix they produce is initiated. This process is driven by environmental stress factors such as temperature, nutrient supply, desiccation, salt concentration, and accumulation of antimicrobials, which trigger the conversion from the planktonic form to

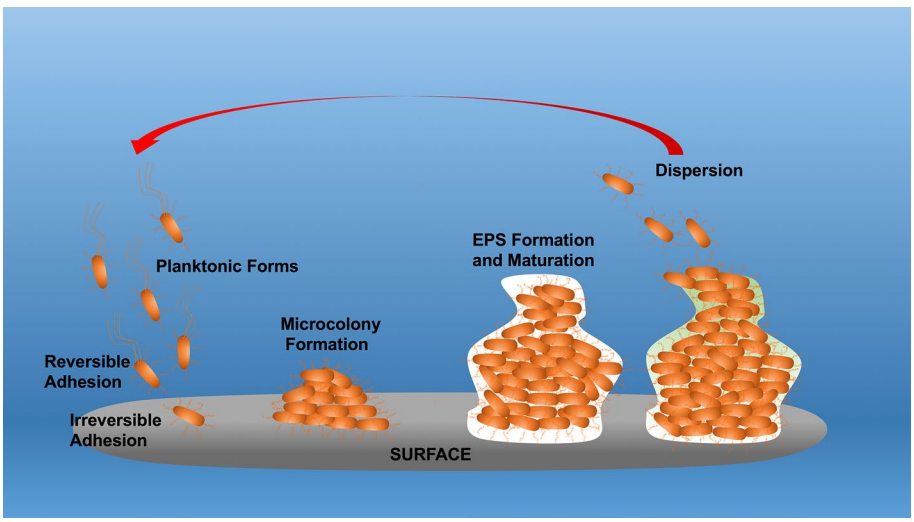

Fig. 1 General steps of bacterial biofilm formation. EPS (Exopolymeric substance matrix). EPS exopolymeric substance

the biofilm form, triggering a global reprogramming that will govern biofilm formation. All known cell-to-cell interactions play a role in the growth and association of microcolonies, which are considered to be the precursors of biofilm structures. These interactions are the main factors that determine the architecture of different biofilm structures and the functionality of cell groups in the biofilm (Flemming et al. 2016; Liu et al. 2020). The typical step affected by the formation of microcolonies is surrounding the colonies in question with the EPS they produce. While EPS production ensures that the formed bacterial community is protected from the environmental stress conditions mentioned above (by acting as a nutrient reservoir, forming a liquid phase, resisting phagocytosis and environmental antimicrobials), it also determines its limits. The most abundant macromolecules in the bacterial EPS matrix, depending on the species are; carbohydrates, proteins, extracellular DNA (eDNA), and lipids. Cell-to-cell interactions, which are essential in the formation and maturation of microcolonies, are predominantly driven by exopolysaccharides. In summary, exopolysaccharides constitute the main force that holds together the components of the structure, which we define as the strongholds of bacterial communities (Flemming et al. 2016). The elasticity of the matrix exopolysaccharides plays an important role in directing the flow of nutrients and oxygen to the biofilms and protecting the biofilms against especially challenging physical (pressure, temperature), chemical (antimicrobial agents, and toxins), and biological (phagocytic effect) effects. Curli fibrils in protein structure in different bacterial species, especially *Salmonella*, also contribute significantly to this power. Typical examples of exopolysaccharides in the biofilm structure are; cellulose (*E. coli, Salmonella, Mycobacterium tuberculosis*), Pel and Psl, alginate (*P. aureginosa*), poly-β(1,6)-N-acetyl-D-glucosamine (PNAG, *Staphylococcus aureus*, and *S. epidermidis*), xanthan (*Xanthomonas citri*), curdlan (*Cellulomonas* sp.) and dextran (*Streptococcus mutans*) (Irie et al. 2012; Gupta et al. 2019; Singh et al. 2021).

The typical architectural form that defines biofilm maturation is mushroom-like three-dimensional formation. In this process, fimbrial and non-fimbrial adhesives, as well as biofilm matrix-specific exopolysaccharides, play a critical role. The data on the origin and function of eDNA in the EPS matrix of bacterial biofilm structures are highly controversial. Some researchers argue that eDNA is produced by promoting the biofilm structure, while some researchers argue that it is included in the EPS structure by lysis of the cells in the biofilm structure (Akçelik and Akçelik 2021). Again, although it has been suggested that the presence of eDNA in different biofilms promotes biofilm production and acts as the cement of EPS, and contributes to the antibiotic resistance (Whitchurch et al. 2002; Mulcahy et al. 2010), there is also evidence that eDNA does not have a significant role in biofilm formation and EPS functionality. In the studies carried out by *Salmonella* Typhimurium, the determination of eDNA to originate from genomic DNA and to take place in the surface structures of planktonic forms indicates that eDNA is not a biofilm-specific element. It was determined that eDNA is effective in the adhesion of planktonic forms to the surfaces in the first stage of biofilm formation in these bacteria. The most prominent role of eDNA in the biofilm matrix can be expressed as increasing the frequency of horizontal gene transfer between bacteria in the biofilm structure, thus contributing to genetic diversity (Özdemir et al. 2018; Çelik et al. 2020).

The dispersion of biofilms can be identified by physical and chemical effects originating from the external environment (oxidative stress, improvement of nutritional conditions, accumulation of toxins and other antimicrobial compounds and sanitation processes, etc.) or as an active process directly managed by the bacteria that form the biofilm (Hwang et al. 2014; Jiang et al. 2020). A typical example of passive biofilm dispersal in *P. aeruginosa* is the increase in environmental carbon and nitrogen sources. The main forces directing the active process are the degradation of the EPS structure by enzymes and the quorum-sensing (QS) signaling system (Cuttuzzola and Frankenberg-Dinkel 2016; Akçelik and Akçelik 2017). It was determined that the decrease in c-di-GMP signals in *P. aeruginosa*, *S.* Typhimurium, and *E. coli* promoted biofilm dispersal (Ma et al. 2011; Zhang et al. 2013; Cuttuzzola and Frankenberg-Dinkel 2016). Biofilm dispersion is the most critical point of the fight against biofilms formed by pathogens in the food industry and clinical environments. In particular, the determination of the active process that direct biofilm distribution and the genetic and biochemical elements that play a key role in these processes are seen as the basic starting point in defining effective and environmentalist strategies to combat biofilms. However, the main challenge in this field is that the production of heterogeneous biofilms, especially in natural environments, is a highly dynamic process.

