Biofilm's Characteristics and Ways to Inhibit

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Abstract. Most bacteria in nature do not exist as free-floating isolated cells; instead, they usually have to do with surfaces. These microbial communities, or biofilms, are made of bacteria that are encased in self-generated Extracellular Polymeric Substances (EPS). Biofilms can exhibit extreme integrity and resistance to environmental pressures. This is mainly because of factors including their physiological condition, cell density, the presence of quorum sensing, the significant role of drug efflux pumps, mutations and overexpression of genes, and the persistent cells. From the mitigation effect of biofilms to the application of beneficial biofilms, the unique characteristics of biofilms have triggered a lot of discussion and research. In this paper, we combine various reading materials to discuss how the structure and properties of biofilms confer the ability to be utilized. Then, with reference to the research paper of Manisha Mukherjee et al., the prospect of inhibiting biofilm formation by engineering quorum quenching biofilm was discussed.

1. Introduction

Biofilms are at the heart of many of the world's problems today. At the same time, due to the complexity of this subject, many people are attracted to study it from different angles, which helps biofilm to gain increasing academic attention all over the world. The tiny biofilm structures allow people to work out several environmental problems, including purification of groundwater. Today biofilm researchers have already been able to use bioengineering techniques to produce gene-modified biofilm to work on some of the environmental problems people are facing today, which makes us quite interested in the structure and characteristics of Biofilms.

In this work, we use an overview of the main characteristics and compositions of biofilms and how these structures give them various functions at the beginning of the article. Secondly, we explored the mechanisms of current biofilm mitigation approaches in an attempt to consolidate and identify common target areas. By linking the unique properties and goals of biofilms, we hope to suggest potential ways to mitigate biofilms, particularly through bioengineering

2. Biofilms' Characteristics

The following attributes biofilms indirectly contribute to their special responses towards antimicrobials. These characteristics and their influences on antimicrobial resistance are listed below.

2.1 The Extracellular Matrix (ECM)

The ECM is an important characteristic of biofilms, which protects them from harsh environments like antibiotics in the environment or the immune system of their host [1]. Figure 1 shows the components of ECM. It acts as a platform for quorum sensing, providing mechanical support, preventing the entry of antimicrobial agents and aiding the flow of energy and nutrients in and out of biofilms. Conventional antimicrobials widely used today do not work properly because they do not enter the extracellular polymeric substance (EPS) layer effectively. ECMs are mostly built up by polymers such as polysaccharides, nucleic acids, phospholipids, amyloid fibers and extracellular DNA (e-DNA). Here are some of the components we found particularly interesting.

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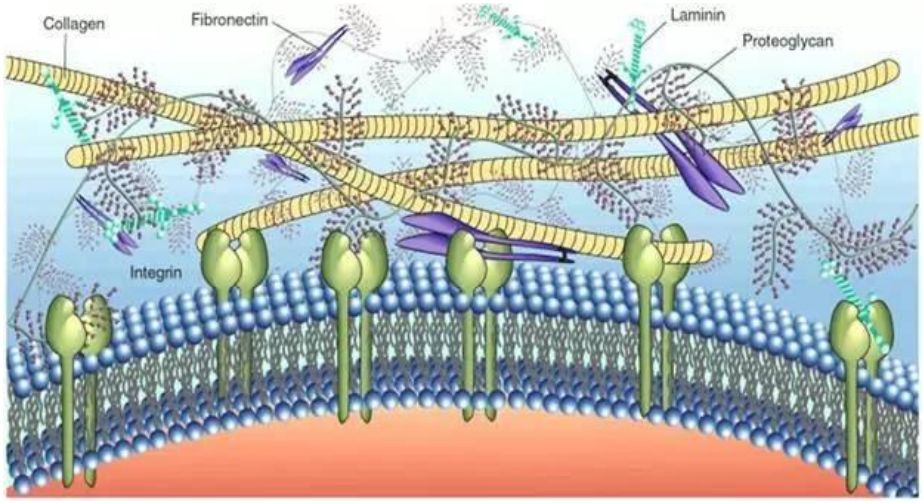


Source: Extracellular matrix and its biological effects_ Encyclopedia TA

Figure 1. Components of extracellular matrix

2.2 Proteins

The proteins found in ECM include flagella, cell surface adhesion protein, and hair protein subunits, exocrine protein and outer membrane vesicle protein. Cell outer membrane proteins like pili are involved in the original surface attachment. In some microbes, it may be in surface migration, thus conducting colonization on that surface [2]. Matrix proteins contribute to the physical support in the structure of biofilms. Most of these proteins have been identified through mutation studies, which have shown that the absence of matrix proteins leads to decreased formation and stability of biofilms, and changes the structure of them. Structural analysis can help to progress understand the function and mechanism of these proteins. Some of these matrix proteins express enzymatic properties to the chemicals in matrix, like glycosylase dispersant B, which can hydrolyze polysaccharides. And DNA enzymes can decompose nucleic acids out of the cells and promote the recombination of biofilm matrix or the degradation and diffusion of biofilm matrix [3].

