2

Bacterial Biofilms and their Regulatory Approaches

AUTHORS DETAIL

Muhammad Adnan Liaqat[1], Muhammad Shahid Mahmood[1], Muhammad Numan Akram[2], Muhammad Imran Arshad[1], Rizwan Aslam[1], Muhammad Kasib Khan[3], Muhammad Umar Zafar Khan[1], Noor Fatima[1] and Sultan Ali[1]*

[1]Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.
[2]Department of Neurology, Allied Hospital, Faisalabad Medical University, Faisalabad, Pakistan.
[3]Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.
*Corresponding author: sultanali@uaf.edu.pk

Received: Sept 22, 2022 Accepted: Nov 05, 2022

INTRODUCTION

Bacteria have been intensively researched in their planktonic condition from the beginning, yet in natural systems they seldom exist in an autonomous, free-swimming planktonic state. Instead, they reside in interdependent complex multicellular surface-attached communities called biofilms that are bound together by a self-secreted extracellular polymer matrix mostly made of polysaccharides, secreted proteins, and extracellular DNAs (Tremblay et al. 2013). Antoine Von Leeuwenhoek initially saw a particular species on his own teeth in the 17th century, a discovery known as a biofilm (Jamal et al. 2018), however, the term "biofilm" was coined in 1978 by a Canadian scientist named "Bill Costerton." According to his definition, a biofilm is a heterogeneous structure made up of several populations of microorganisms encased in an extracellular matrix that allows bacteria to cling to either an abiotic (such as rock, glass, or plastic) or biotic surface (skin, mucosa, or cuticle) (Berlanga and Guerrero 2016).

The myth that the majority of bacteria on Earth are capable of building biofilm is generally believed since microbes are ubiquitous and can be found everywhere and on every surface. In contrast to the matrix, which may make up more than 90% of the dry mass, microorganisms make up less than 10%. In various conditions, between 40 and 80 percent of bacteria on earth are capable of forming biofilms (Flemming and Wuertz 2019). The extracellular matrix is primarily responsible for the resilience of bacterial biofilm. It has structural and functional roles in the production of vital nutrients, extracellular enzymes, cell communication, and defense against various hazardous substances such as antimicrobials and disinfectants (Galié et al. 2018).

Bacteria develop biofilms to survive under certain environmental circumstances. Depending on their composition, biofilms may be made up of a single homogeneous species (monomicrobial biofilms) or a variety of heterogeneous/diverse species (polymicrobial biofilms), sometimes crossing several biological kingdoms. In particular, cross-kingdom polymicrobial biofilms have been shown to be more resistant to antimicrobial therapy. In general, they can be conceptualized as porous, soft viscoelastic materials similar to hydrogels (Raghupathi et al. 2018).

Reason of Biofilm Formation

Bacteria develop biofilms in response to environmental stresses such as insufficient/low nutrients, high temperatures, high pH, high salt concentrations, high pressure, ultraviolet radiation, desiccation, and antimicrobials. In contrast to their planktonic cell life, microorganisms may thrive in oligotrophic environments with greater ease, have better access to nutrients, are protected from biocides, have increased productivity and interactions with other organisms, and have more stable environments due to the development of biofilms. Under certain situations, biofilms protect them and make them more adaptable to the external environment (Galié et al. 2018).

Biofilm Formation Process

A central belief of biofilm formation is that it is a dynamic process. It depends on how the bacterium cells, substrates, and nearby environments interact (Hall-Stoodley and Stoodley 2002). Biofilm formation models depict it as a linear process, however, the events involved in its formation are complex having multiple steps and being regulated by the intracellular and intercellular signaling systems. Free-floating bacterial cells initially connect reversibly to a surface with the help of intermolecular forces and hydrophobicity, followed by an irreversible attachment that is typically assisted by bacterial adhesive structures and close proximity interactions and finally the creation of EPS (extracellular polymeric substances) that allow cells to cling firmly to a

Citation: Liaqat MA, Mahmood MS, Akram MN, Arshad MI, Aslam R, Khan MK, Khan MUZ, Fatima N and Ali S, 2023. Bacterial biofilms and their regulatory approaches. In: Abbas RZ, Saeed NM, Younus M, Aguilar-Marcelino L and Khan A (eds), One Health Triad, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. 2, pp: 8-17.
https://doi.org/10.47278/book.oht/2023.35

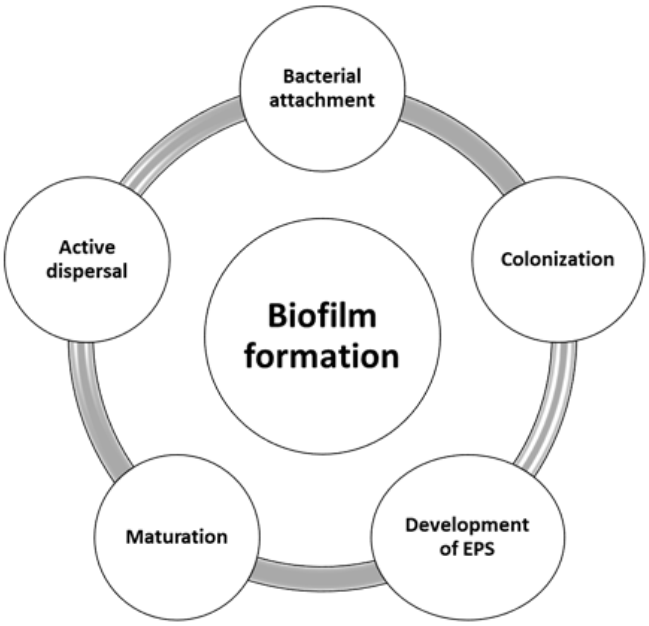

Fig. 1: Steps involved in biofilm formation

medium's surface. Eventually, they become a structured entity contained within an EPS grid. Lastly, bacteria can break free from the developed biofilm and spread in the environment to inhabit fresh niches. So in between initiation and termination of biofilm formation there are defined specific biofilm stages, but the currently available evidence suggests that these transitions are mainly governed by adaptive responses, and not by specific genetic programs (Yu et al. 2016).