Molecular basis of transition from planktonic form to biofilm form

The main reason of survival and adaptation of bacteria to almost all environmental conditions on earth is their high genetic adaptation abilities. The main forces governing this adaptation are mutations, genetic recombination, horizontal gene transfer frequency, and high elasticity in the regulation of gene expression. The main strategy used by bacteria for survival and adaptation to biotic or abiotic environments is the transition from independent life forms to a biofilm form. In this way, in the first stage, a common and multicellular life form that is much more resistant to new environmental

conditions than planktonic forms is developed, and "reproductive fitness", which is the main force of environmental permanence and evolution, is ensured. The strong resistance characters of biofilm forms to extreme chemical, physical and biological effects has been the main strategy for the spread of bacteria by occupying new niches since the earliest stages of life on earth. The transition from planktonic forms to biofilm forms is an active process directly managed by bacteria, or vice versa, however if compelling environmental effects are excluded, it is managed by the cells forming the biofilm or their metabolites (Steenackers et al. 2016). It is imperative to determine the regulation strategies that play a role in the formation of biofilm forms, especially to solve the serious problems caused by pathogen biofilms in food and health industries. Here, the practical expectation of scientists and practitioners is that bacteria use common regulation strategies in the transition from planktonic to biofilm form. Thus, co-agents can be developed to combat the vast majority of bacterial biofilms. However, first of all, it is necessary to answer the question of whether this optimistic expectation is possible or, if so, how far the "light at the end of the tunnel" is.

Numerous genes effective in biofilm formation in different bacterial species and the differences in expression of these genes between planktonic and biofilm forms have been described. At the same time, the regulation characteristics of most of the active genes in biofilm formation were determined at certain levels. Although these genes and their regulation characteristics show some similarity with closely related species, they were found to be quite different with distant relatives. However, the fact that many new genes affecting the biofilm formation are still being defined in different studies, especially even in the same species of bacteria (Casper-Lindley and Yildiz 2004; Cue et. al 2009; Irie et al. 2010; Fazli et al. 2014; Fechter et al. 2014; Tan et. al 2014; Uğur et. al 2018; Eran et al. 2020), makes the possibility of identifying a common strategy in biofilm formation almost a dream. Is this the real situation? The answer to this question lies within the studies mentioned in the question. First of all, the above-mentioned studies are carried out with biofilms that are promoted in laboratory environments and usually consist of a single species (homogeneous). In addition, the main starting point in these studies is to determine the differences in expression of major environmental stress regulators, biofilm forms, and their planktonic forms. In such a case, it is impossible to obtain a data to reach an integrative level to describe the regulation of biofilm, not only among all bacteria but even in a single species. However, since all these genetic regulation systems in biofilm formation are activated as a response to environmental signals, quorum sensing systems (QS), cAMP: CRP complexes and C-di-GMP stand out as basic elements that trigger bacterial biofilm formation. These systems, which we can define as regulators of regulators, are global regulatory systems that regulate almost all biofilm regulators in Gram-positive and Gram-negative bacteria.

QS systems

The QS system was first discovered over 40 years ago in the luminescent marine bacteria *Vibrio fischeri* (*V. fischeri*) and *Vibrio harveyi* (*V. harveyi*). In both species, luminescence was determined to occur only at high cell population density in response to the accumulation of secreted AI signaling molecules. This was the first evidence showing that the bacteria communicate with each other through signal molecules they produce, meaning they have a socialization network (Nealson and Hastings 1979). It is now known that these systems depend on the cell density of both Gram-negative and Gram-positive bacteria.; It has been determined that QS systems in bacteria regulates regulators that control many features such as biofilm formation, horizontal gene transfer, antibiotic resistance, pathogenicity, stress response, expression of secretion systems, motility, and toxin production. QS has been described not only between the cells of the same species (within species) but also between the species, as well as between the bacteria and the higher organization organisms (eg, mammalian paraoxonases) (Diggle et al. 2007). Gram-negative bacteria predominantly use AHL (acyl homoserine lactone) molecules (AI-1) in their QS system, while Gram-positive bacteria generally use translationally modified peptides (AIP). The second type of AI molecules, AI-2 molecules, are autoinducer-2 (AI-2, Vibrio harveyi), PQS (*Pseudomonas* quinolone signal), DSF (diffusible signaling factor, *Xanthomonas campestris*), indole (*E. coli*) and PAME (hydroxyl-palmitic acid methyl ester, *Ralstonia solanacearum*) is synthesized and detected by both Gram-negative and Gram-positive bacteria and provides interspecies communication (Verbeke et al. 2017; Pena et al. 2019) (Fig. 2).

Thanks to the QS system, which is an important mechanism in the biofilm formation process and dispersal, bacteria can measure the population density by communicating with the signal molecules they produce. As the number of bacteria attached to the surface increases, the concentration of these signal molecules increases, and with this increase, several processes directly lead to the initiation of biofilm formation (Akçelik and Akçelik 2021). Three types of QS molecules have been identified in *Salmonella*. These are AHLs designated AI-1, furanosyl borate diesters called AI-2, and host cell-associated AI-3 molecules (pyrazinone metabolites). In the first system, *Salmonella* uses a protein called SdiA to respond to AHLs produced by other bacterial species. SdiA is essentially a transcription factor belonging to the LuxR family. Although a direct link between SdiA

