REVIEW PAPER

Extracellular Polymeric Substances as Driver for Biofilm Development and Microbial Resistance

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ABSTRACT

Biofilms are microbial communities embedded within extracellular polymeric substances, which are crucial for their development, stability, and ability to adhere to surfaces. The rising cases of pathogenic and multidrug-resistant microbes in food and clinical environments push for better understanding of biofilm associated resistance. The extracellular polymeric substances (EPS) composed of extracellular DNA, lipids, proteins and polysaccharides and its compositions varies according to the species of microbes and environmental factors. The modern analytical tools have advanced the characterization, structure and functions of EPS which plays major role in biofilm formation by microorganisms. The synthesis of EPS is highly regulated process and contribute to resistance by acting as barriers against various kinds of biotic and abiotic stresses. To overcome these challenges various strategies like EPS disrupting agents, nanocarrier-based delivery systems and approaches such as photodynamic therapy, magnetic fields and ultrasound in combinations with antibiotics could enhanced the controlling methods. Moreover, further deeper research is required to develop tools in imaging, polymicrobial biofilms and mixed-species EPS interactions for effective control of biofilms.

Keywords: Biofilms, Extracellular Polymeric Substances, EPS disrupting agents, photodynamic therapy, Polymicrobial biofilms

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I. INTRODUCTION

The biofilms are the microbial communities that allow microbes to exist for long term on any surfaces and enable them to survive in harsh environmental conditions including resistance to antibiotics. Most of the microbe form biofilms in their natural environment and is mode of life for protective growth. Moreover, mediate the development of resistance and make them very difficult to remove and more prone to redevelopment of biofilms. The biofilms formed by microbes can be beneficial by forming a safety layer against pathogenic microbes or harmful leading to resistance against antimicrobial agents[1&2]. For development of effective detection methods and medical regimens, we need to understand the formation of biofilms, its structure and along with its respective functions. This is thus important in managing and controlling their potential benefits and their health implications[3]. A hydrated and three-dimensional matrix, known as the extracellular polymeric substances (EPS) is a function of biofilms that is basic to the structure and also a function of other microbial communities. Mainly polysaccharides, proteins, extracellular DNA, lipids, and other biopolymers sum up to the combination of this matrix. These together create a network that is complex in general, interferes interactions with the environment and balances the biofilm architecture[4]. There is the facilitation of nutrient capture, provision of physical cohesion,

protection of the embedded cells from various environmental issues like the immune responses and the antimicrobials along with the and enabling of biofilms to adhere to surfaces is what the EPS is responsible for [3]. The continuous modification through enzymatic activity influencing the development of biofilm, acrimony, and flexibility is the active nature of EPS[4&5]. EPS also play major roles in natural ecosystems beyond microbial communities that affects sediment cohesion and moisture upholding in soils. Along with that, wastewater treatment impacting flocculation and removal of contamination are its technological applications[6]. In order to control biofilms that are harmful or tackle their advantageous properties in environmental and industrial circumstances, it is important to understand the biosynthesis pathways and composition of molecules of the EPS[7&8].

II. CHEMICAL COMPOSITION AND CHARACTERIZATION OF THE EXTRACELLULAR POLYMERIC SUBSTANCES

The EPS matrix mainly composed of exopolysaccharides, proteins, extracellular DNA, lipids and enzymes. These components are the backbone of overall architecture of microbial biofilms. Moreover, it also contains water channel proteins that mediate transportation of materials like gases, nutrients and wastes inside the biofilms[9]. The exopolysaccharides of EPS are synthesised both intracellularly and extracellularly and composed of common sugars like glucose, mannose, galactose and N-acetyl-glucosamine. But there is variation among species and production increases in stressed conditions[10]. The extracellular proteins form the major fraction of EPS matrix that helps in adhesion and integrity of the biofilms and amyloid proteins improve structural stability of biofilms[11]. The various enzymes associated with EPS the promote biofilms spreading on surfaces and provides nutrients for biofilm development[12]. Moreover, the extracellular DNA helps in attachment, motility and maturation of biofilms. It also enhances antibiotics resistance in some microbes like *P. aeruginosa* and *Salmonella* due to net negative charge[13]. The techniques employed for the characterization of extracellular polymeric substances (EPS) are numerous and vital for understanding their complex composition and structure. Fourier-transform infrared (FTIR) and nuclear magnetic resonance (NMR) are two spectroscopic methods that deliver in-depth insights into the chemical bonds and molecular structures found within EPS, which aids in identifying polysaccharides, proteins and other biomolecules[14,15&16].The three-dimensional architecture and surface morphology of biofilms can be visualized by using confocal laser scanning microscopy (CLSM) and field emission scanning electron microscopy (FE-SEM), maintaining the spatial relationships between EPS components and microbial cells[15&17]. Raman mapping reveals the interaction of polysaccharides, proteins, and humic substances in layered biofilms and presents a non-destructive technique of spatially resolving chemical variability within EPS layers[14]. Molecular methods such as chromatographic techniques along with mass spectrometry support thorough examination of monosaccharide composition and molecular weight distribution, which is essential to understand the EPS biosynthesis and its functions[15&18]. Even with these improvements, challenges continue to exist due to interference in colorimetric assays and the requirement for standardized protocols to ensure reproducibility and comparability in studies[19&20]. The composition of extracellular polymeric substances (EPS) show variation based on different environmental conditions such as temperature, nutrient availability, light intensity, and pollution exposure. According to studies, the primary determinants of EPS composition in harsh environment are environmental factors rather than microbial phylogeny where certain monosaccharides are associated with stress resistance mechanisms such as temperature tolerance and heavy metal toxicity[21]. Nutrient sources play an important role in the production and composition of EPS; for example, while growing on starch as compared to glycerol soil bacteria and fungi generate more carbohydrate to protein ratio suggesting that the quality of the substrate influences the composition of EPS[22]. Temperature and light intensity are key factors that affect the both quantity and quality of EPS in phototrophic biofilms, influencing their resistance to disruptions through varying protein to polysaccharide ratios in diverse species[23]. The presence of contaminants such as microplastics or heavy metals can lead changes in EPS composition which include variations in polysaccharide to protein ratio as well as the prevalence of functional group, possibly improve the biofilm protection or metal adsorption capacity[23&24]. Also, sugar content and biosynthetic pathways in cyanobacterial EPS can be changed due to the nutrient deficiencies of sulphur or magnesium, which illustrate the complex regulatory responses of EPS synthesis to environmental factors[25]. These results highlight the adaptive qualities of EPS as a