Thus, there are five different stages that plays pivotal role in biofilm formation process

1. Reversible attachment
2. Irreversible attachment
3. Extra-cellular Polysaccharide (EPS) production
4. Biofilm Maturation
5. Detachment/Dispersal

Through these stages, free-living planktonic bacteria transition into "biofilm" lifestyle bacteria. Fig. 1 illustrated the steps involved in the formation of biofilm.

1- Reversible Attachment

The preliminary step of biofilm formation is reversible attachment of bacteria to a substrate. It occurs after an interaction with the abiotic or biotic substrate surface and few bacterial cells under specific environmental conditions. Bacterial cells migrated to substrate surface via chemotaxis, sedimentation, and Brownian motion (Palmer et al. 2007). Chemotaxis enables the enhanced interactions between bacteria and surface by directing the movement of bacteria towards the nutrient source via chemical signals (Vladimirov and Sourjik 2009). Once the bacteria interact with the surface, attachment of bacteria depends on the forces which are either attractive or maybe repulsive. Bacteria will adhere to surfaces if appealing forces outweigh repelling forces, and vice versa (Carniello et al. 2018). The main forces behind the preliminary attachment of bacteria and the surfaces are Van der Waals molecular interactions, electrostatic forces, and hydrophobic interactions (Dunne 2002). This attachment of bacteria is reversible and occurs when bacteria adhere to the surface of medium in a way that bacteria stay in a 2d Brownian motion. At this stage, bacteria can be detached from the surface either by their own motion or movement or by the flow of any fluid or liquid over the surface of substrate (Li and Tang 2009).

Any surface (biotic or abiotic) coming in the contact with bacteria can act as a substrate for the bacterial attachment (Tuson and Weibel 2013). Bacterial attachment is majorly affected by hydrophobicity, roughness, and charge of surface. It is observed that hydrophobic surfaces tend to have more bacterial colonies than hydrophilic surfaces. This may be due to less repulsive forces on hydrophobic surfaces than hydrophilic surfaces (Yu et al. 2016). Roughness of substrate surface also plays a major role in the bacterial attachment. The spaces and irregularities between substrate surface can promote the bacterial attachment because of more surface area to which bacteria can adhere (Zhao et al. 2014). Thirdly, surface charge can also affect the adherence of bacteria to the surface of substratum. The significant quantity of amino, carboxyl, and phosphate groups found in bacterium cells contributes to the cells' increased negative charge. That's why surfaces with positive charge promotes the adhesion of negatively charged bacterial cell (Dziubakiewicz et al. 2013).

2- Irreversible Adhesion

The second step in biofilm formation is irreversible attachment or adhesion of bacterial cells to the substrate surface. This step is accomplished with the help of short-range interactions namely;

- Covalent bonding
- Hydrophobic interactions
- Ionic bonding
- Hydrogen bonding
- Dipole-dipole forces (Bos et al. 1999)

The bacterial surface is covered with many adhesive projections that are projected away from the extracellular environment. In biofilm formation, many bacterial adhering structures take part mainly; pili, flagella, fimbriae, and adhesion initiated by non-fimbrial structures. The first physical contact of bacterial cells with the substrate surface is achieved using these adhesive structures (Berne et al. 2015).

Irreversible attachment using flagella is one of the most common ways of forming biofilm in bacteria. Flagella is a whip like filamentous thread plays a role in the locomotion of bacterial cell. Flagellar movement can be of either swarming (on solid surface) or swimming (in liquids). A wide

range of bacterial species shows both type of movement for the attachment on the surface of substrate (Hintsche et al. 2017). Flagella plays a major role in overcoming the repulsive forces that can obstruct the bacterial attachment and biofilm formation and initiates the initial adhesion of cells (Terashima et al. 2008).

Another filamentous appendage used by bacteria for the attachment of cells with each other and initial adhesion is pili or fimbriae. Some bacteria can utilize a pili mediated movement known as twitching motility that can play a role in initial adhesion (Maldarelli et al. 2016). On their exterior, some bacteria have type 1 fimbriae and type 3 fimbriae. These fimbriae are very helpful for biofilm formation and adherence (Murphy et al. 2013). In addition, curli fimbriae, which are thin and flexible, aid in bacterial attachment in the onset (Carter et al. 2016).

3- EPS Production

Third step in the biofilm formation is production of EPS (extracellular polysaccharide). Bacterial cells produce and secrete extracellular polysaccharide for biofilm formation. EPS is a very important constitute of biofilm extracellular matrix. EPS regulates both adhesion of biofilms to substrate surface and cohesion of bacterial cells by using two main forces i.e., Ion bridging interaction and hydrophobic interaction. Bacteria produce EPS because of many reasons including; attachment to the surface of substrate, intra-cell recognition, formation of biofilm, structural constitute of biofilm, cell signaling, protection of cells from desiccation, trapping of nutrients, and for exchanging genetic information to other bacterial cells (Fahs et al. 2014). A secondary messenger known as c-di GMP functions as a stimulant to control the transition of bacterial cells from reversible attachment to permanent adherence. The production and release of EPS and other cell structures enable this change (Toyofuku et al. 2016).

EPS produced by bacterial cell to form biofilm is made up of polysaccharides, DNA, lipid, proteins, and other compounds. The most important and prominent constitute of bacterial EPS is polysaccharide which plays a major role in the maturation of bacterial biofilm and their growth (Bacosa et al. 2018).

In Gram-negative bacteria, polysaccharide present in the EPS is either neutral or polyanionic. Uronic acid present in the polysaccharide is considered to be the reason of this anionic nature. This nature of EPS clears the way for attachment of divalent cation molecules like calcium and magnesium which facilitates in the formation of biofilm. On the other hand, Gram-positive bacteria produce EPS which is cationic in nature (M. Donlan 2002) . The extracellular matrix (EPS) of gram-positive bacteria is made up of many different types of molecules, including proteins, DNA, enzymes, and even structures like pili and fimbriae. DNA present in the EPS acts as intracellular connector (Flemming et al. 2016).