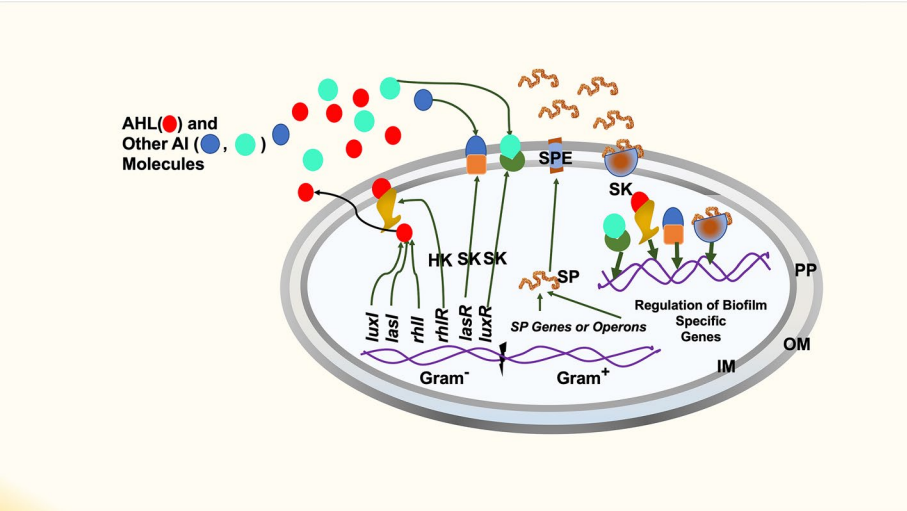

Fig. 2 QS systems in bacteria. AHL acyl homoserine lactone, AI autoinducer, HK histidine kinase, SK sensor kinase, SP signal peptide, SPE signal peptide exporter, IM ınner membrane, OM outer membrane, PP periplasmic space, Gr- Gram-negative, *P. Aeruginosa*, Gr + Gram-positive

and *Salmonella* biofilms has not been reported, it has been suggested that genes regulated by SdiA may also have a role in biofilm formation. Because SdiA can indirectly affect the expression and assembly of fimbrial proteins (Ahmer et al. 1998; Michael et al. 2001).

The second QS system uses the LuxS enzyme (S-ribosylhomocysteinase) for the synthesis of *Salmonella* AI-2 (Surette et al. 1999). The Lsr transport system is responsible for the recognition and transportation of the synthesized AI-2 signal molecules by the cell. AI-2 signaling molecules are a common language used by both Gram-negative and Gram-positive bacteria (Xue et. al 2009). Jesudhasan et al. (2010) found that biofilm formation in *Salmonella* is affected by mutation of the *luxS* gene which encodes the enzyme S-ribosylhomocysteinase, which breaks S-ribosylhomocysteine thioether bonds to form L-homocysteine and 4,5-dihydroxy-2,3-pentanedione. *S.* Typhimurium *luxS* mutant strains were observed to form an impaired biofilm structure on polystyrene. Microarray analyses revealed that the expression of biofilm-related genes, as well as some motility genes, was decreased in the *luxS* gene deletion mutant compared to the wild type. In *E. coli*, on the other hand, it was determined that external AHL signals, that is, interspecies communication, also contribute to biofilm production by promoting exopolysaccharide production (Zhou et al. 2020). Pathogenic *E. coli* strains use five types of QS signals. These are (i) AI-2 signal produced by the LuxS enzyme, (ii) SdiA, the transcriptional regulator of the LuxR homologous receptor for homoserine lactone, (iii) AI-3/epinephrine/norepinephrine signaling pathway involved in host-bacteria communication, (iv) its own indole signaling mediated by the self-produced effector indole; and (v) extracellular death factor (EDF) carried by a self-produced peptide that triggers the activation of the toxin-antitoxin systems (Zohar et al. 2015). Overall, QS in *E. coli* is involved in the regulation of virulence genes related to biofilm production, motility, type III secretion system (T3SS), toxicity, and curli fimbria production (Witse et al. 2016). QS in *Salmonella* species is involved in the regulation of the pathogenicity island SPI-1 (invasion phenotype), the expression of flagellar genes, the *pefI-srgC* plasmid operon that regulates *rck* (resistance to complement killing) genes, and *srgE* (Abed et al. 2014; Habyarimana et al. 2014).

It was determined that QS signals induce biofilm formation by attaching to the LuxR regulatory protein of N-(3-oxohexanoyl)-L-homoserine lactone in *Vibrio fischeri* as well as *Salmonella* (Azimi et al. 2020). Apart from this, it was identified that two different AHL-mediated systems in *P aeruginosa* contribute to biofilm production by promoting swarming motility and production of bacterial extracellular DNA (Overhange et al. 2008). In *E. coli,* on the other hand, AI-3 signals were found to induce biofilm formation by promoting flagellum and adhesin production (Witsø et al. 2016). In addition, it was determined that QS signals regulate biofilm formation in many genera such as *Actinobacillus, Aggregatibacter, Bacillus, Haemophilus, Moraxella, Mycobacterium, Staphylococcus and Streptococcus* (Escobar-Muciño et al. 2022). This regulation is accomplished by different QS signals and in different ways, as exemplified above. Acyl homoserine lactone (AHL) signals activate the expression of critical genes in biofilm production, often by leading to the activation of LuxR-like regulatory proteins, major regulators that regulate the expression of genes controlled by QS systems, in *V. fischeri*. In addition, peptide signals, and even some AHLs, are typically sensed by membrane-associated receptors to initiate a phosphorylation cascade that leads to target biofilm gene expression (Sturme et al. 2002; Kumari et al. 2016). Biofilm formation is promoted in *Vibrio harveyi* by detecting an AHL (HAI-1) and a furanone (AI-2) by different surface receptors (LuxN and LuxP/Q, respectively) (Henke and Bassler 2004; Yu et al. 2020). The presence of more than one QS system in many

bacteria is the main point that complicates the mechanism of action of the system. For example, it was determined that biofilm formation was completely inhibited in *csrA* mutants in which the production of CsrA protein, a global carbon storage regulator, was blocked in *S*. Typhimurium. In the same study, it was determined that the synthesis of AI-2 was significantly reduced in *csrA* mutants, but the uptake of AI-2 was more efficient. Based on these data, it was interpreted that the *csrA* mutation up-regulated the *lsrA* gene, encodes Lsr transport apparatus, leading to an increase in the expression of the lsr operon and causing more AI-2 transfer to the cell. According to this; Up-regulation of *lsrK,* encodes a kinase, naturally enhances AI-2 phosphorylation and compensates for up-regulation of *lsrR* by phospho-AI-2 binding to LsrR and inactivating it. On the other hand, the fact that CsrA remains a positive regulator of c-di-GMP synthesis indicates that this regulation may involve a more complex mechanism with the participation of cAMP: CRP and c-di-GMP (Bakkheda and Akçelik, unpublished data).