A functional protein we came across is the GlcNAc-binding protein A in V. cholerae. It is mainly involved in the adhesion of V. cholerae onto chitinous surfaces. In the natural water environment, this allows V. cholerae to colonise these surfaces and hence its survival [4]. Chitin is an important carbon and energy sources for V. cholerae; hence V. cholerae GbpA, being a protein bind with chitin, should be crucial for V. cholerae to survive. In fact, studies have shown that a mutant strain of gbpA exhibited reduced attachment to zooplankton structures like their chitinous exoskeletons, and decreased adherence to chitin-coated beads. Additionally, GbpA can also bind to intestinal mucin that contains GlcNAc. In that case, GbpA functions as an adhesin in attachment on chitinous surfaces [5-7].

2.3 Nucleic Acids

DNA is an important part of bacterial biofilm and participates in horizontal transfer of genes through transformation, transduction and coupling. Transformation is when bacteria take up foreign DNA exposed to the environment and replenish receptors on the cell surface. The foreign DNA enters the bacterial chromosome through two homologous regions on the chromosome to form recombinant cells. Transduction is the process by which a bacteriophage introduces bacterial DNA from one host into another bacterial cell in the biofilm due to a distortion in the reproductive cycle of the bacteriophage. In generalized transduction, foreign bacterial DNA can replace the homologous region on the chromosome of the recipient cell through homologous recombination, while in specific transduction, when the defective phage enters the lysogenic cycle, the new allele in the proto-bacterial cell can also enter the genome of the new host through the integration of phage-bacterial hybrid DNA. Last but not least, during binding, plasmid DNA is transferred from the F+ donor cell to the F-recipient bacterial cell via the cytoplasmic mating bridge between them. This allows the exchange of different alleles, resulting in permanent changes in genotype and phenotype. Lateral gene transfer and gene exchange are the driving forces behind the spread of antibiotic resistance genes (ARGs), allowing them to accumulate in biofilms.

Other than coding DNA and RNA, small non-coding RNAs (sRNA) are also quite significant in biofilms, particularly in biofilm formation. This is achieved by regular system of sRNA. Such network of sRNA regulation enables concentration-specific responses, by activating or antagonizing protein regulators in response to environment, or through directly interfering the production of proteins in order to further affect the production of biofilms [8].

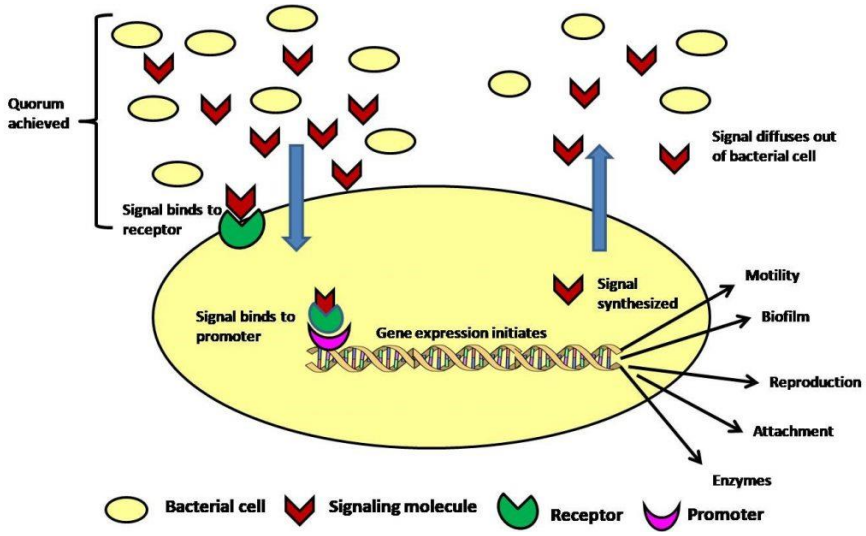
2.4 eDNA

A cationic extracellular polysaccharide Pel crosslinked eDNA in Pseudomonas aeruginosa biofilm ensure the integrative structure of the matrix. Meanwhile, DNABII-binded protein makes it possible for biofilm formation to occur by solidifying the secondary structure of eDNA. In an aquatic bacterium whose substrate contained water

(97%), soluble jelly-forming polysaccharides, proteins, eDNA, and other insoluble components, eDNA support the formation of filamentous network structures. This allows void in the matrix to be set up by pores and channels between microcolonies, facilitating the transport of liquids and inspiring the perspective of a "basic circulatory system" for biofilms.

