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# Synthesis and Role of Biofilm as a Matrix for Bacterial Protection

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**A B S T R A C T**

Bacterial biofilms consist of structured groups of microorganisms that are surrounded within a self-produced extracellular polymeric substance (EPS) matrix, adhering to both living and non-living surfaces. Biofilms represent a fundamental mode of bacterial life across natural, industrial, and clinical settings. The formation of biofilms enhances the survival of bacteria by providing a shield against environmental challenges, antimicrobial agents, and the immune responses of the host. Understanding the molecular mechanisms that govern biofilm formation, maintenance, and dispersal is essential for developing effective strategies to control harmful biofilms and harness beneficial ones. Biofilm formation involves the production of extracellular polymeric substances (EPS), which play a crucial role in the structural integrity and functionality of the biofilm matrix. Additionally, chaperones are essential for protein folding and stability within these complex microbial communities.

## Introduction

Biofilms are intricate microbial communities in which diverse strains and species collaborate efficiently to protect themselves from environmental stresses while improving nutrient uptake and utilization. Biofilms often pose significant challenges, linked to a range of problems including microbially induced corrosion in oil rigs, contamination of food and drinking water, as well as dental caries and periodontal diseases (Marsh and Martin, 1992). These factors also play a role in various infections associated with biomaterials in humans, along with diminished heat transfer efficiency in pipelines (Turetgen, 2004). Biofilms often serve a vital function in the cycling of nutrients, encompassing carbon and key minerals such as nitrogen, phosphorus, and sulfur ions. In microorganisms capable of nitrogen fixation, biofilms act as a protective barrier for the nitrogenase enzyme, preventing its inactivation by atmospheric oxygen (Bazylinski *et al*., 2000). Bacterial colonization occurs on a variety of surfaces, encompassing both living and non-living entities. This includes glasses, ship hulls, dairy and petroleum pipelines, rocks in streams, and an array of biomedical implants and transcutaneous devices.

Three essential components of biofilm have been identified: microorganisms, extracellular polymeric substances, and surfaces. The formation of a biofilm is contingent upon the presence of all necessary elements in the mixture; the absence of any one of them will prevent

its development. The use of phase-contrast microscopy to examine a set of mutants deficient in surface attachment demonstrated that flagella and type-IV pili play a crucial role in the early phases of biofilm formation in *P. aeruginosa*. The study showed that in *P. aeruginosa* strains that produce two types of lipopolysaccharides (A and B band), a mutation causing the loss of B-band LPS led to a reduction in attachment to hydrophilic surfaces, while simultaneously increasing attachment to hydrophobic surfaces. Watnick *et al*. (1999) indicate that the mannose-sensitive type-IV pilus encoded by the mshA locus, flagella-mediated motility, and the synthesis of the main EPS are all essential for the formation of a wild-type biofilm in *V. cholerae*. Furthermore, it has been shown that flagella and type-IV pili are not absolutely required for the initial attachment to the surface; nonetheless, they considerably improve this process in *V. cholerae*. *Enterococci* are important pathogens in infections linked to medical devices, and like other Gram-positive bacteria, the formation of enterococcal biofilms on implants is emerging as a critical clinical issue. In comparison to other organisms, the formation of a biofilm offers *Enterococcus* sp. demonstrating improved resistance to antibiotics (Gilbert *et al*., 1997).

Primary contact generally takes place between an organism and a conditioned surface, with the hydrophobicity of the latter potentially varying considerably depending on the molecules present in the conditioning film. Wang *et al*. (1993) demonstrated that the initial adhesion. The adhesion of epidermidis to polyethylene disks was enhanced in the presence of surface-activated platelets, whereas the presence of adsorbed plasma proteins resulted in a reduction of adhesion compared to uncoated polyethylene. The use of polymethylmethacrylate as a substratum resulted in a significant enhancement of coagulase-negative Staphylococci adhesion when the surface was coated with various plasma proteins, including fibronectin.

The net repulsion between two surfaces can be mitigated through specific molecular interactions enabled by adhesins present on structures that extend from the cell surface, such as pili. The length of primary adhesion is influenced by the combined impact of multiple factors. Nonetheless, surface chemistry has impacted the equilibrium regarding adhesion by indicating that organic substances in solution are likely to concentrate near surfaces, whereas microorganisms tend to congregate in nutrient-rich environments.