Finally, biofilm regulation can be achieved as a result of the interaction of CRISPR-Cas systems with bacterial QS systems. Cui et al. (2022) determined that the CRISPR-Cas3 system provides inhibition of the activity of the LsrR (repressor of the *lsr* operon) protein by downregulating *lsrFGBE* and subsequently delaying the degradation of p-AI-2, ultimately increasing the active form of AI-2. In this case, since the expression of *lsrFGBE* is still suppressed, the genes involved in biofilm formation are induced. This research is the first to demonstrate biofilm regulation in *Salmonella* by the interaction of CRISPR-Cas systems and QS systems.

c-di-GMP and cAMP-CRP

The role of the signal molecule, known as bis-(3′–5′) cyclic dimeric guanosine monophosphate, or c-di-GMP for short, in biofilm formation process was first demonstrated by the discovery of its role as an allosteric control factor in the biosynthesis of cellulose, an important component of the biofilm matrix in *Gluconacetobacter xylinus* (Ross et al. 1987). Determining that intracellular c-di-GMP levels play a critical role in the formation of rdar and rugose biofilm morphotypes in *S.* Typhimurium and *Vibrio cholerae*, respectively, and has brought c-di-GMP into the focus of attention in understanding the global regulation of biofilm structures (Bomchil et al. 2003; Beyhan et al. 2006). c-di-GMP is an extremely important signaling molecule that plays a role not only in the regulation of the formation of biofilms, which is a multicellular behavior, but also in the expression of motility and virulence (Lamprokostopoulou et al. 2010). In response to various extracellular signals, c-di-GMP containing the GGDEF protein domain is synthesized by diglucan cyclase (DGCs) (Hengge 2009). The synthesis process begins with two molecules of guanosine triphosphate (GTP), and GTP is then degraded by phosphodiesterase (PDEs) (protein domains EAL or HD-GYP) specific for 5' phosphoguanylguanosine (pGpG). In this step, pGpG is converted to two molecules of guanosine monophosphate (GMP) by phosphodiesterase. Many diglucan cyclase enzymes have the RxxD motif. This motif regulates the allosteric control activity of the enzyme by binding to c-di-GMP. Three other classes of c-di-GMP effectors contain the PilZ protein domain. The GGDEF, EAL, and HD-GYP protein domains associated with the regulation of signaling molecules are found in many bacteria. For example; *S*. Typhimurium contains five types of GGDEF, seven types of EAL, and seven types of GGDEF/EAL proteins. The c-di-GMP ratio in the cell depends on the activities of diglucan cyclase and phosphodiesterase enzymes (Galperin et al. 2001).

The regulation of various cellular functions by c-di-GMP, including life-type changes such as the transition from planktonic to sessile (biofilm) form, Allosteric regulation of enzymes or proteins in these pathways, can be achieved in different ways, such as modulation of transcription factors or regulation of gene expression as a result of direct interaction with regulatory RNA molecules (Valentini and Filoux 2016). The first step in triggering all these processes is the detection of the levels of cellular c-di-GMP by c-di-GMP effector proteins and thus its interaction with c-di-GMP when it reaches a certain intracellular concentration. It was determined that c-diGMP signaling in *Pseudomonas aureginosa* suppresses this process by sequestering RsmA, a regulator that induces the planktonic life form, and triggers the transition from the planktonic form to the biofilm form by activating the expression of two regulatory miRNA molecules that promote biofilm formation. Also, when c-diGMP reaches high levels in *P. aureginosa*, it binds to FleQ, an enhancer binding protein, and activates genes that control the production of exopolysaccharides and adhesins. This activation occurs as a result of blocking the ATPase activity of FleQ protein, which is the repressor of genes responsible for adhesin and exopolysaccharide production, by the c-diGMP allosteric effector (Hickman and Harwood 2008). On the other hand, it is also known that c-di-GMP is an allosteric inhibitor of enzymes that catalyze metabolic processes suppressed during biofilm formation, such as FliI (flagellar ATPase), which is an important protein in flagellar movement (Trampari et al. 2015). As a result of blocking the *bcsE* gene, which encodes the c-di-GMP binding protein in *Salmonella*, it was determined that the motility and cellulose biosynthesis, and thus biofilm formation, were highly reduced (Özdemir et al. 2021). In studies conducted on *Salmonella*, c-di-GMP has been identified as an activator of *csgD*, which is the main regulator of biofilm. However, the molecular mechanisms of this activation remain a mystery. It

was determined that extracellular c-di-GMP inhibits biofilm formation in *Staphylococcus aureus* by preventing cell-to-cell interactions (Richter et al. 2019), while high intracellular c-diGMP levels stimulate biofilm formation by inducing exopolysaccharide production (Yan et al. 2020). Likewise, in *Streptococcus mutant* strains, it was determined that cellular c-di-GMP promoted biofilm formation, but extracellular c-di-GMP inhibited biofilm formation. On the other hand, Ahmad et al. (2020) suggested that the signaling of different c-di-GMP effectors in *Acetobacter baumanii* produces opposite effects (activator-repressor) on biofilm formation and surface motility. The research that most completely describes the role of c-di-GMP in bacterial biofilms is by Liu et al. (2022a, b). According to this research, when the intracellular concentration of c-di-GMP is at a high level, it activates the effector protein BpfD, and as a result of BpfD activation, it binds a periplasmic protease, BpfG, leading to its inactivation. In this case, the BpfG protease blocks the degradation and release of the BpfA adhesion protein precursor by breaking down the proteins involved in these processes. However, when intracellular c-di-GMP levels are low, blocking of BpfD cannot occur, so this process is reversed and BpfA is not released (Liu et al. 2022a, b) (Fig. 3).

