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An Overview of Biofilm as a Virulence Factor for Bacteria to Survive in the Harsh Environment

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ABSTRACT

Microbial biofilms are collections of grouped microbial cells enmeshed in an extracellular polymeric substances (EPS) matrix that they have self-assembled. Biofilms are resistant to harsh environments and can serve as "protective clothing" for bacteria by shielding them from ultraviolet (UV) radiation, extreme temperatures, pH ranges, high salinity, high pressure, inadequate nutrition, antibiotics, etc. Research on biofilms in recent years has mostly concentrated on biofilm-associated illnesses and methods for eradicating microbial biofilms.

KEYWORDS: microorganism, harsh environment, biofilm,extracellular polymeric substances (EPS), adaptative mechanism

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1. INTRODUCTION

There are a number of highly harsh settings in the natural world, including intense high alkalinity, high acidity, high salt, high or low temperatures, high pressure, inadequate nourishment, ultraviolet (UV) radiation, and an abundance of antibiotics, among others.These severe conditions were once thought to be uninhabitable, but recent research has revealed that they are really teeming with microbes. They are not only alive, but they also thrive under harsh conditions that were once believed to be uninhabitable by life. Extremophiles are microorganisms that can endure these harsh conditions, and they consist of thermophiles, psychrophiles, alkaliphiles, acidophiles, halophiles, piezophiles, radiation-resistant extremophiles, and other types. The role of biofilm is thought to be one of the distinct resistance mechanisms that each microbe uses to survive.

2. BIOFILMS IN EXTREME ENVIRONMENTS

Previous research has demonstrated that the capacity to produce biofilms is crucial for bacteria to develop in a variety of harsh conditions. Biofilm creation is a distinct growth pattern that microorganisms choose in response to varied environmental stressors. Planktonic cells are initially attached in an irreversible manner (brown ovals), and then they adhere to the surface (grey) to create a biofilm (1). The bacteria subsequently create an extracellular matrix, which allows them to adhere to one another permanently (2). Multilayers then arise where a microcolony has established (3). The biofilm matures at a later stage and develops distinctive "mushroom" structures as a result of the polysaccharides (4). Finally, some cells begin to separate, and the biofilm will disperse (seen in yellow) (5)[1]. Figure1.

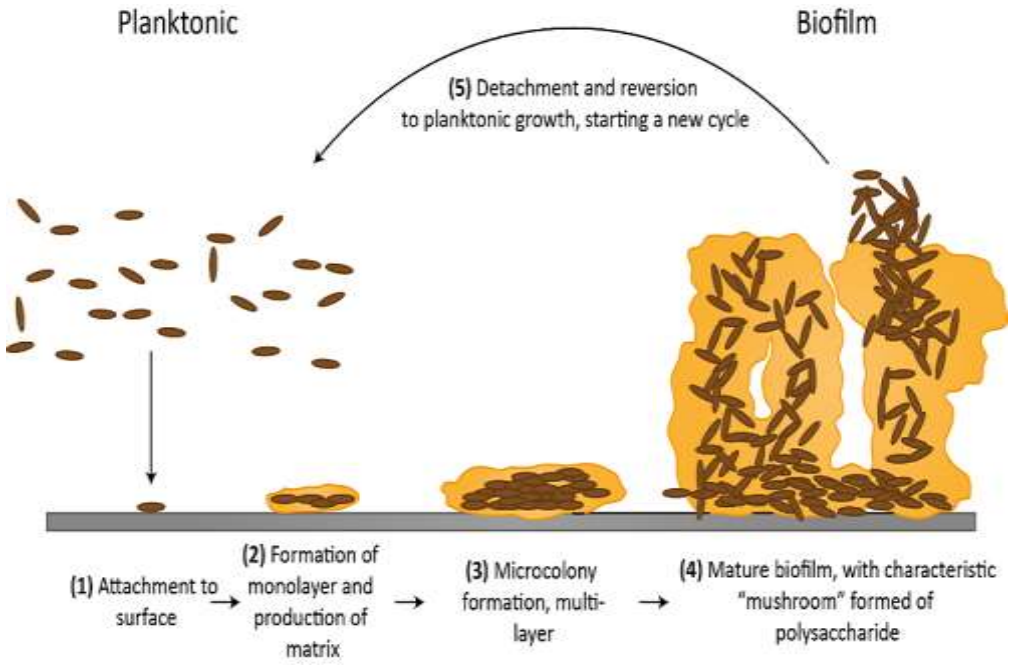

Figure 1. Schematic representation of a biofilm formation.