Numerous methods exist to determine the formation of biofilm, with the tube test and the microtiter plate test (Christensen *et al*., 1985) being the most prevalent. A cationic dye is employed in the tube test to stain the bacterial film that coats the culture tube, after which the film is assessed visually. The optical density of the stained bacterial film is measured spectrophotometrically in the microtiter plate test. Stepanovic *et al*. (2000) measured the biofilm formation of *Staphylococcus* sp. utilizing tube tests and microtiter plate test wells. The growth of 31 coded *Listeria monocytogenes* strains was assessed using PVC microtiter plate wells. The formation of biofilm was evaluated indirectly through the application of a 1% crystal violet staining technique, subsequently measuring the absorbance of crystal violet with a destaining solution. The rates of cellular growth and the final densities of cells showed no correlation with biofilm formation, indicating that the differences in biofilm formation observed under the same environmental conditions were not due to variations in growth rates. As noted by Vasudevan *et al*. (2003), the different strains of S. aureus displayed variations in their ability to form biofilms within the microtiter plate wells. Narisawa *et al*. (2005) evaluated biofilm formation by measuring the cell count of *Escherichia coli* in microtiter plate wells.

A significant number of microorganisms, including prokaryotes (such as bacteria and archaea) and eukaryotes (like phytoplankton and fungi), primarily thrive and multiply in aggregated formations such as biofilms that attach to natural or artificial surfaces, flocs, and in a free planktonic state (Bhaskar and Bhosle, 2005). Modern microscopy techniques have demonstrated that these microorganisms are located within a complex matrix of extracellular polymeric substances (EPS). Individual EPS macromolecules display a fibrillar architecture, with a thickness of a few nanometers and a length extending to several micrometers (Decho, 1990; Leppard, 1995, 1997; Passow, 2002). The analysis of these polymers uncovers a diverse composition that includes polysaccharides and proteins, as well as smaller amounts of lipids, nucleic acids, and other polymers such as flagella, phages, debris from lysed cells, outer membrane vesicles, and pili, among others (Kumar *et al*., 2004; Hunter and Beveridge, 2005). Upon secretion, these substances can remain strongly bound to the cell surface, either through interactions between the carboxyl groups of EPS and the hydroxyl groups of lipopolysaccharides (LPS) or through a covalent bond involving phospholipids and

glycoproteins. As a result, the capsules that are densely packed and exhibit lower diffusibility, distinguished by a more structured polymeric arrangement, are termed "capsular" EPS, or "attached" EPS. Conversely, polymers that exhibit a looser adherence, creating slime that can easily be released into the extracellular environment, along with those already existing in the medium, are referred to as "non-attached" EPS.

EPS provide a protective mechanism for bacteria. The buffering zone against micro-environmental fluctuations is formed through the secretion of extracellular mucilaginous compounds, especially in reaction to low nutritional levels, high concentrations of toxins and metals, or excessive salinity, pH, and temperature. In Arctic winter sea ice, elevated levels of exopolymeric substances have been recorded, showing a significant correlation with bacterial abundance and diatom activity (Krembs *et al*., 2002). This further supports the idea that EPS are produced in response to different growth stresses and may act as an essential self-protective mechanism for microorganisms, especially in harsh winter conditions marked by high salinity and the risk of ice-crystal damage. Exoenzymes attached to the cell surface can effectively break down significant foreign compounds into more easily usable forms, like amino acids and monosaccharides, for bacterial uptake. This approach effectively leverages the limited food resources found in aquatic environments, given that it is rare for all essential chemicals required to support microorganisms to be available in sufficient amounts in natural conditions. Microbial EPS are primarily composed of water, accounting for about 97% of their mass, which provides a protective barrier for cells against drying out (Hunter *et al*., 2005; Bhaskar and Bhosle, 2005).

The strong binding capacity of EPS has led to their widespread application in the extraction of heavy metals and radionuclides from wastewater and natural water sources (Philippis *et al*., 2001). Choppin (1992) proposed, based on binding tests with humic substances, that organic material plays a significant role in the mobilization and immobilization of plutonium and other actinides in natural waters. Moreover, studies indicate that certain bacterial species, including Pseudomonas sp., can aid in the transformation of uranium, neptunium, and plutonium into less soluble, reduced forms. This process allows them to act as "living" backfill, effectively immobilizing actinides through mechanisms such as surface binding or metabolic uptake. Microorganisms in coastal waters play a variety of ecologically important roles. The primary role of heterotrophic bacteria is to serve as a carbon source for different grazers, as well as to decompose and mineralize both aqueous and particulate organic materials. Changes in microbial activities due to variations in environmental factors can have a profound impact on coastal marine ecosystems. Temperature plays a vital role in influencing the growth and metabolism of aquatic organisms. Nitrogen serves as a crucial element in a variety of biological metabolites and structural frameworks, encompassing amino acids, proteins, and nucleic acids. The availability of nitrogen in marine environments is often regarded as a key element that limits development and production (Ryther and Dunstan, 1971). Studies conducted in both freshwater and marine environments suggest that bacteria may rely on NH4+ and NO3- for their growth and biomass production, indicating that they are significant consumers of inorganic nitrogen. Documented mean consumption levels are 30% for NH and 40% for NO, as noted by Kirchman in 2000. The assimilation of nitric oxide by bacteria is currently absent from models of pelagic carbon and nitrogen cycles (Bissett *et al*., 1999). Microbial activity can lead to the depletion of nitrogen in ecosystems by utilizing oxidized nitrogen forms as electron acceptors. Paerl and Zehr (2000) reported on bacterial respiration resulting in the release of nitrous oxide and N2 gas, alongside nitrogen fixation to offset this depletion. Denitrifiers utilize nitrate and nitrite as respiratory substrates, using these nitrogen oxides as electron acceptors in place of oxygen. This process converts nitrate and nitrite into nitric oxide and nitrous oxide, ultimately producing dinitrogen gas (Tiedje, 1989). Denitrification involves several semi-independent stages that may not function simultaneously; denitrifiers begin the process with nitrate and produce varying amounts of subsequent products depending on environmental conditions (Palaniappan and Krishnamurthy, 1985).