4- Biofilm Maturation

Changes in gene expression are triggered during microcolony formation and EPS accumulation, and the products of these genes are used to produce EPS, which acts as biological "glue" between implanted bacterial cells (Karimi et al. 2015). The creation of the matrix is succeeded by the production of water-filled channels that function as circulatory systems, transporting nutrients to the cell communities and eliminating waste. According to a structural study, microcolonies have a pyramid/mushroom-shaped multicellular structure (Garnett and Matthews 2013). During the maturation process, mobility within microcolonies is limited because surface structure development in bacteria is inhibited. Additionally, differences between the gene expression patterns of sessile and planktonic cells are striking (Hall-Stoodley and Stoodley 2002). Furthermore, QS allows bacteria of the same or different species to communicate by secreting and detecting AIs. Bacteria employ these signaling molecules to detect one another presence and to control gene expression in response to variations in population density (Wei and Ma 2013).

5- Dispersal/Detachment

Detachment of bacterial cell from biofilm is also called dispersal of biofilm. It is the final and terminal phase of biofilm formation. Bacteria detach from the already developed biofilm to continue a new cycle of biofilm (Singh et al. 2017). Bacterial cell dispersal from the adhered biofilm surface is due to environmental factors under which bacteria form new microcolonies on another substrate surface. This final step is rather complex because it is influenced by signal transduction pathways and environmental signals and factors (Díaz-Salazar et al. 2017).

The process of dispersal differs among different bacterial species but in general the whole process of detachment and dispersal can be classified into three steps i.e., cell detachment, movement of cells towards a new substrate and bacterial cell adhesion to new substrate Detachment of bacterial cells can be either active (seeding) or passive (erosion or sloughing). Seeding action is the detachment of bacterial cells from biofilm under the signals from environmental factors like nutrient deficiency, degradation from enzymes, antimicrobial actions. The biofilm's core is quickly evacuated by bacterial or planktonic cells during the seeding dissemination process, leaving a hollow interior. Passive detachment of cells is done under the action of external force also known as shear forces. The rapid dispersal of a large portion of bacterial cells from biofilms is known as sloughing, whereas the sudden dispersal of a tiny portion of bacterial cells from biofilms is known as erosion (Lee and Yoon 2017).

During the detachment process the genes responsible for EPS production, adhesion, and pili production are downgraded while genes responsible for cell motility and movement such

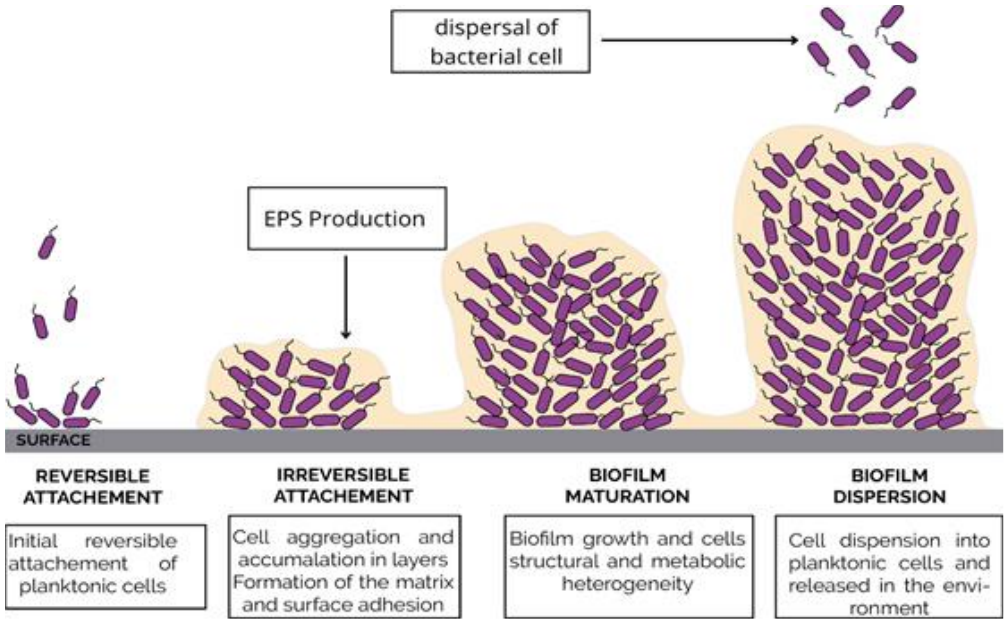

Fig. 2: Process of biofilm formation

as production of flagella and EPS degradation are upgraded. Environmental factors like temperature, pH change, nutrient deficiency and oxygen scarcity plays a major role in the dispersal of bacterial cells from biofilms. Environmental factors like limited supply of oxygen contributes in the degradation of c-di-GMP which facilitates in the dispersal of biofilm (Raghupathi et al. 2018). Additionally, a physiochemical process also known as autolysis assists in the biofilm dispersal (Lee and Yoon 2017). Fig. 2 highlights the complete process of biofilm formation.

Impacts of Bacterial Biofilm

There are many harmful effects of bacterial biofilm. Bacteria can form biofilm on any surface including biotic or abiotic surfaces (Hall-Stoodley and Stoodley 2002) . There are too many impacts of bacterial biofilms like nosocomial illness, food spoilage, dental carries, ship hull fouling issues, spoilage of sea foods as well as industrial pipe-fouling (Khatoon et al. 2018).

Public Health Hazards

In hospital environment, bacteria can form biofilm on every surface including medical equipment (prosthetic heart valves, machines, pacemakers, lenses, and catheters), dead tissues and part of bones, and on living tissues (teeth and lungs surface) (Alav et al. 2018).

In health care environment both Gram-negative and Gram-positive bacteria are found attached with the surface of medical equipment and form biofilm, but the most prominent bacteria reported in the hospital environment forming biofilm are; *Staphylococcus (S.) aureus*, *S. epidermidis*, and *Pseudomonas (P.) aeruginosa*. Out of these bacteria, most of the infections caused by the staphylococcal species (Hall-Stoodley and Stoodley 2002). *P. aeruginosa* is mostly found in the water system of health care hospital (Loveday et al. 2014). Moreover, biofilm forming bacteria are main contributors of life-threatening diseases in the hospital environment like; Osteomyelitis, chronic wounds, infective endocarditis (IE), and cystic fibrosis (Masters et al. 2019). It is reported that biofilm producing bacteria accounts for about 65% of all the bacterial infection in hospital environment because they show high resistance against antibiotics and host defense system (Ciofu and Tolker-Nielsen 2019).