2.1. UV Radiation and Biofilm

Depending on the wavelength range, Solar UV is composed of three types: ultraviolet A (320 to 400 nm), ultraviolet B (290 to 320 nm), and ultraviolet C (100 to 290 nm)

In addition to the effect of UV-A radiation in degradation of proteins and membranes, it can damage DNA by generating reactive oxygen to stimulate single-stranded DNA differentiations [3, 4], unlike DNA, which can directly absorb UV-B radiation and change or modify nucleotides[5]. However, compared to UV-A or UV-B radiation, UV-C radiation is the most active source and generates more photovoltaic products [6].

Pseudomonas aeruginosa FRD1 derivative RM4440 displays plasma-based plasma-based recA-luxCDABE fusion, acting as a full-fledged biosensor for the organism to track DNA damage[7]. To explore how biofilms react to UV exposure, Elaser et al. suppressed RM4440 in a melginous matrix to imitate biofilm development[8

The findings showed that the PCW matrix appears to be protective in the physical protection of microorganisms against UV-C, UV-B, and UV-A rays, and transfers only 13%, 31%, and 33% of UV radiation, respectively, to microorganisms. This is in contrast to plankton bacteria. As a result, biofilms are useful for shielding microbial cells from UV light.

Listeria may also be shielded from UV light by the biofilm's composition [9]. Only one isolated strain of Listeria N53-1 from the salmon smoking house was given seven days to build a biofilm, and this strain had stronger UV resistance than others that had only been incubated for an hour. Ennedy et al. have discovered that the biofilms of naturally luminous hydrothermal spring caves, which have been uniquely adapted to the environment of high subsurface radioactivity, contain a great diversity of microorganisms [10]. They also discovered that the microbial communities in biofilms were less diversified but more resilient the greater the radioactivity.

Geothermal dinococcus represents the highly radiation-resistant dynocoxia family [11]. According to research by Frosler et al., the geothermal biofilm DSM 11300 appears to be more resistant to UV radiation than plankton cells. They hypothesized that this might be because of the production of reactive oxygen species from the photosynthesis of water molecules that are kept in cells or the array of plankton sedimentation in the biomembrane[12].

2.2. Biofilm at High Temperatures

Bacteria are significantly impacted by temperature, and biofilm can effectively explain how bacteria react to temperature fluctuations. Jihan et al. examined the biomembrane composition of thermophilic bacteria in the Bacillus family at various temperatures and found that for the genera Thermobacilli, Aerobacilli, Geobacilli, and Anoxybacillus thermoplastic, incubation at 65°C is more effective at producing biofilms than 55°C[13].

Some Sulfops species prefer a temperature of 75 °C for optimum growth [14, 15]. According to research by Koerdt et al. conducted at temperatures ranging from 60 to 85 °C, biofilm quantities rose at these temperatures in both Yellowstone National Park in the United States and Sulphopus sulvatarikus (a European territory cut off from Italy). They demonstrated a fivefold and a fourfold increase in biofilm composition, respectively, at 60 ° C.

Bacteriovorax studies have revealed that at temperatures below 10°C, the amount of bacteria in the water column greatly decreases, but not in surface biofilms[16]. Williams et al.'s other research has revealed that bacteria live 50% longer

in biofilms than in stranded cultures at a temperature of 5°C[17]. Polystyrelylene-chlorophylline in biofilms taken from Antarctic bacteria (Winogradskyella CAL384 and CAL396, psychoparasite Colwellia GW185, and Shewanella CAL606) have also shown the capacity to create stable emulsions, which shield cells from repeated cycles of freezing and thawing and improve microbial cells' ability to adapt to cold settings[18]. Microorganisms in hostile settings might therefore grow more resistant to harm brought on by heat stress because to the makeup of biofilm.

2.3. Extreme pH Environments and Biofilm

Due to the fact that biofilms often include both acid and alkali fibers, they also aid in the resistance of microbes to the effects of high pH[19, 20, 21].