Heat shock proteins (HSPs) play a crucial role in cellular metabolism under various growth conditions, aiding in the folding, assembly, and translocation of cellular proteins (Georgopoulos and Welch, 1993; Hartl *et al*., 1994). Molecular chaperones are defined as proteins that assist in the proper non-covalent assembly of other protein-containing structures in vivo, without being integral components of these structures during their typical biological functions. Hendrick and Hartl (1993) described HSP as a molecular chaperone that interacts with and stabilizes an otherwise unstable conformer of another protein, thereby guiding its appropriate fate in

vivo. This may involve processes such as folding, oligomeric assembly, transport to different subcellular compartments, or regulated transitions between active and inactive conformations, achieved through controlled binding and release of the substrate protein. Alongside heat shock, various physiological events also trigger the synthesis of heat shock proteins. The production of HSP in *Listeria mesenteroides* was observed to increase after ethanol treatment. Ethanol induced the expression of heat shock proteins in various organisms, including E. Escherichia coli, yeast, and mammalian cells (Travers and Mace, 1982; Lindquist, 1986)

Capsular exopolymers are tightly bound to the cells, forming a protective envelope, whereas slime exopolymers are more loosely connected and often spread into the surrounding environment. Capsular, intricate extracellular polymorphic compounds offer a wide range of industrial applications. *Xanthomonas campestris* produces xanthan gum, which finds use in a range of food and industrial applications. Marine biopolymers represent a substantial source of untapped potential, given that only a small percentage of marine prokaryotes has been successfully cultured. EPS serves various functions, such as aiding in surface adherence, offering protection from predation and desiccation, acting as stabilizers or concentrators for enzymes through ionic interactions, providing storage or nutritional reserves, and functioning as dispersants to release bacteria from nutrient-depleted surfaces (Weiner *et al*., 1998). Frolund *et al*. (1996) showed that extracellular polymeric substances (EPS) consist of a range of organic compounds, primarily featuring carbohydrates and proteins, along with smaller quantities of humic substances, uronic acids, and nucleic acids. EPS are generally composed of high molecular weight capsular polysaccharides and play various important roles in nature, such as protecting parasitic organisms from phagocytosis. Recent studies have drawn considerable interest to their essential function in the composition and physiology of biofilms.

Biofilm formation on metallic and non-metallic surfaces was reported by Sonak and Bhosle (1995), who reported the growth of biofilm on four different surfaces. It was noted that rough surfaces, due to their inherent properties, consistently facilitated superior bacterial attachment compared to smooth surfaces. Bott (1993) found that electro polished stainless steel and fluorinated enzyme propylene and glass tubes exhibited reduced susceptibility to biofilm growth compared to untreated stainless steel. The analysis focused on biofilm formation on both hydrophilic (stainless steel) and hydrophobic Poly tetra fluoroethylene (PTFE) surfaces across varying temperatures and growth phases. The findings indicated that biofilm growth occurred more rapidly on hydrophilic surfaces in comparison to hydrophobic ones, with a negative correlation observed with growth temperature (Chavant *et al*., 2002).

In Conclusion, Bacteria possess the capability to adhere to various surfaces, both natural and artificial, resulting in the development of sessile multicellular communities known as biofilms. The colonization of bacteria on non-living materials such as suspended particles, metal surfaces, and concrete, in addition to living surfaces, is regarded as a crucial survival strategy for microorganisms. This process provides numerous important advantages to bacteria, enhancing their survival within hosts and in challenging environmental conditions.

## Author Contributions

Karuppiah Parthiban: Formal analysis, writing—original draft and editing. G.Buvaneswari: Validation, writing—reviewing.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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