There are many findings that indicates cAMP-CRP (cyclic adenosine monophosphate-catabolite repressor protein), a secondary messenger like c-di-GMP, is also a regulator of biofilm regulators in bacteria. The data obtained to date has determined that cAMP-CRP activates biofilm formation in some bacterial species while inhibiting it in others. It was suggested that this difference is due to carbon source preferences in these bacteria (Krasteva et al. 2010; Liu et al. 2016; Matsuyama et al. 2016; Liu et al. 2019; Liu et al 2020). In studies conducted on *E. coli*, it was determined that cAMP-CRP makes a critical contribution to biofilm formation as a result of the activation of *csgD* regulator, which is the main regulator of curli fibrils, and flagella and cellulose biosynthesis genes, and suppression of *rpoS,* which is the main regulator of the stress response (Ahmad et al. 2017). However, it was determined that cAMP-CRP inhibits biofilm formation on solid surfaces by downregulating csgD in another enteric bacterium, *S*. Typhimurium (Sokaribo et al. 2020). On the other hand, in *P. aureginosa*, unlike *E. coli*, prefers complex carbohydrates such as acetate instead of simple sugars and uses a different mechanism of catabolite repression, therefore, the mechanism of biofilm formation of cAMP in this bacterium has some differences (Liu et al. 2022a, b). The allosteric activator of cAMP in *P. aureginosa* is the protein Vfr. While the cAMP-Vfr complex, which is formed in response to environmental signals such as calcium and high osmolarity, promotes biofilm formation, it was determined that the cAMP-CbpA complex plays a role in the dispersion of the biofilm (Coggan and Wolfgang 2011). It was concluded that cAMP-CRP directly promotes biofilm formation in *Yersinia pestis* and *Klebsiella pneumoniae*, similar to *E. coli*. In *Yersinia pestis*, this activation is achieved by promoting the production of biofilm exopolysaccharides due to carbon-derived metabolic pathways, and by promoting fimbriae production and capsular polysaccharides in *K. pneumoniae* (Liu et al. 2017; Ou et al. 2017). However, on the contrary, it was determined that cAMP: CRP suppressed biofilm formation in different ways and indirectly in *V. cholera* (Fong et al. 2008).

Finally, Sharma et al. (2022) specified that *Salmonella* CRISPR-Cas systems promote the formation of surface-attached biofilm structures through the activation of *csgD, fliC and flgK* genes, while suppressing pellicle biofilm structures in the same organism. Researchers have suggested that CRISPR-Cas systems perform the induction of surface biofilms by suppressing the expression of cAMP receptor protein (CRP), which is the negative regulator of the biofilm master regulator *csgD* gene. In a comparative

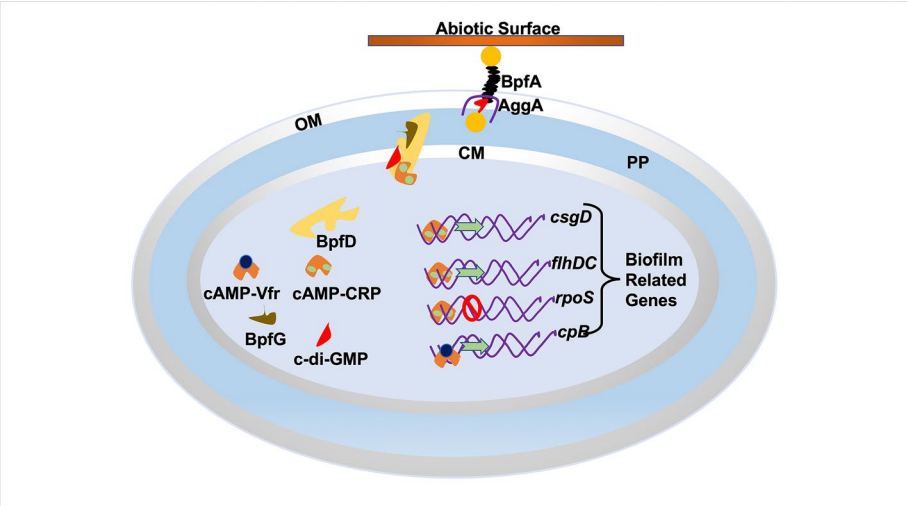

Fig. 3 Biofilm regulation by cAMP-CRP and c-di-GMP signaling in bacteria. OM outer membrane, IM inner membrane, PP periplasmic space

study conducted by our research group on *S.* Typhimurium serovariaty and its deoxy adenine methylase enzyme (*dam*) mutant, similar to the findings of Sharma et al. (2022), it was identified that the *dam* gene promotes biofilm production by suppressing the *crp* gene (Akçelik, unpublished data).

All these literature data indicate that biofilm formation in bacteria is a general global regulator of cAMP-CRP, but the type of regulation may vary depending on the specificities of carbon metabolism in these bacteria.

Conclusion

The bacterial biofilm forms have become the focus of intense scientific research due to the serious health problems and economic losses they cause, especially in medical and industrial terms, as well as showing a community behavior consisting of single or mixed species. The biofilms are a mode of socialization created by bacteria as a result of environmental signals. Thereforethe transition from planktonic to biofilm form, or vice versa, reveals the big picture; the signals triggering biofilm formation, the perception, and the transmission of these signals, and the generation of cellular biofilm responses require a detailed answer. The studies carried out to date in this field have clearly shown that the key elements of the answer to these questions are the bacterial QS systems, as well as the secondary messengers cAMP-CRP and c-di-GMP. On the other hand, the fact that biofilms, which are a life-type change formed as a response to internal and external environmental signals, are highly correlated with the presence and preference of carbon sources, necessitates the need to consider that QS systems are in a relationship with cAMP: CRP and c-di-GMP in biofilm regulations. As a result, QS, cAMP: CRP and c-di-GMP stand in a key position in understanding and combating biofilm structures.

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Declarations

Competing interest The authors declare no competing interests.

