Exploitation of Biofilms for Dental Microbes and Use in Industries for Bioremediation and Biofertilizers in Agriculture

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Abstract: ***Biofilm production by the microorganisms is for and against the human beings. It is being exploited in microbial infections in tools and lungs as well as industries for use in bioremediation against industrial effluents and organic farming for enhancing agricultural productivity through biofilm - biofertilizer production. Biofilms are one of the lifestyles of microbial organisms wherein they are attached to an inert or living surface by polymeric matrix surfaces. In this review efforts have been made to highlight the use of biofilm associated infections against the human diseases, solving ground contamination from an oil spill and healthcare as well as for bioremediation and also biofertilizer production in agriculture for which multidisciplinary research efforts are warranted.***

Keywords: Biofilms, bioremediation, biofertilizers

1. Introduction

Anthony van Leeuwenhoek first observed microorganisms on tooth surfaces by using his simple microscope. It is a complex process by which communication takes place between cells called as quorum sensing or signaling. Leeuwenhoek first observed microorganisms on tooth surfaces and credited with the discovery of micro - biofilms. However, the word Biofilm was not defined until a manuscript by Costerton et al., (1978). The microorganisms living in groups have benefit of biofilms e, g., *Pseudomonas and* *Klebsiella* can coexist in a biofilm. A biofilm comprises of microbial cells and produce extra cellular polymeric substance (EPS) matrix which provides an optimal environment for the exchange of genetic material among cells. This type of biofilm has disadvantage to human health in causing infectious diseases and other type of infections. Biofilm is protective against antibiotics and disinfectants.

In recent years, the increasing temperature resulting from global warming has significantly impacted plant growth and development. Abnormal climatic conditions induced by global warming have aggravated plant disease problems both from abiotic and biotic stresses thus adversely affecting crop quality and productivity. These stresses can inhibit plant growth and development by prompting plants to respond in various ways viz., altering cell metabolism, reducing growth rates and yields, and changing gene expression as part of their adaptation mechanisms. Stress is categorized into biotic and abiotic types based on its origin. Biotic stress arises from biological factors like diseases and pests, while abiotic stress stems from physical and chemical environmental factors such as variations in radiation, floods, drought, salinity, heavy metals, and extreme temperature and moisture conditions. Roots release various metabolites into the rhizosphere and provide nutrients that attract microbes and form microbial biofilms which are essential for plant development and soil health. Microbial biofilms in the root system play a crucial role in these processes.

Biofilms have their use in waste water treatment i. e., bioremediation and metal leaching. Electro - active biofilm forms the basis of bio - electrochemical systems. Initially bacteria move toward the surface followed by reversible attachment of the planktonic bacteria on the surface. Finally, there is detachment of microbes from the biofilms. The importance of biofilms was acknowledged by the American Society for Microbiology in 1993. Biofilms have their importance in human life and industrial use as well as agriculture in the biofertilizer production.

Van Leeuwenhoek first observed microorganisms on tooth surfaces and is credited with the discovery of microbial biofilm. Heukelekian and Heller (1940) observed the bottle effect for marine microorganisms. The biofilm was investigated in detail with an invent of electron microscope. Jones et al., examined biofilm on trickling filters in a waste water treatment plant and showed them to be a mixture of microorganisms. Characklis studied microbial slimes in industrial water system during the year 1973 and showed that they were not only tenacious but also resistant to disinfectants. Biofilm in industrial and ecologic setting has been worked out through scanning electron microscopy and standard microbiologic culture techniques. Biofilm is a complex microbiome structure having different colonies adhering to the surface. Bacterial biofilm is as serious health concern throughout the world due to their abilities to tolerate antibiotics in a host defense system and other external stresses leading to chronic infections. Biofilm is immobile microbial communities that grows and colonizes on the surfaces of medical tools such as sutures, catchers and dental implants by self - produced extracellular polymeric substances and cause infections which can only be treated after their removal. It gives the additional resistance to bacteria to tolerate unfavorable conditions and thus become multi - drug resistant. Degradation of such type of biofilm matrix is possible through antibiofilm strategy. DNase I, Dispersin B and α - amylase degrade biofilm matrix and exopolysaccharide thus inhibiting biofilm formation in various type of microbes like *S. aureus*, *Vibrio cholerae* and *Pseudomonas aeruginosa.*