Impact on Food Industry

The existence of biofilm and bacteria in food industry and food processing units can have very consequential risks in food industry and for food (Galié et al. 2018). Food industry is highly affected by the biofilm forming bacteria. Biofilm producing bacteria can be attached to any surface in the food industry including food surface. Approximately 60% of all the foodborne infections arises due to the bacteria producing biofilms (Han et al. 2017). In food industry, biofilm producing bacteria mainly come from the surrounding air, contaminated equipment, and spoiled food surfaces (Coughlan et al. 2016). The biofilms formed on food surface results in the spoilage of food which is a very serious concern for consumers and can have economic and financial consequences. The most common biofilm forming agent present in the food industry and on the surface of food is *Listeria monocytogenes* which is found everywhere and can cause serious complications in the pregnant women and other

infections in the immunocompromised patients. After that a major spoiler of food in food industry *Salmonella* spp. can cause foodborne diseases and leads to Reiter's syndrome or in severe cases lead to death (Galié et al. 2018).
Many technical problems also occur due to the presence of biofilm in the food industry like prevention of heat flow across the surface of equipment, increase in the resistance of fluid, increase in the rate of corrosion of surfaces, and loss of food production (Meesilp and Mesil 2019).

Benefits of Bacterial Biofilm

There are many harmful effects of biofilm in the environment, but it has some benefits too. The benefits of biofilms are primarily seen in the agricultural, bioremediation, wastewater treatment, corrosion protection, and other sectors (Singh et al. 2019).

1- Bioremediation

In the process of bioremediation, living organisms, mostly bacteria, interact with harmful and toxic substances in the environment to change them into less dangerous forms (Van Dillewijn et al. 2009). In terms of cost and safety, it is a better solution than traditional cleanup including various chemicals and physical removal of contaminants (Mangwani et al. 2015). Furthermore, biofilm-mediated remediation approaches are more efficient in changing hazardous wastes due to greater pollutant bioavailability to degrading organisms and enhanced adaptability of degrading bacteria to diverse toxic chemicals (Dos Santos et al. 2018). The process is generally a step of bacterial metabolism and depends on enzymatic assault by bacteria to transform environmental contaminants into harmless chemicals (Rodríguez-Martínez et al. 2006). Microbial bioremediation can occur at the site of contamination (in situ) or elsewhere (ex situ) (Azubuike et al. 2016). It may be accomplished by incorporating limited nutrients and electrons (bio stimulation) or by introducing microorganisms into contaminated areas (bioaugmentation) to boost the transformation process (Rodríguez-Martínez et al. 2006). Microorganisms residing in biofilms have better tolerance to toxins, a higher likelihood of survival and adaptability and stronger capacities to degrade various pollutants through catabolic pathways than their planktonic counterparts. Additionally, biofilms provide an essential habitat for bacterial chemotaxis, adherence, metabolite dispersion, and intercellular gene transfer (Dos Santos et al. 2018).
Numerous aerobic and anaerobic bacteria may thrive in biofilms during remediation processes, and these bacteria frequently use the decomposition of pollutants as a source of energy. Bacteria may employ oxygen as the ultimate electron acceptor during aerobic degradation to convert harmful pollutants into benign products, mostly carbon dioxide and water. When pollutants are transformed into less harmful or innocuous byproducts in anaerobic settings, electron acceptors like nitrate and sulphate, which function like oxygen, may be necessary for the byproduct's survival (Azubuike et al. 2016). Pesticides, dyes, heavy metals, explosives, medicinal products, and chronic organic pollutants, includes compounds such as PAHs, PCBs, and PCEs (polychlorinated biphenyls and polychlorinated ethenes) are all potential candidates for bacterial biofilm-mediated cleaning (Edwards and Kjellerup 2013).

2- Wastewater Treatment

Water contamination is a significant concern worldwide as a result of industrialization, population expansion, and urbanization (Daud et al. 2017). There are many different types of organic and inorganic pollutants found in wastewater, which are brought on by industrial sewage, domestic and commercial sewage, storm water, and other sources. The protection of aquatic environments depend on wastewater purification (Naidoo and Olaniran 2013). Electrochemical treatment, membrane filtering devices, and coagulation/flocculation are all physicochemical wastewater treatment techniques (Favero et al. 2020). These processes are effective, but they struggle to remove organic matter because filtering and sanitation are the two main elements of most water treatment systems (Hlihor et al. 2017). Biofilm-based wastewater treatment systems have made use of microbial communities to degrade and neutralize organic and inorganic contaminants. To stop marine eutrophication, which results in anoxia, extra nutrients in effluent must also be eliminated. Typically, nitrogen and phosphate are the main nutrients in effluent. As a consequence, denitrifying or phosphorous-neutralizing bacteria are frequently used in wastewater purification (Yamashita and Yamamoto-Ikemoto 2014).
The biologically active carbon (BAC) method, which is one of the biotechnologies used to purify water, physically removes pathogenic bacteria, organic matter, and inorganic pollutants from water by using granular activated carbon (GAC) as a water filtering medium (Shirey et al. 2012). To degrade organic carbon and other contaminants in the influent water, bacteria colonize and form biofilms on the rough, porous surfaces of the GAC after the media granules have been depleted. Some of the biofilm reactors being developed at the moment for the remediation of wastewater include revolving contactors, fluidized beds, and membrane reactors (Simpson 2008).

Regulatory Approaches of Biofilms

Unlike planktonic bacteria, biofilms are not effectively eliminated by normal cleansing, washing, and disinfection methods, however, the creation of biofilm may also be helpful (Somers and Lee Wong 2004). As a result, a variety of elements have been investigated to encourage the creation of beneficial biofilms. Bacterial biofilm matrix, quorum sensing interference, and regulation of bacterial attachment

Table 1: Regulating strategies of bacterial biofilm and their mechanism (Chung and Toh 2014)