References

Abed N, Grépinet O, Canepa S, Hurtado-Escobar GA, Guichard N, Wiedemann A, Velge P, Virlogeux-Payant I (2014) Direct regulation of the *pefI-srgC* operon encoding the Rck invasin by the quorum-sensing regulator SdiA in *Salmonella Typhimurium*. Mol Microbiol 94:254–271

Ahmad I, Cimdins A, Beske T, Römling U (2017) Detailed analysis of c-di-GMP mediated regulation of csgD expression in *Salmonella typhimurium*. BMC Microbiol 17(1):27. https://doi.org/10.1186/s12866-017-0934-5

Ahmad I, Nygren E, Khalid F, Myint SL, Uhlin BE (2020) A Cyclic-di-GMP signaling network regulates biofilm formation and surface-associated motility of *Acinetobacter baumannii* 17978. Sci Rep 10(1):1991. https://doi.org/10.1038/s41598-020-58522-5

Ahmer BMM, van Reeuwijk J, Timmers CD, Valentine PJ, Heffron F (1998) *Salmonella typhimurium* Encodes a SdiA Homolog, a Putative Quorum Sensor of the LuxR Family, That Regulates Genes on the Virulence Plasmid. J Bacteriol 180(5):1185–1193

Akçelik N, Akçelik M (2017) Bacterial biofilms and their interactions with the host defence system. Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi 33:15–28

Akçelik N, Akçelik M (2021) Characteristics and Regulation of biofilm formation in *Salmonella.* 2021. Post Mikrobiol Adv Microbiol 60:113–119. https://doi.org/10.21307/PM-2021.60.2.09

Azimi S, Klementiev AD, Whiteley M, Diggle SP (2020) Bacterial quorum sensing during infection. Annu Rev Microbiol 74:201–219

Beyhan S, Tischler AD, Camilli A, Yildiz FH (2006) Transcriptome and phenotypic responses of *Vibrio cholerae* to increased cyclicdi-GMPlevel. J Bacteriol 188:3600–3613

Bomchil N, Watnick P, Kolter R (2003) Identification and characterization of a *Vibriocholerae* gene, *mbaA*, involved in maintenance of biofilm architecture. J Bacteriol 185:1384–1390

Casper-Lindley C, Yildiz FH (2004) VpsT is a transcriptional regulator required for expression of vps biosynthesis genes and the development of rugose colonial morphology in *Vibrio cholerae* O1 El Tor. J Bacteriol 186:1574–1578

Çelik ÇE, Akçelik M, Akçelik N (2020) Inhibition of early stages of *Salmonella* Typhimurium biofilms by extracellular DNA (eDNA) and genomic DNA (gDNA). J Microbiol Biotech Food Sci 10:441–444

Coggan KA, Wolfgang MC (2011) Global regulatory pathways and cross-talk control *Pseudomonas aeruginosa* environmental lifestyle and virulence phenotype. Curr Issues Mol Biol 14:47–70

Cue D, Lei MG, Luong TT, Kuechenmeister L, Dunman PM, O'Donnell S, Rowe S, O'Gara JP, Lee CY (2009) Rbf promotes biofilm formation by *Staphylococcus aureus* via repression of *icaR*, a negative regulator of *icaADBC*. J Bacteriol 191:6363–6373

Cui L, Wang X, Huang D, Feng J, Liu Q, Pu Q, Wang Y, Cheng G, Wu M, Dai M (2022) CRISPR-cas3 of *Salmonella* upregulates bacterial biofilm formation and virulence to host cells by targeting quorum-sensing systems. Pathogens. https://doi.org/10.3390/pathogens9010053

Cutruzzolà F, Frankenberg-Dinkel N (2016) Origin and impact of nitric oxide in *Pseudomonas aeruginosa* biofilms. J Bacteriol 198:55–65. https://doi.org/10.1128/jb.00371-15

de la Fuente-Núñez C, Reffuveille F, Fernández L, Hancock REW (2013) Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opin Microbiol 16(5):580–589

Diggle SP, Gardner A, West SA, Griffin AS (2007) Evolutionary theory of bacterial quorum sensing: when is a signal not a signal? Philos. Trans R Soc Lond B Biol Sci 29:1241–1249

Eran Z, Akçelik M, Yazıcı BC, Özcengiz G, Akçelik N (2020) Regulation of biofilm formation by marT in *Salmonella* Typhimurium. Mol Biol Rep 47:5041–5050. https://doi.org/10.1007/s11033-020-05573-6

Escobar-Muciño E, Arenas-Hernández MMP, Luna-Guevara M (2022) Mechanisms of inhibition of quorum sensing as an

alternative for the control of *E. coli* and *Salmonella*. Microorganisms 10:884–898. https://doi.org/10.3390/microorganisms10050884

Fazli M, Almblad H, Rybtke ML, Givskov M, Eberl L, Tolker-Nielsen T (2014) Regulation of biofilm formation in *Pseudomonas* and *Burkholderia* species. Environ Microbiol 16:1961–1981

Fechter P, Caldelari I, Lioliou E, Romby P (2014) Novel aspects of RNA regulation in *Staphylococcus aureus*. FEBS Lett 588:2523–2529

Flemming HC, Neu TR, Wozniak DJ (2007) The EPS matrix: the "house of biofilm cells." J Bacteriol 189:7945–7947

Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S (2016) Biofilms: an emergent form of bacterial life. Nat Rev Microbiol 14:563–575. https://doi.org/10.1038/nrmicro.2016.94

Fong JC, Yildiz FH (2008) Interplay between cyclic AMP-cyclic AMP receptor protein and cyclic di-GMP signaling in *Vibrio cholerae* biofilm formation. J Bacteriol 190:6646–6659. https://doi.org/10.1128/JB.00466-08

Galperin MY, Nikolskaya AN, Koonin EV (2001) Novel domains of the prokaryotic two-component signal transduction systems. FEMS Microbiol Lett 203(1):11–21

Gupta P, Pruthi PA, Pruthi V (2019) Role of exopolysaccharides in biofilm formation. Introduction to biofilm engineering. American Chemical Society, Washington

Habyarimana F, Sabag-Daigle A, Ahmer BM (2014) The SdiA-regulated gene srgE encodes a type III secreted effector. J Bacteriol 2014(96):2301–2312

Hengge R (2009) Principles of c-di-GMP signaling in bacteria. Nat Rev Microbiol 7(4):263–273