microbial strategy to deal with various environmental challenges.

III. ROLE OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) IN DEVELOPMENT OF BIOFILMS.

The early stages of biofilm formation and maturation on surfaces is mediated by EPS by forming bridge between functional groups of cell surfaces thereby promote aggregation and stabilization of newly formed biofilms[26,27&28]. During maturation of biofilms the chemical composition of EPS changed and polysaccharides concentration increases along with protein, lipids and nucleic acids[27,29&30.] The increased in concentration of proteins and polysaccharides ratios enhance the stability and organisation of biofilms[31]. The irreversible attachment and metabolic cooperation among heterogenous microbial populations is facilitated by EPS matrix[26,31&32]. The extracellular environment formed by biofilms maintain cohesion, localisation of nutrients, acts protective barriers and three-dimensional structure of the biofilms[33&34]. Therefore, the EPS matrix improve functions and resistance by biofilms thereby making key target to control biofilms[35,36&37].

IV. THE CONTRIBUTION TO BIOFILM RESISTANCE DEVELOPMENT BY EPS.

The EPS plays significant role in development of biofilms and resistance in microorganisms and few examples are mentioned below in the table 1.

Table 1: The Examples that Highlight the Role of EPS in Development of Biofilms and Resistance in Microorganisms.

Examples	Outcomes	Contribution	Reference
The interaction of nanoparticle-biofilm.	Nanoparticles help in disruption of EPS and enhance antimicrobial activities.	EPS blocks physically the antimicrobial agents and reduces the effects.	38
Microbial biofilms formed on devices of medical fields.	EPS reduces the penetration of antibiotics and promotes dormant state of bacteria.	EPS protects bacteria by limiting the actions of drugs like antibiotics.	39
Pseudomonas biofilms at low temperature	Increase the regulation of EPS synthesis genes.	Increased EPS results more thicker biofilms that enhance resistance and stresses.	40
Biofilms of *Bacillus subtilis* exposure to H_2O_2.	Mutants that lacked EPS showed reduced resistance to stresses.	Oxidative stress can be protected by EPS of microbes.	41
The biofilms of *Staphylococcus epidermidis.*	The proteins, polysaccharides and DNA present in EPS influences mechanical properties.	Modulating individual components of EPS can alter the structure and stability of biofilms.	42

V. MECHANISMS OF ENHANCED RESISTANCE BY BIOFILMS IN MICROBES

The microbes can survive very hostile conditions by forming biofilms which enable them by various mechanisms. The mechanisms are given in the figure 1.

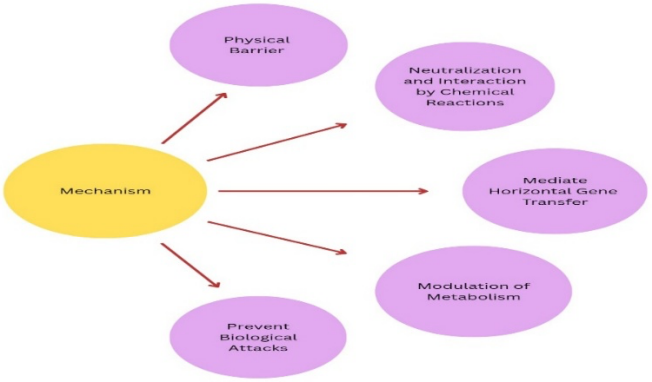

Figure 1: Mechanisms that Enhance Resistance in Microorganisms by Forming Biofilms