Strategy	Mechanism
1. Prevention and promotion of bacterial attachment	
• Use of polythene glycol (antifouling surface)	Repel bacterial coating
• Use of silver (antimicrobial surface)	Bacterial killing while in contact with surface
• Use of aryl rhodanines and other small molecules	Non-adhesive property
• Use of curlicides and pilicides	Non-adhesive property
Modifying surface of substratum	
• Oxygen plasma on carbon materials	Promotes bacterial adhesion and bacterial biofilms
• Nitrogen plasma with carbon anode	Enhance bacterial biofilm production
• Use of polythene membrane	Enhance nitrifying bacteria forming biofilm
2. Promotion and Control of bacterial signal transduction	
• Quorum quenchers	Degradation of signal molecules using enzymes
• Quorum sensing inhibition	Inhibit synthesis of signals
• Natural agents (Furanone, ajoene. Etc.)	Degrade biofilm formation
• AIs and QS genes	Encourage the formation of beneficial biofilm
• Increased expression of QS gene	Enhance EPS formation
3. Dispersal of biofilm matrix	
• Targeting matrix enzyme	Degradation of EPS
• Use of Bacteriophages	Degradation of EPS
• C_2DA (Cis-2 decenoic acid)	Enhance biofilm dispersal

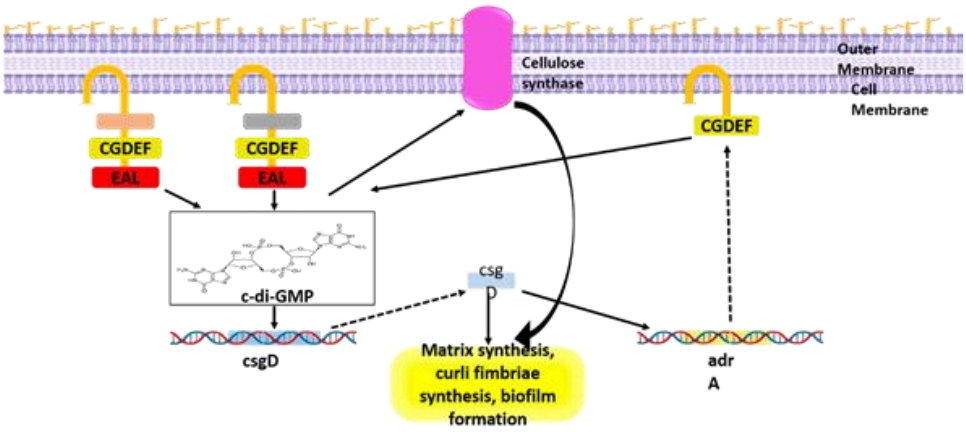

Fig. 3: Regulation pathway of biofilm formation by DGCSs.

are all closely linked (Chung and Toh 2014). Various strategies for avoiding, regulating, or encouraging bacterial biofilm development are enlisted in Table 1. Regulation pathway of biofilm formation by DGCSs is shown in Fig. 3. Various diguanylatecyclases act both upstream and downstream in the signaling cascade that regulates biofilm formation. Broken lines connect the genes to their gene products.

Prevention and Control of Bacterial Attachment

The best way to stop the production of biofilms in their early stages is to inhibit cell attachment. As a result, redesigning the surface or covering the surface with compounds that inhibit bacterial adherence may limit the creation of bacterial biofilm (Chung and Toh 2014). There are two kinds of antibiofilm surfaces i.e., antifouling surfaces and antimicrobial surfaces. In contrast to the latter, the former prevents pathogens from sticking to surfaces and prevents the growth of microbes there (Xu et al. 2005). Antifoulants in the form of nanoparticles of titanium oxide, graphite, arsenic, mercury oxide, silver, copper oxide, and zinc oxide have been manufactured and employed successfully in coating agents and coatings (Kuang et al. 2018). Recently, the marine and healthcare industries have started to use polyethylene glycol (PEG) on a large scale as an antifouling coating. PEG-coated surfaces have been discovered to inhibit bacterium adhesion due to their hydrophilic surface characteristics. Numerous bacterial species, such as *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *E. coli*, may be rejected by PEG coverings (Zhang et al. 2017). Antibacterial surfaces are created for indwelling medical devices (like catheters and intubation tubes) that may be infiltrated by bacteria that form biofilms so that pathogens including *S. aureus*, *E. coli*, and *P. aeruginosa* can be combated by releasing antibiotics, bacteriocins, metal ions, plant extracts, or nanoparticles (Chung and Toh 2014). Silver coating may be used to prevent biofilm growth on medical device surfaces. Quaternary ammonium compounds (QACs) are also utilized in contact

killing surfaces functioning as antimicrobial agents. Unlike silver ions, which release antibiotics slowly over time, the QACs coatings provide a durable contact-based antibacterial action (Francolini et al. 2017). One drawback of contact killing surfaces is that some bacteria can get resistant to them (Achinas et al. 2019). Aryl rhodanines are tiny substances that prevent bacteria from adhering to surfaces and may stop Gram positive diseases from forming biofilms in their early phases (Chung and Toh 2014). Furthermore, tiny synthetic chemicals known as pillicides and curlicides may disrupt bacterial adherence by blocking the formation of bacterial pili and fimbriae. Honey and tea are natural items that may help hinder bacterial adhesion (Kuang et al. 2018).

Promotion of Bacterial Attachment

However, surface modification of materials, including both physical and chemical-based alteration, or electrochemical oxidation treatment, may encourage the adhesion of beneficial microorganisms (Kang et al. 2014). Changes and management of surface properties can promote bacterial adherence and the production of useful biofilms for BESs and the yeast fermentation sector. It has been shown that nitrogen or oxygen ions increase the hydrophilicity and surface energy of carbon-based materials, such as graphite electrodes, which in turn encourages bacterial adherence, the development of biofilms, and the production of electricity in BESs. Additionally, the application of nitrogen plasma to the carbon anode may modify its surface roughness and hydrophobicity to encourage the growth of biofilms and increase the generation of power in MFCs. Additionally, UV/O3-treated carbon felt electrodes may promote *Shewanella oneidensis* MR-1 adhesion and biofilm growth, resulting in an increase in electron transfer rate and a rise in the amount of current produced in MFCs (Berlowska et al. 2013). Ammonium is converted to nitrate as a crucial step in the treatment of wastewater (nitrification). However, the nitrifying organisms have relatively slow growth rates and do not produce robust biofilms. As a result, efforts must be taken to keep these nitrifiers in reactor systems. It has been claimed that the connection between adhering bacteria and the surface of the material determines biofilm strength and shear resistance (Lackner et al. 2009). To encourage nitrifying bacteria attachment to a membrane surface, a variety of techniques have been employed. It was found that nitrifying biofilms quickly formed on the PE membrane that had its surface altered with positively charged graft polymer chains. This membrane had a higher level of bacterial adhesion than the original, unmodified membrane. They used a combination of plasma polymerization and wet chemistry to add PEG strands with two different functional groups (-PEG-NH2 and -PEG-CH3) to the membrane surfaces. They discovered that adding methoxy-PEG-amine (-PEG-NH2) to both a smooth PE surface and a rough PP surface greatly accelerated the development of biofilms. Methoxy – PEG - amino amine's