Biofilm formation

Biofilms are formed by microbes that grow in association with other organisms on the surfaces of inert materials by adhering through exopolysaccharide (EPS). Natural biofilms contain microorganisms like bacteria, fungi, algae, protozoa. Microbes in biofilms communicate among each other by means of Quorum sensing. There are some beneficial uses of biofilm in bioremediation of waste water treatment and metal leaching. During the process of biofilm formation, bacteria first move toward the surface like some planktonic microorganisms while other organisms reach the surface by Brownian motion, gravitational and Vander walls forces. It is then followed by reversible attachment of the planktonic bacteria on the surface. In this process pili, fimbriae and flagella drive the adhesion of the cells toward the surface. pH, temperature and motility affect the attachment of microbes on the surface to mark the beginning of biofilm process. Colanic acid is the major constituent of the *Escherichia coli* while alginate forms the major component of *Pseudomonas aeruginosa* biofilm. A biofilm has a three - dimensional structure, defined water channels and considerable thickness. Biofilm formation is a gradual process starting with bacterial cells initially adhering reversibly to a surface. This is followed by irreversible adherence, leading to the expansion of microcolonies and the production of an extracellular polymeric substance (EPS) matrix. As the biofilm matures, it develops a three - dimensional architecture, becoming more resistant to host immune defenses and antibacterial agents. Dispersal of the biofilm occurs as cells undergo lysis and are released from the community. Within a host, bacteria can form biofilms on either living or non - living surfaces, with the latter often coated in proteins or other biological molecules, altering cell adhesion. Host cells can also play a crucial role in biofilm formation, with their components assimilated into the biofilm matrix. This process is detailed by Lynch and Robertson (2008) and Romling and Balsalobre (2012). Batoni et al., 2016; Nazir et al., 2019 reported Biofilms consist of microorganisms that generate extracellular polymeric substances (EPS), including proteins (less than 1–2%), polysaccharides (1–2%), DNA (less than 1%), and RNA (less than 1%). Water, comprising up to 97% of the biofilm, is crucial for facilitating nutrient movement within the matrix, although it is distributed unevenly. The extracellular polymeric substances (EPS) within the biofilm matrix serve as a governing factor for biofilms. They provide protection to the inhabitants against various environmental factors such as competing microbes, temperature fluctuations, host cells, antimicrobial agents, and desiccation, while also facilitating access to nutrients and enabling adaptation to environmental changes. Bacteria produce diverse types of EPS to fulfill these requirements in different ways. EPS play roles in bacterial adhesion to various surfaces and hosts, offering environmental protection and serving as reservoirs for nutrient acquisition.

Quorum Sensing (QS) Blockage Strategy

Li et al., 2020 conveyed that bacterial pathogens within the host can activate QS signals for biofilm formation and the production of virulence factors. Therefore, inhibiting this bacterial communication through QS inhibitors renders bacterial pathogens more susceptible to the host immune system and antibiotic responses. Inhibiting the Quorum Sensing (QS) system in Gram - negative bacterial pathogens typically entails three main approaches: i) Blocking AHL molecule biosynthesis, ii) Inactivating or breaking down AHL, and iii) Interfering with the signal receptor. This results in reducing or inhibiting the activity of the Quorum Sensing (QS) system. Additional research conducted by Masevicius et al. (2016) has demonstrated that certain Gram - negative bacterial pathogens are capable of producing S - adenosyl - L - methionine (AdoMet) as the primary methyl donor for various methylation processes. AdoMet can also serve as a precursor for the synthesis of two distinct QS signal molecules. Consequently, disrupting AdoMet production could potentially inhibit biofilm formation in diverse Gram - negative bacterial pathogens. This suggests that targeting AdoMet hindrance could lead to the development of QS inhibitors aimed at AHL molecule biosynthesis.

Biofilm in Human Diseases

Wounds are injuries to living tissue caused by trauma like cuts, abrasions, burns, or surgical procedures and can also occur due to diseases like diabetes. While the majority of wounds that harbour microorganisms typically heal without complications. There are instances where microorganisms proliferate excessively and disrupt the healing process resulting in an infection (Percival et al., 2012). It is widely acknowledged that chronic wound infections often host a diverse array of microorganisms, and the actual number of species may be underestimated due to the limitations of traditional culturing techniques. To overcome this challenge, molecular methods offer a solution by enabling the identification of viable bacteria that may not be culturable using conventional means. Moreover, cutting - edge microscopy techniques, such as confocal scanning electron microscopy, fluorescence microscopy, and electron microscopy, not only facilitate the visualization of bacteria within wounds but also aid in their precise identification. Brackman et al., (2013) revealed chronic wounds are frequently colonized by multiple bacterial species such as *Staphylococcus aureus* which is the most commonly isolated organism. Among these, *S. aureus*, *S. epidermidis* and *Pseudomonas aeruginosa* are commonly detected on the surface of chronic wounds, while anaerobic species tend to predominate in the deeper tissue layers. Detecting biofilm infections in wounds often relies on macroscopic evaluation, especially in clinics lacking advanced microscopy equipment and expertise. One proposed approach involves combining macroscopic examination of wound slough with microscopy, which can serve as a clinical indicator for the biofilm presence. Additionally, characteristics like a shiny or sheen appearance in wounds have been used to suggest biofilm presence. However, these macroscopic observations are highly subjective. There is a pressing need for a rapid and straight forward diagnostic method for identifying biofilm infections in wounds.