The abundance of species generally drops off in extremely acidic environments, but there are still plenty of acid lovers shielded by biofilms that are common[22]. In reality, below the highly acidic pH, it was discovered that the solubility of heavy metals increases, and thus the toxicity index increases. It has been found that combining extreme pH and heavy metals together significantly alters the composition of biofilm-formed polystyrene, which regards a crucial to how well bacteria adapt in harsh settings. It is not only inhibit the toxicity of heavy metals, but it can also trap and enrich trace elements [23].

In addition, the contents of inositol and 3-O-methylglucose in EPS were shown to have a positive correlation with the toxicity index. Early studies revealed that inositol polyphosphates can prevent ferric iron from producing hydroxyl radicals, reducing its toxicity [24], and polysaccharides extracted from marine microorganisms that include methylglucose have also been used to get rid of heavy metals from solutions[25]. According to these evidence, biofilms have a protective role in severely acidic environments that is at least partially mediated by certain sugars.

Alkali-loving societies have also been demonstrated to develop biofilms under alkaline circumstances to surround the microbes in an EPS matrix [26, 27]. By demonstrating that Alishewanella and Dietzia can maintain internal pH values of 10.4 and 10.7 under a thick layer of EPS, Charles et al. show that biofilm formation can significantly increase the ability of alkaliphilic communities to withstand hyper alkaline stress [28]. Clinical isolates of *Enterococcus faecalis*, *Lactobacillus paracasei*, *Olsenella uli*, *Streptococcus anginosus*, *Streptococcus gordonii*, *Streptococcus oralis*, and *Fusobacterium nucleatum* from infected root canals were stressed at pH 10.5 for 4 hours to compare the responses of bacteria in biofilm or planktonic state to alkaline stress. The findings demonstrated that bacteria can withstand alkaline changes more successfully in a biofilm than they do in a planktonic condition [29]. Furthermore, higher EPS production in biofilm shields *Enterococcus faecalis* against 20 millimeters of Ca(OH)2, according to van der Waal et al. [30].

In general, microorganisms protected by biofilms are better able to withstand high pH stress than they are while in a planktonic stage.However, further research is needed to determine the precise processes behind the persistence of bacteria in biofilms under acidic and alkaline stress. It is still unknown if the biofilm is connected to the biological evolution of bacteria despite acting as an acid- and alkali-resistant "strong protective clothing". It will be fascinating to learn more about the distinctive qualities and composition of this "protective clothing".

2.4. Biofilm in Extremely Salinity Environments

The majority of halophiles on Earth are found in high-salinity areas like salt lakes, oceanic settings, and inland saline soils. Increasing osmotic pressure in these locations considerably contributes to microbial cytoplasmic degradation and cell death [31, 32].

Indeed, microorganisms can form a biofilm that is highly resistant to salt damage [33, 34]. In fact, a halophilic strain of *Halomonas stenophila* HK30 was discovered by Amjres et al. in a salty marsh near Brikcha (Morocco) and has the ability to build a biofilm in a medium containing 5% w/v salt [35]. Mallic et al. demonstrated that the salt-loving individuals of *Bacillus vietnamensis* AB403and *Kocuria flava* AB402, which were isolated from mangroves rhizosphere of Sundarban, can not only form biofilms effectively but also produce a significant amount of EPS under salt stress. Additionally, they can employ EPS to create inherent resistance, absorb a lot of metal components, etc. etc. [36].

To examine the resistance mechanisms of non-halophiles, several researchers have tested their salt tolerance in a number of ways. The impacts of the salinity on the biofilm composition of *Vibrio* sp B2 isolated from brine, seawater slip and biofilm, and found that bacteria with low salinity still maintained good cellular activity and excess production of formed polystyrene, which indicates a significant ability to induce formation of biofilms have been evaluated by Kim et al. In addition, Zhao et al. investigated the composition of biofilm-based microbial PSAIDs at various salinity levels and discovered that the generation of both protein and polysaccharides from precipitated, unsealed or sealed polystyrene in biofilms increased with increasing salinity [37].

On other hand, the biofilm's major constituents, EPS, function as a gel-like matrix that holds cells together to form aggregates and protects microorganisms from excessive salt stress [38]. Even if saline or non-saline, the biofilm they form may be crucial to agricultural operations since it may be utilized to adsorb various metal elements to support crop development and encourage soil bioremediation in salt-stressed environments.