2.5 Quorum Sensing

Quorum sensing refer to the process of microorganisms collaborating their activities and exert information exchanging in a crowd-dependent group by producing a particular kind of chemical signaling molecules named quorum sensing molecules (QSMs). Figure 2 shows the general mechanism of quorum sensing in bacteria. QS works as a significant component in several activities of biofilm cells [9]. Such a process includes expression production of toxin and disease-causing gene and extracellular polysaccharide synthesis. It also has a critical role of regulation in the formation of biofilms and drug efflux pumps. QSMs in bacteria could be Gram-positive or Gram-negative. Other molecules involved in the synthesis of self-induced N-acyl-hyperserine lactone (AHL) and S-adenosine-methionine are known as QSMs of Gram-negative bacteria, while self-induced peptides are Gram-positive QSMs responsible for interacting with the two-component histidine kinase signal pathway [10,11]. Cao et al. demonstrated the relationship between QSMs and drug resistance. They reported that two Candida albicans anti-fungal resistance genes were up-regulated in the presence of exogenous farnitol.



Source: https://thescientificfactor.com/quorum-sensing-bacterial-language-of-communication/

Figure 2. General mechanism of quorum sensing in bacteria

3. Formation of Biofilms

In the early stages of biofilm's formation, planktic bacterial cluster at gas-liquid interfaces to form floating or membrane-like biofilms or attach to a solid surfaces to form adhesive biofilms. These cells multiply and form small colonies composed of a few cells in total. During the formation of such kind of microcolony structure, cells begin to secrete EPS, which supports cell attachment. EPS is biofilm's main component, which occupy more than 90% biofilm mass [12]. Production of EPS is related to various and complex factors such as temperature and nutrition in the environment and genetic regulation. Chemical components of dead cells are also used for stabilizing biofilm's physical state. For example, eDNA from lytic cells is a main source of EPSs for biofilms. Microcolonies grow into mature biofilms by continuing growth of cells and producing larger amount of EPSs. During the built-up of biofilm, some cells are separated from the biofilm attributed to complex factors. For example, parts of the biofilm can be separated by external physical forces such as pressure, and intense water flow. On the other hand, Subpopulations in biofilms can be transformed into producers of motion-cell or EPS degrading enzymes by internal factors like mutation on gene or external factors like signal ligands in the environment. The isolated cells return to a floating state and begin searching for a new niche

4. Current Approaches to Mitigate Biofilms

4.1 Chemical Method

Antimicrobial peptides (AMPs) are short (<100 amino acids), positively charged peptides capable of interacting with biofilms. They penetrate into microorganisms to form pores, destroying cell membranes, and inhibiting cell wall, enzyme, nucleic acid, and protein synthesis.

4.2 Physical Method

Electricity is a potential therapeutic that involves the application of direct current to reduce biofilm formation on indwelling medical devices. The application of DC will exacerbate the electrostatic repulsion forces to disrupt the adherence of microorganisms as well as alter the

physical conditions such as pH and temperature to further impede biofilm formation; therefore, Biofilms are initiated when the attraction forces between microorganisms and surface area are greater than the repulsion force [13].

4.3 Antimicrobial Coating

In addition to electrical methods, antibacterial coatings are promising alternative therapies for eradicating biofilm-associated infections. Medical devices with biofilm formation are coated with antibiotic membranes to prevent microbes from adhering to surfaces [14]. These coated surfaces contain surface charge that will prevent microbial adhesion.

5. Conclusion

In this work, we use specific evidence to show the important structures and chemicals in biofilms, including the macromolecules like specific protein structures and the eDNA. After that, we show the process of Quorum sensing, which is an essential step before the formation. With these characteristics above, we then concentrate on the formation process of biofilm and how these characteristics allow such formation to go smoother. Lastly, we introduce two specific ways of inhibiting biofilms from formation.

Biofilms are continuing to attract researchers to work on them. Today loads of gene-modification programs of biofilms have already been set up all over the world. In the future, these engineered biofilms might help people to deal with hundreds of problems in nature.

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