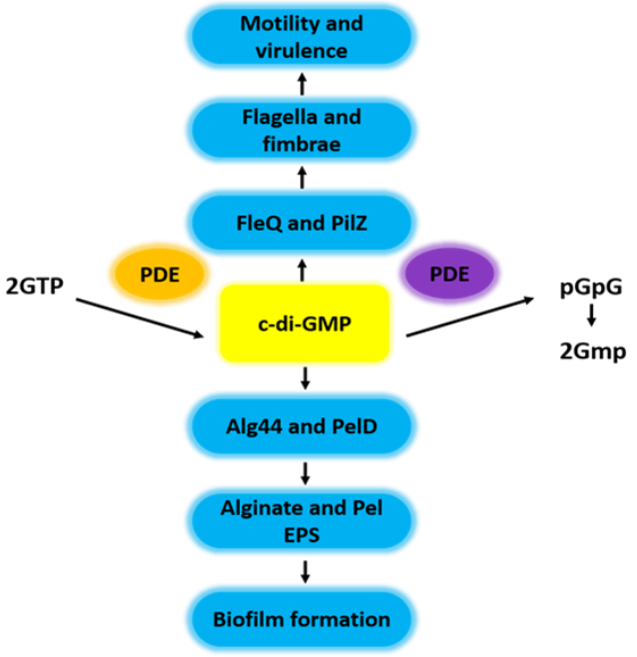

Fig. 4: Schematic presentation of Physiological functions of c-di-GMP

group draws nitrifiers like *Nitrosomonas europea* and *Nitrobacter winogradskyi*, facilitating the growth of biofilms (Lackner et al. 2009). Fig. 4 illustrated the schematic presentation of physiological functions of c-di-GMP.
High levels of c-di-GMP however favours sessility and stimulate synthesis of various EPS, such as Pel (mediated by PelD) and alginate (mediated by Alg44).

Control of Bacterial Signal Transduction (Quorum Sensing)

Bacterial QS is dependent on a variety of processes, including signal generation, diffusion, receptors, and recognition, as well as gene expression and signaling reaction. Because of this, quorum quenchers (QQs) or quorum sensing inhibitors (QSIs) that thwart these procedures may be able to lower bacterial QS and eventually stop the development of biofilms (Rémy et al. 2018). Healthcare settings and medicine, as well as commercial membrane bioreactors, agricultural production, and aquaculture, all depend on the quorum-quenching method, which uses quorum quenching enzymes to inactivate quorum sensing signals. Different kinds of animals have been found to contain quorum-suppressing enzymes, including lactonase, acylase, oxidoreductase, and paraoxonase. Aryl homoserine lactone molecules are rendered inactive by QQs, which is their well-known mode of action. Another method is the QSI N-octanoyl-L-HSL (C8-HSL), which blocks the production of signal molecules (like AHLs), by inhibiting the enzymatic activity of the Lux operon protein. Among the natural QSIs that are known to stop bacterial colonies are furanone, ajoene, naringin,

musaceae and curcumin (Fong et al. 2018). Additionally, using honey as a natural preservative can stop the growth of biofilms by blocking the activity of genes like AI-2 and LsrA that are involved in bacterial transmission when used in significant quantities. Additionally, more second mediator c-di-GMP encourages the development of bacterial biofilms. To stop the growth of biofilms, it may therefore be advantageous to inhibit the c-di-GMP pathway (Sharahi et al. 2019).

Promotion of Bacterial Signal Transduction

However, genetically modifying AIs can promote the growth of advantageous biofilms, which are essential to produce wastewater treatment, bioremediation, and power generation. For instance, by incorporating a specific type of AIs, the start-up time for a dual chamber MFC was reduced from 10 days to 4 days (Chen et al. 2017). The extreme halophile Halanaerobium boosts its biofilm mass on the MFC anode in response to an AI (100 mM quinolone), which improves power production and the effectiveness of high salinity wastewater treatment. Additionally, QS bacteria have the ability to degrade a wide range of pollutants. The QS genes *las*I and *rhl*I, which assist in accelerating the breakdown of polycyclic aromatic hydrocarbons, can be upregulated in order to increase the development of maritime *P. aeruginosa* N6P6 biofilms and EPS production (Mangwani et al. 2015).

Disruption of Bacterial Biofilm Matrix

It's crucial to eliminate the EPS' structural elements in order to disperse bacterial biofilms (Wei and Ma 2013). Therefore, degradation of the EPS matrix may be a useful technique to prevent the formation of bacterial biofilms. The enzymes which play a major role in EPS matrix-degradation like hydrolases, glycosides, restriction endonucleases, dispersin B, and proteases can promote the spread of pre-existing biofilm populations while inhibiting the formation of bacterial biofilms. The bacteria are released as planktonic cells after the biofilm matrix is enzymatically broken down, and these cells are quickly destroyed by phages, immune systems, antibacterial agents, and other disinfectants (Parrino et al. 2019). Both diffusion and phage-derived enzymes can help phages enter the EPS matrix. In terms of removing biofilms, an engineered lytic phage with a biofilm-degrading enzyme worked better than a non-enzymatic equivalent (Simmons et al. 2018). The capacity of phage SAP-26 to penetrate biofilm matrix when coupled with the antibiotic rifampicin led to the disruption of biofilm structures. In addition to successfully causing biofilm dispersal, C2DA may also prevent the beginning of biofilm formation (Chung and Toh 2014).

Conclusion

The formation of bacterial biofilm is very organized and highly regulated process which is related to ubiquitous nature of bacteria in natural and manmade environment. The ability of bacteria to colonize on surfaces and form biofilms are considered serious issues and is related to very disastrous consequences in many branches related to food, water, pharmacy, and healthcare. Various techniques and approaches concerning the interferences against bacterial attachment, QS and destruction of biofilm matrix have been developed to get rid of harmful biofilms. However, bacterial biofilms disrupt the bio-environments beyond risk. There are numerous beneficial applications of bacterial biofilms. Biofilm-associated bacteria play pivotal roles in the transformation of health hazardous pollutants to harmless substances, the protection of plants against phytopathogens, the plant growth promoters, as well as the removal of excess nutrients from wastewater.