2.5. High-Pressure Environments and Biofilm

Piezophiles can be defined as microorganisms that live, reproduce, thrive when subjected to intense pressures, such as those seen in deep-sea habitats. There is very few studies on the formation of their biofilms as a result of the difficulty of separation and culture, and coexistence with the limited distribution of these organisms [39]. Several research have demonstrated that elevated stress enhances the expression of the outer membrane protein gene [40], despite the fact that there are few investigations on how their biofilms are generated. Particularly in microorganisms, high hydrostatic pressure (HHP) can change a variety of macromolecules as well as their intracellular translation and transcription, leading to the generation of defective proteins [41, 42]. Microorganisms in biofilms are more resistant to high pressure than microorganisms suspended, according to studies on HHP biofilms. Additionally, compared to gram-negative bacteria, gram-positive bacteria's biofilms are more resistant to HHP [41].

Thus, research into the structural build and process of the "pressure-resistant garments" that develop in high-pressure situations will be crucial for the disciplines of biotechnology, industry, and medicine.

2.6. Oligotrophic Conditions and Biofilm

Due to inadequate nutrients, microbial development may be hampered under oligotrophic settings. However, it is also shown that biofilm production at this period contributes to the microbial tolerance to the constrained microbial growth.

Regarding the nutritional requirements in the living conditions, bacteria may be divided into two broad groups: oligotrophs and copiotrophs, which respectively, thrive well under low and high nutritional circumstances.

With regard to autotrophic organisms, several researches have verified that biofilm development is enhanced in nutrient-poor media [43, 44]. For instance, in a rich medium (brain heart pump, BHI) or 10-fold diluted vibrio cholera (BHI/10), Cherifi et al. examined the biomembrane composition of single-celled genes of the genus copiotroph *listeria monocytogenes* (isolated from hog slaughterhouses and cutting facilities) [45]. In order to create a cAMP-cAMP receptor protein complex, *Vibrio cholerae* A1552 appears to induce the creation of cyclic adenosine monophosphate. To control the expression of genes involved in the latter stages of nutrient intake and utilization, which will encourage the creation of bioshells [46, 47].

In oligotrophic conditions, oligotrophic strains predominate and are more prevalent in clear water. Non-tuberculous mycobacteria are oligotrophs and may thrive in pure water, artificial water, or in soils systems and capable of growing at low concentrations of carbon [48]. As they grow, biofilms help oligotrophic organisms resist sparsely nutritious surroundings. Although there is few studies which have focused on oligotrophic biofilms, it is not hard to predict that in sparsely nutritious circumstances, biofilms are a perfect existence strategy for autotrophic organisms.

In an undernourished environment, these "protective clothing" take a number of actions to maintain the reproduction and normal metabolism of bacteria, allocating the few resources preferentially within these "protective clothing" [49]. As a result, biofilms appear to be crucial for bacterial survival in the severe oligotrophic environment. No matter what kind they are, biofilms serve as a "protective garment" for microorganisms and are in charge of ensuring their survival and procreation.

2.7. Antibiotic tolerance and resistance in Biofilms

Biofilm-forming microorganisms appear to have a high level of antibiotic tolerance and resistance. The formation of biofilms, which is a temporary and non-heritable trait, is typically linked to microbial tolerance [50]. Microorganisms' acquired resistance to drugs in a genotype is known as antimicrobial resistance [50]. The tolerance and resistance of certain biofilms are regulated by a variety of molecular processes. The following forms of biofilms mostly impart bacteria resistance to antibiotics [52].

First, biofilms can serve as physical barriers, and their chemical structure and thickness can inhibit antibiotic spraying [53]. The EPS of biofilms contains a large number of anionic and cationic molecules, including proteins, uronic acids, glycoproteins, glycolipids, and eDNA. They can also provide a good shelter for microbes by binding to charged antibiotics [54], to aid bacteria that are enmeshed in biofilms withstand antibiotics [55]. According to research by Singh et al., *Staphylococcus aureus* and *epidermal staphylococcus* biofilms greatly limit the penetration of oxyacillin, cefotaxime, and vancomycin. [56]. Another factor affecting antibiotic penetration is the adsorption of antibiotics by biofilm components [57] or the breakdown of antibiotics by hydrolases such -lactamase [58, 59]. Adsorption of antibiotics by biofilm components or hydrolysis by hydrolase, such as beta-lactase [57, 58, 59], can limit antibiotic penetration. Exogenous polysaccharides, which binds via with negatively charged electron DNA in a *Pseudomonas aeruginosa* matrix of EPS [60], can also play a protective and structural role in reducing the body's susceptibility to antibiotics as aminoglycosides [61].