Biofilm - Biofertilizers

Agroecosystems are composed of interconnected plant, soil, and microbial communities, all of which are influenced by farmers' management practices. These complex interactions determine soil health, plant growth, and overall productivity. However, land management practices that rely heavily on chemical fertilizers, pesticides, and intensive tillage can disrupt the natural processes of soil microorganisms, particularly beneficial ones. Soil hosts a vast array of microorganisms, including soil fauna, algae, bacteria, fungi, and actinomycetes. These organisms coexist, interacting with each other and with plants. Some of these interactions are beneficial to plants, such as nitrogen - fixing bacteria and phosphorus - solubilizing microbes, while others can be harmful, involving soil - borne pathogens that cause diseases. Hassani et al., (2018) confirmed the microbial biofilm formed in root systems serves as an interface for the interaction of various microbial groups from different kingdoms. Parsek et al., (2005) revealed that within the biofilms, microbial cells engage in a form of social activity known as "Socio - Microbiology". This social behaviour involves specific interactions such as the exchange of metabolic products and communication of alerts between cells. The formations of bacterial biofilms is a crucial aspect of the relationship between plant roots and the microbial community in the biosphere, driven by their intricate surface chemistry. The diverse composition of root exudates suggests the existence of sophisticated mechanisms that promote the development of beneficial biofilms while suppressing pathogenic organisms. This regulation is achieved through the modulation of gene expression in both plants and microorganisms. Pandit et al., (2020) put forward ideas on Biofilm Biofertilizer (BFBF) is a type of fertilizer enriched with a robust microbial community that has developed in biofilm form, offering significant potential benefits. Beneficial biofilm - forming microbes can be cultivated *in vitro* as biofertilizer inoculum to create BFBF.

Premarathna et al., (2018) reported that using a mixed culture in BFBF in a consortium is more effective for crop plants than using a single microbial strain. Climate change intensifies both biotic and abiotic stress on plants thus negatively impacting crop quality and yield while making plants more vulnerable to insect pests and diseases. Interactions among microbes in a microbiomic system facilitated by biofilms can improve plants resilience to various environmental stresses. Although microbial biofilms naturally occur in the soil, their abundance and effectiveness are often insufficient for optimal plant growth. Therefore, the application of microbial biofilm biofertilizers is imperative in plant disease control and organic farming. Research is essential to develop high - quality microbial biofilm biofertilizers and to evaluate their effectiveness on different crops across various soil types and environmental conditions so as to protect our ecosystem and for welfare of humanity.

Future Prospects

Biofilm is of major concern in healthcare industry. There are anti - biofilm techniques available i. e., isolation of Quorum Quenching compounds and their use in combination with antibiotics. It is a promising hope for the development of new drugs for the control of biofilm forming pathogens. There is a need to investigate genes in biofilm associated organisms and to work out control strategy for preventing their colonization on medical tools. Chronic diseases need to be investigated especially in relation to biofilms perspective. The future prospects of biofilm research and applications in medical microbiology, bioremediation and Biofilm biofertilizers are quite promising. Understanding how biofilms form and function could lead to improved methods for prevention, detection, and treatment of infections in medical microbiology. Researchers are exploring innovative approaches like disrupting biofilm formation or enhancing the immune defense system. Biofilms are employed in bioremediation to clean up contaminated environments.

Future advancements might involve engineering biofilms with enhanced capabilities to degrade pollutants to efficiently capture heavy metals from river effluents in wastewater near industries. Biofilms are used in various industrial processes too viz., waste water treatment, food production, and biofuel synthesis. Future developments may focus on optimizing biofilm - based processes for higher efficiency and reliability. Additionally, biofilm - based bioreactors could be designed for pharmaceutical production. Biofilms offer inspiration for the development of bio - inspired nanomaterials and nanotechnologies. Future advancements may involve leveraging the self - assembly and structural properties of biofilms to create novel nanomaterials for drug delivery, sensing etc.

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