VI. THE DIFFERENT STRATEGIES TO CONTROL BIOFILMS BY TARGETING EPS.

Many different types of agents can be used to control biofilm formation that can degrade EPS matrix of microbes that protect the them. The enzymes like protease, DNases, and enzymes that degrade polysaccharides increase the potential of inhibition of microbes by increasing the killing capacity of antibiotics[43&44]. Moreover, another effective method can be the use of carrier of nanoparticles that deliver antimicrobial agents along with agents that disrupt EPS matrix of microorganisms[45&46]. However, the nanoparticle carries embedded with various active enzymes that break down EPS allow penetration of antibiotics deep into the biofilms[43]. The physical techniques like photodynamic therapy, magnetic field and ultrasound help in overcoming the problems of biofilm formation and biofilm associated resistance that disrupt EPS matrix which is critical for microbial biofilms formation. The photodynamic therapy that oxidises and damage biofilm components thereby generate reactive oxygen species which promote microbial killing by disturbing the integrity of the EPS[47,48&49]. The efficiency of magnetic field increases when combined with magnetic nanoparticles and sonic effects of ultrasounds by producing synergistic effects that attack the biofilms mechanically and chemically[50&51]. The main advantages of these techniques are the non-invasiveness and targeted that rectify the limitation of commonly used conventional techniques. These approaches expose bacteria enclosed in biofilms thereby increase the efficiency of antimicrobial agents[52]. The quorum sensing the communication mechanism of microbial population and helps in formation of microbial aggregation (biofilms). So, EPS matrix disrupting approaches and agents that interfere in quorum sensing could be powerful approach to fight antimicrobial resistance[53&54].

VII. CHALLENGES AND FUTURE PERSPECTIVES ON BIOFILMS

The microbial biofilms consisting of different species or strains of microbes are called polymicrobial biofilms. The structural organization, stability and metabolic cooperation of polymicrobial biofilms is mediated by EPS matrix of microbial populations[55]. In polymicrobial biofilms the resilience, tolerance and pathogenicity of the microbes due to presence of mixed microbial species communities, combined interaction of components of EPS matrixes and amyloid like materials of microbial species[56]. Moreover, the interaction of different species results modification of EPS compositions and different patterns of glycans and proteins developed thereby enhance the stability of biofilms and resistance to oxidative stress. The polymicrobial biofilms also mediate transfer of antibiotic resistance genes via horizontal gene transfer and make it difficult in treatment different healthcare systems[57,58&59]. So, the approaches that disrupt amyloid like materials assembly and pattern of EPS matrix can weaken the overall structure of biofilms and improve antimicrobial efficiency[60&61]. The synergistic approaches of physical strategies combined with conventional techniques have potential to limit the development of resistance and matured biofilms. However, the challenges in eradicating biofilms are the mixed species that limit the development of highly specific therapies [62&63]. The advanced microscopy and molecular tools can be emerging approaches in study of architecture of biofilms and antimicrobial resistance. The omics approaches like genomics, proteomics and metabolomics can reveal the metabolic and genetic basis of biofilms and resistance development[64&65]. The recent emerging tools like volumetric super-resolution imaging and microfluidics coupled with artificial intelligence reveal heterogeneity of biofilms in terms of distribution and responses and provide deep insight

into polymicrobial biofilms behaviour that help to design targeted antibiofilm therapies[66&67].

VIII. CONCLUSIONS

The extracellular polymeric substances (EPS) are the backbone of microbial biofilms and play significant role in developing resistance to environmental stresses and diverse antimicrobial agents. The dynamic composition of EPS enables resistance to antimicrobial agents, promote dormant state thereby enhanced the survival in different setting of healthcare and food industries. The advanced techniques of imaging and molecular can reduce the gap of understanding the structure and function of biofilms. Therefore, the future efforts should be on advanced techniques in the fields of microscopy and omics based for deeper knowledge about the dynamics of biofilms to prevent the prevalence of resistance due to formation of biofilms by microorganisms.

AUTHORSHIP CONTRIBUTION STATEMENT

SL: Review of literature & editing, original draft, Resources, Data curation**.** Conceptualization, **TS:** Review writing – review & editing, Investigation, Formal analysis, critical check**, DS:** Review of literature, Initial draft, editing, data analysis**, LS:** Checking the original draft, Supervision, Resources, Investigation, Formal analysis, Data curation, Coordination. **SD:** literature Review, data collection, help in drafting**, RD:** literature Review & editing, initial draft**; BM:** Drafting the MS, editing and formatting the manuscript**, JS:** critical review, editing and formatting the manuscript, **MS**: literature Review & editing, data collection, **GA:** literature Review & editing, data collection, **RS:** literature Review & editing, data collection & compilation, **AS:** literature Review & editing, data collection & compilation, validation.

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