Second, physiological restrictions such as the pace of development [62,63,64], lifespan of the biofilm [65], malnutrition [66], etc., may reduce the sensitivity of the biofilm to antibiotics. Williamson et al. showed that a subset of dormant bacteria found in *Pseudomonas aeruginosa* biofilms is resistant to ciprofloxacin and tobramycin, while the population that is actively growing is still susceptible to these antibiotics [67]. In addition, a small fraction of microorganisms in biofilms and persistent cells that enter a state of impaired growth or starvation are also more tolerant of being killed by antibiotics. [68].

Antibiotic resistance is higher in biofilm-forming microorganisms than in their planktonic counterparts. A relationship between the growth of biofilms and antibiotic resistance has also been noted by a number of authors [69, 70].

First, the genetic diversity of microbes in biofilms can result in antibiotic resistance [71].

Second, the biofilm is thought to represent a major genetic diversity reserve that helps microbes survive in harsh settings and acquire antibiotic resistance. It has been shown that the production of biofilms in *Enterococcus faecalis* cells results in an increase in the average number of plasmid copies as well as an increase in the transcription of plasmid-borne resistance genes [72]. This finding suggested that biofilm growth could reduce microbial susceptibility to antibiotics.

Third, Antibiotics can be transported by multi-drug flow pumps in biofilms to reduce toxin development. [73, 74, 75]. Antibiotic resistance in *Pseudomonas aeruginosa* is caused by the PA1874-1877 flush pump, which is more prevalent in biofilms than in plankton. [76]. In *Pseudomonas aeruginosa* PA14, deletion of the genes encoding this pump improves microbiological susceptibility to tobramycin, gentamicin, and ciprofloxacin, particularly when this mutant strain is present in a biofilm.

Fourth, antibiotic sub-minimal inhibitory concentrations (sub-MICs) can also result in antibiotic resistance. Sub-MICs of erythromycin were found in clinical isolates of *Staphylococcus epidermidis* [77], tetracycline, and quinopristin- dalfoprestin [78] appear to boost the expression of the intercellular adhesion cluster gene, increasing the expression of EPS and invasion. Semi-middle-income organisms are also stimulated by thicker beta-lactam biofilm antagonists by higher genes implicated in the *Haemophilus influenzae* strains' glycogen biosynthesis which have been identified in patients who suffer chronic bronchitis and otitis [79].

Additionally, antibiotic resistance is increased in polymicrobial biofilms [80, 81, 82]. For instance, *Escherichia coli* cells embedded inside the *Candida albicans* biofilm demonstrate enhanced resistance to ofloxacin compared to the monomicrobial *Escherichia coli* biofilm. This is due to the *Candida albicans* -1, 3-glucan's capacity to bind to ofloxacin [83]. Additionally, *Staphylococcus albicans* and *Candida albicans* frequently form polymicrobial biofilms in a variety of illnesses, and *Staphylococcus albicans* coated in the matrix generated by *Candida albicans* exhibits improved vancomycin resistance [84, 85, 86, 87].

Microorganisms can find protection from a variety of severe settings in biofilm. In addition to the factors already mentioned, biofilms can shield microorganisms from a variety of acute environmental stresses, including oxidative stress, heavy metal pollution, and drought [80]. It was discovered that biofilms had colonized the Mir space station severely and had shattered quartz panes and struck several metal surfaces [81]. It is not only a scientific research, but also beneficial to human life since it will be an effective method of service creation, to understand the structure and defense mechanisms of these many and magical "protective clothing" in hostile settings.

3. CONCLUSIONS

The creation of specialized enzyme preparations for the pharmaceutical business, food industry, agricultural production, environmental protection, energy utilization, and other fields of industry, as well as scientific study, depends critically on protection by microbial biofilms. Numerous protective benefits of biofilm may be either physical or genetic. current scientific studies has shown that bacteria preferentially bind to many types of surfaces, and that groups of bacteria exhibit characteristics, behaviors, and survival strategies that far exceed their capabilities in the form of a single bacterial cell. For example, microbial biofilms can withstand doses of antibiotics up to 1,000 times more than those that kill bacteria in plankton.

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