Henke JM, Bassler BL (2004) Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. J Bacteriol 186:6902–6914

Hickman JW, Harwood CS (2008) Identification of FleQ from *Pseudomonas aeruginosa* as a c-di-GMP-responsive transcription factor. Mol Microbiol 69:376–389. https://doi.org/10.1111/j.1365-2958.2008.06281.x

Hwang G, Klein MI, Koo H (2014) Analysis of the mechanical stability and surface detachment of mature streptococcus mutans biofilms by applying a range of external shear forces. Biofouling 30:1079–1091. https://doi.org/10.1080/08927014.2014.969249

Irie Y, Starkey M, Edwards AN, Wozniak DJ, Romeo T, Parsek MR (2010) *Pseudomonas aeruginosa* biofilm matrix polysaccharide Psl is regulated transcriptionally by RpoS and post-transcriptionally by RsmA. Mol Microbiol 78:158–172

Irie Y, Borlee BR, O'connor JR, Hill PJ, Harwood CS, Wozniak DJ, Parsek MR (2012) Self-produced exopolysaccharide is a signal that stimulates biofilm formation in *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 109:20632–20636. https://doi.org/10.1073/pnas.1217993109

Jesudhasan PR, Cepeda ML, Widmer K, Dowd SE, Soni KA, Hume ME, Zhu J, Pillai SD (2010) Transcriptome analysis of genes controlled by luxS/autoinducer-2 in *Salmonella enterica* serovar Typhimurium. Foodborne Pathog Dis 7(4):399–410

Jiang Y, Geng M, Bai L (2020) Targeting biofilms therapy: current research strategies and development hurdles. Microorganisms 8:1222. https://doi.org/10.3390/microorganisms8081222

Krasteva PV, Fong JC, Shikuma NJ, Beyhan S, Navarro MV, Yildiz FH (2010) Vibrio cholerae VpsT regulates matrix production and motility by directly sensing cyclic di-GMP. Science 327:866–868. https://doi.org/10.1126/science.1181185

Kreve S, Reis ACD (2021) Bacterial adhesion to biomaterials: what regulates this attachment? A review. Jpn Dent Sci Rev 57:85–96. https://doi.org/10.1016/j.jdsr.2021.05.003

Kumari S, Mangwani N, Das S (2016) Synergistic effect of quorum sensing genes in biofilm development and PAHs degradation by a marine bacterium. Bioengineered 7:205–211

Lamprokostopoulou A, Monteiro C, Rhen M, Römling U (2010) Cyclic di-GMP signaling controls virulence properties of *Salmonella enterica* serovar Typhimurium at the mucosal lining. Environ Microbiol 12(1):40–53

Lee CK, De Anda J, Baker AE, Bennett RR, Luo Y, Lee EY et al (2018) Multigenerational memory and adaptive adhesion in early bacterial biofilm communities. Proc Natl Acad Sci USA 115:4471–4476. https://doi.org/10.1073/pnas.1720071115

Lin CT, Lin TH, Wu CC, Wan L, Huang CF, Peng HL (2016) CRP-cyclic AMP regulates the expression of type 3 fimbriae via cyclic di-GMP in *Klebsiella pneumoniae*. PLoS ONE 11:e0162884. https://doi.org/10.1371/journal.pone.0162884

Liu C, Yang J, Liu L, Li B, Yuan H, Liu W (2017) Sodium lactate negatively regulates shewanella putrefaciens CN32 biofilm formation via a three-component regulatory system (LrbS-LrbA-LrbR). Appl Environ Microbiol 83:e00712-e717. https://doi.org/10.1128/AEM.00712-17

Liu C, Sun D, Zhu J, Liu W (2019) Two-component signal transduction systems: a major strategy for connecting input stimuli to biofilm formation. Front Microbiol 9:3279. https://doi.org/10.3389/fmicb.2018.03279

Liu C, Sun D, Zhu J, Liu J, Liu W (2020) The regulation of bacterial biofilm formation by cAMP-CRP: a mini-review. Front Microbiol 11:802. https://doi.org/10.3389/fmicb.2020.00802

Liu C, Sun D, Liu J, Chen Y, Zhou X, Ru Y, Zhu J, Liu W (2022a) cAMP and c-di-GMP synergistically support biofilm maintenance through the direct interaction of their effectors. Nat Commun 13:1493. https://doi.org/10.1038/s41467-022-29240-5

Liu D, Huang Q, Gu W, Zeng XA (2022b) A review of bacterial biofi, lm control by physical strategies. Crit Rev Food Sci 63:3453–3470

Luo A, Wang F, Sun D, Liu X, Xin B (2022) Formation, development, and cross-species interactions in biofilms. Front Microbiol. https://doi.org/10.3389/fmicb.2021.757327

Ma Q, Yang Z, Pu M, Peti W, Wood TK (2011) A novel c-di-GMP-binding protein for biofilm dispersal. Environ Microbiol 13:631–642

Matsuyama BY, Krasteva PV, Baraquet C, Harwood CS, Sondermann H, Navarro MV (2016) Mechanistic insights into c-di-GMP-dependent control of the biofilm regulator FleQ from *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 113:E209–E218. https://doi.org/10.1073/pnas.1523148113

Michael B, Smith JN, Swift S, Heffron F, Ahmer BMM (2001) SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. J Bacteriol 183(19):5733–5742

Mulcahy H, Charron-Mazenod L, Lewenza S (2010) *Pseudomonas aeruginosa* produces an extracellular deoxyribonuclease that is required for utilization of DNA as a nutrient source. Environ Microbiol 12:1621–1629

Nealson KH, Hastings JW (1979) Bacterial bioluminescence: its control and ecological significance. Microbiol Rev 43(4):496–518

Ou Q, Fan J, Duan D, Xu L, Wang J, Zhou D, Yang H, Li B (2017) Involvement of cAMP receptor protein in biofilm formation, fimbria production, capsular polysaccharide biosynthesis and lethality in mouse of *Klebsiella pneumoniae* serotype K1 causing pyogenic liver abscess. J Med Microbiol 66:1–7. https://doi.org/10.1099/jmm.0.000391