REFERENCES

Garnett J and Matthews S, 2013. Interactions in Bacterial Biofilm Development: A Structural Perspective. Current Protein and Peptide Science 13: 739–755.

Achinas S et al., 2019. Brief Recap for Bacteria Adhesion. Applied Sciences 9: 1–15.

Alav I et al., 2018. Role of bacterial efflux pumps in biofilm formation. Journal of Antimicrobial Chemotherapy 73: 2003–2020.

Azubuike CC et al., 2016. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World Journal of Microbiology and Biotechnology 32: 1–18.

Bacosa HP et al., 2018. Extracellular polymeric substances (EPS) producing and oil degrading bacteria isolated from the northern Gulf of Mexico. PLoS One 13: 1–19.

Berlanga M and Guerrero R, 2016. Living together in biofilms: The microbial cell factory and its biotechnological implications. Microbial Cell Factories 15: 1–11.

Berlowska J et al., 2013. Enhancing adhesion of yeast brewery strains to chamotte carriers through aminosilane surface modification. World Journal of Microbiology and Biotechnology 29: 1307–1316.

Berne C et al., 2015. Adhesins Involved in Attachment to Abiotic Surfaces by Gram-Negative Bacteria. Microbiology Spectrum 3: 1–27.

Bos R et al., 1999. Physico-chemistry of initial microbial adhesive interactions- its mechanisms and methods for study. Microbiology Reviews 23: 179–230.

Carniello V et al., 2018. Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth. Advances in Colloid and Interface Science 261: 1–14.

Carter MQ et al., 2016. Curli fimbriae are conditionally required in Escherichia coli O157: H7 for initial attachment and biofilm formation. Food Microbiology 57: 81–89.

Chen S et al., 2017. Quorum sensing signals enhance the electrochemical activity and energy recovery of mixed-culture electroactive biofilms. Biosensors and Bioelectronics 97: 369–376.

Chung PY and Toh YS, 2014. Anti-biofilm agents: Recent breakthrough against multi-drug resistant Staphylococcus aureus. Pathogens and Disease 70: 231–239.

Ciofu O and Tolker-Nielsen T, 2019. Tolerance and resistance of

pseudomonas aeruginosabiofilms to antimicrobial agents-how P. aeruginosa Can escape antibiotics. Frontiers in Microbiology 10: 1–15.

Coughlan LM et al., 2016. New weapons to fight old enemies: Novel strategies for the (bio)control of bacterial biofilms in the food industry. Frontiers in Microbiology 7: 1–21.

Daud MK et al., 2017. Drinking Water Quality Status and Contamination in Pakistan. BioMed Research International 1: 1–18.

Díaz-Salazar C et al., 2017. The stringent response promotes biofilm dispersal in Pseudomonas putida. Scientific Reports 7: 1–13.

Van Dillewijn P et al., 2009. Bioremediation, a broad perspective. Microbial Biotechnology 2: 125–127.

Dunne WM, 2002. Bacterial Adhesion : Seen Any Good Biofilms Lately ? Bacterial Adhesion : Seen Any Good Biofilms Lately ? Clinical Microbiology Reviews 15: 155–166.

Dziubakiewicz E et al., 2013. Study of charge distribution on the surface of biocolloids. Colloids and Surfaces B: Biointerfaces 104: 122–127.

Edwards SJ and Kjellerup BV, 2013. Applications of biofilms in bioremediation and biotransformation of persistent organic pollutants, pharmaceuticals/personal care products, and heavy metals. Applied Microbiology and Biotechnology 97: 9909–9921.

Fahs A et al., 2014. In situ analysis of bacterial extracellular polymeric substances from a pseudomonas fluorescens biofilm by combined vibrational and single molecule force spectroscopies. The Journal of Physical Chemistry B 118: 6702–6713.

Favero BM et al., 2020. Evaluation of the efficiency of coagulation/flocculation and Fenton process in reduction of colour, turbidity and COD of a textile effluent. Environmental Technology (United Kingdom) 41: 1580–1589.

Flemming HC and Wuertz S, 2019. Bacteria and archaea on Earth and their abundance in biofilms. Nature Reviews Microbiology 17: 247–260.

Flemming HC et al., 2016. Biofilms: An emergent form of bacterial life. Nature Reviews Microbiology 14: 563–575.

Fong J et al., 2018. Combination Therapy Strategy of Quorum Quenching Enzyme and Quorum Sensing Inhibitor in Suppressing Multiple Quorum Sensing Pathways of P. aeruginosa. Scientific Reports 8: 1–11.

Francolini I et al., 2017. Antifouling and antimicrobial biomaterials: an overview. APMIS 125: 392–417.

Galié S et al., 2018. Biofilms in the food industry: Health aspects and control methods. Frontiers in Microbiology 9: 1–18.

Hall-Stoodley L and Stoodley P, 2002. Developmental regulation of microbial biofilms. Current Opinion in Biotechnology 13: 228–233.

Han Q et al., 2017. Removal of foodborne pathogen biofilms by acidic electrolyzed water. Frontiers in Microbiology 8: 1–12.

Hintsche M et al., 2017. A polar bundle of flagella can drive bacterial swimming by pushing, pulling, or coiling around the cell body. Scientific Reports 7: 1–10.

Hlihor RM et al., 2017. Bioremediation: An Overview on Current Practices, Advances, and New Perspectives in Environmental Pollution Treatment. BioMed Research International 2017: 3–5.

Jamal M et al., 2018. Bacterial biofilm and associated infections. Journal of the Chinese Medical Association 81: 7–11.

Kang CS et al., 2014. Enhanced current production by Desulfovibrio desulfuricans biofilm in a mediator-less microbial fuel cell. Bioresource Technology 165: 27–30.

Karimi A et al., 2015. Interplay of physical mechanisms and biofilm processes: Review of microfluidic methods. Lab on a Chip 15: 23–42.

Khatoon Z et al., 2018. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4: 1–36.