Overhage J, Bains M, Brazas MD, Hancock RE (2008) Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. J Bacteriol 190:2671–2679. https://doi.org/10.1128/JB.01659-07

Özdemir C, Akçelik N, Akçelik M (2018) The role of extracellular DNA in *Salmonella* biofilms. Mol Genet Microbiol Virol 33:60–71

Özdemir C, Akçelik N, Neslihan Özdemir F, Evcili İ, Kahraman T, Gürsel İ, Akçelik M (2021) The role of *bcsE* gene in the pathogenicity of *Salmonella*. Pathog Dis. https://doi.org/10.1093/femspd/ftab037

Pena RT, Blasco L, Ambroa A, González-Pedrajo B, Fernández-García L, López M, Bleriot I, Bou G, García-Contreras R, Wood TK, Tomás M (2019) Relationship between quorum sensing and secretion systems. Front Microbiol. https://doi.org/10.3389/FMICB.2019.01100

Petroff AP, Beukes NJ, Rothman DH, Bosak T (2013) Biofilm growth and fossil form. Phys Rev 3(4):1–14

Richter AM, Fazli M, Schmid N, Shilling R, Suppiger A, Givskov M, Eberl L, Tolker-Nielsen T (2019) Key players and individualists of cyclic-di-GMP Signaling in *Burkholderia cenocepacia*. Front Microbiol 9:3286. https://doi.org/10.3389/fmicb.2018.03286

Ross P, Weinhouse H, Aloni Y, Michaeli D, Weinberger-Ohana P, Mayer R, Braun S, de Vroom E, van der Marel GA, van Boom JH, Benziman M (1987) Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. Nature 325(6101):279–281

Sharma N, Das A, Raja P, Matarhe SA (2022) The CRISPR-Cas system differentially regulates surface-attached and pellicle-biofilm in 2 *Salmonella enterica* serovar Typhimurium. bioRxiv. https://doi.org/10.1101/2022.01.20.477050;t

Singh S, Datta S, Narayanan KB, Rajnish KN (2021) Bacterial exopolysaccharides in biofilms: role and antimicrobial treatments. J Genet Eng Biotechnol 19:140–158. https://doi.org/10.1186/s43141-021-00242-y

Sokaribo AS, Hansen EG, McCarthy M, Desin TS, Waldner LL, MacKenzie KD, Mutwiri G, Herman NJ, Herman DJ, Wang Y, White AP (2020) Metabolic activation of CsgD in the regulation of *Salmonella* biofilms. Microorganisms 8(7):964

Steenackers HP, Parijs I, Dubey A, Foster KR, Vanderleyden J (2016) Experimental evolution in biofilm populations. FEMS Microbiol Rev 40:373–397

Sturme MH, Kleerebezem M, Nakayama J, Akkermans AD, Vaugha EE, de Vos WM (2002) Cell to cell communication by autoinducing peptides in gram-positive bacteria. Antonie Van Leeuwenhoek 81:233–243

Surette MG, Miller MB, Bassler BL (1999) Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and Vibrio harveyi: a new family of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA 96(4):1639–44

Tan SY, Chew SC, Tan SY, Givskov M, Yang L (2014) Emerging frontiers in detection and control of bacterial biofilms. Curr Opin Biotechnol 26:1–6

Trampari E, Stevenson CE, Little RH, Wilhelm T, Lawson DM, Malone JG (2015) Bacterial rotary export ATPases are allosterically regulated by the nucleotide second messenger cyclic-di-GMP. J Biol Chem 290:24470–24483

Uğur S, Akçelik N, Yüksel FN, Karatuğ NT, Akçelik M (2018) Effects of *dam* and *seqA* genes on biofilm and pellicle formation in *Salmonella*. Pathogens Global Health 112:368–377

Valentini M, Filoux A (2016) Biofilms and cyclic di-GMP (c-di-GMP) signaling: lessons from *Pseudomonas aeruginosa* and other bacteria. J Biol Chem 291:12547–12555

Verbeke F, De Craemer S, Debunne N, Janssens Y, Wynendaele E, Van de Wiele C, De Spilegeer B (2017) Peptides as quorum sensing molecules: measurement techniques and obtained levels in vitro and in vivo. Front Neurosci 11:183. https://doi.org/10.3389/fnins.2017.00183

Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS (2002) Extracellular DNA required for bacterial biofilm formation. Science 295:1487–1495

Witsø IL, Rukke HV, Benneche T, Scheie AA (2016) Thiophenone attenuates enteropathogenic *Escherichia coli* O103:H2 virulence by interfering with AI-2 signaling. PLoS ONE 11:e0157334

Xue T, Zhao L, Sun H, Zhou X, Sun B (2009) LsrR-binding site recognition and regulatory characteristics in *Escherichia coli* AI-2 quorum sensing. Cell Res 19(11):1258–1268

Yan J, Qu T, Zhao H, Su L, Yu Q, Gao J, Wu B (2020) The effect of c-di-GMP (3′–5′-cyclic diguanylic acid) on the biofilm formation and adherence of *Streptococcus mutans*. Microbiol Res 165:87–96. https://doi.org/10.1016/j.micres.2008.10.001

Yu Z, Hu Z, Xu Q, Zhang M, Yuan N, Liu J, Meng Q, Yin J (2020) The LuxI/LuxR-type quorum sensing system regulates degradation of polycyclic aromatic hydrocarbons via two mechanisms. Int J Mol Sci 21:5548. https://doi.org/10.3390/ijms21155548

Zhang W, Sikleika T, Packman AL (2013) Effects of fluid flow conditions on interactions between species in biofilms. FEMS Microbiol Ecol 84:344–354

Zhou L, Zhang Y, Ge Y, Zhu X, Pan J (2020) Regulatory mechanisms and promising applications of quorum sensing-inhibiting agents in control of bacterial biofilm formation. Front Microbiol. https://doi.org/10.3389/fmicb.2020.589640

Zohar BA, Kolodkin-Gal I (2015) Quorum sensing in *Escherichia coli*: interkingdom, inter-and intraspecies dialogues, and a suicideinducing peptide. Quorum sensing vs quorum quenching: a battle with no end in sight. Springer, New Delhi, pp 85–99