Kuang X et al., 2018. Novel Approaches to the Control of Oral Microbial Biofilms. BioMed Research International 2: 1–13.

Lackner S et al., 2009. Enhancing the formation and shear resistance of nitrifying biofilms on membranes by surface modification. Water Research 43: 3469–3478.

Lee K and Yoon SS, 2017. Pseudomonas aeruginosa Biofilm, a Programmed Bacterial Life for Fitness. Journal of Microbiology and Biotechnology 27: 1053–1064.

Li G and Tang JX, 2009. Accumulation of microswimmers near a surface mediated by collision and rotational Brownian motion. Physical Review Letters 103: 1–4.

Loveday HP et al., 2014. Association between healthcare water systems and Pseudomonas aeruginosa infections: A rapid systematic review. Journal of Hospital Infection 86: 7–15.

M. Donlan R, 2002. Biofilms: Microbial Life on Surfaces. Emerging Infectious Diseases 8: 881–889.

Maldarelli GA et al., 2016. Type IV pili promote early biofilm formation by Clostridium difficile. Pathogens and Disease 74: 1–10.

Mangwani N et al., 2015. Involvement of quorum sensing genes in biofilm development and degradation of polycyclic aromatic hydrocarbons by a marine bacterium Pseudomonas aeruginosa N6P6. Applied Microbiology and Biotechnology 99: 10283–10297.

Masters EA et al., 2019. Evolving concepts in bone infection: redefining “biofilm”, “acute vs. chronic osteomyelitis”, “the immune proteome” and “local antibiotic therapy.” Bone Research 7: 2–18.

Meesilp N and Mesil N, 2019. Effect of microbial sanitizers for reducing biofilm formation of Staphylococcus aureus and Pseudomonas aeruginosa on stainless steel by cultivation with UHT milk. Food Science and Biotechnology 28: 289–296.

Murphy CN et al., 2013. Role of klebsiella pneumoniae type 1 and type 3 fimbriae in colonizing silicone tubes implanted into the bladders of mice as a model of catheter-associated urinary tract infections. Infection and Immunity 81: 3009–3017.

Naidoo S and Olaniran AO, 2013. Treated wastewater effluent as a source of microbial pollution of surface water resources. International Journal of Environmental Research and Public Health 11: 249–270.

Palmer J et al., 2007. Bacterial cell attachment, the beginning of a biofilm. Journal of Industrial Microbiology and Biotechnology 34: 577–588.

Parrino B et al., 2019. Synthetic small molecules as anti-biofilm agents in the struggle against antibiotic resistance. European Journal of Medicinal Chemistry 161: 154–178.

Raghupathi PK et al., 2018. Synergistic interactions within a multispecies biofilm enhance individual species protection against grazing by a pelagic protozoan. Frontiers in Microbiology 8: 1–11.

Rémy B et al., 2018. Interference in bacterial quorum sensing: A biopharmaceutical perspective. Frontiers in Pharmacology 9: 1–17.

Rodríguez-Martínez EM et al., 2006. Microbial diversity and bioremediation of a hydrocarbon-contaminated aquifer (Vega

Baja, Puerto Rico). International Journal of Environmental Research and Public Health 3: 292–300.

Dos Santos ALS et al., 2018. What are the advantages of living in a community? A microbial biofilm perspective! Memórias do Instituto Oswaldo Cruz 113: 1–7.

Sharahi JY et al., 2019. Advanced strategies for combating bacterial biofilms. Journal of Cellular Physiology 234: 14689–14708.

Shirey TB et al., 2012. Composition and stability of bacterial communities associated with granular activated carbon and anthracite filters in a pilot scale municipal drinking water treatment facility. Journal of Water and Health 10: 244–255.

Simmons M et al., 2018. Phage mobility is a core determinant of phage-bacteria coexistence in biofilms. The ISME Journal 12: 532–543.

Simpson DR, 2008. Biofilm processes in biologically active carbon water purification. Water Research 42: 2839–2848.

Singh HM et al., 2019. Microbial fuel cells: a sustainable solution for bioelectricity generation and wastewater treatment. Biofuels 10: 11–31.

Singh PK et al., 2017. Vibrio cholerae Combines Individual and Collective Sensing to Trigger Biofilm Dispersal. Current Biology 27: 3359-3366.

Somers EB and Lee Wong AC, 2004. Efficacy of two cleaning and sanitizing combinations on Listeria monocytogenes biofilms formed at low temperature on a variety of materials in the presence of ready-to-eat meat residue. Journal of Food Protection 67: 2218–2229.

Terashima H et al., 2008. Flagellar motility in bacteria: structure and function of flagellar motor. International review of cell and molecular biology 270: 39-85.

Toyofuku M et al., 2016. Environmental factors that shape biofilm formation. Bioscience, Biotechnology, and Biochemistry 80: 7–12.

Tremblay YDN et al., 2013. Method to grow Actinobacillus pleuropneumoniae biofilm on a biotic surface. BMC Veterinary Research 9.

Tuson HH and Weibel DB, 2013. Bacteria-surface interactions. Soft Matter Journal 9: 4368–4380.

Vladimirov N and Sourjik V, 2009. Chemotaxis: How bacteria use memory. Journal of Biological Chemistry 390: 1097–1104.

Wei Q and Ma LZ, 2013. Biofilm matrix and its regulation in Pseudomonas aeruginosa. International Journal of Molecular Sciences 14: 20983–21005.

Xu Q et al., 2005. Assessment of antifouling effectiveness of two natural product antifoulants by attachment study with freshwater bacteria. Environmental Science and Pollution Research 12: 278–284.

Yamashita T and Yamamoto-Ikemoto R, 2014. Nitrogen and phosphorus removal from wastewater treatment plant effluent via bacterial sulfate reduction in an anoxic bioreactor packed with wood and iron. International Journal of Environmental Research and Public Health 11: 9835–9853.

Yu P et al., 2016. Influence of Surface Properties on Adhesion Forces and Attachment of Streptococcus mutans to Zirconia In Vitro. BioMed Research International 2016.

Zhang X et al., 2017. A brief review of recent developments in the designs that prevent bio-fouling on silicon and silicon-based materials. Chemistry Central Journal 11: 1.

Zhao B et al., 2014. Soft tissue integration versus early biofilm formation on different dental implant materials. Dental Materials 30: 716–727.