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EXTRACELLULAR POLYMERIC SUBSTANCES-WONDER MACROMOLECULES IN MICROBIAL MATS (*GENERAL ARTICLE*)

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Abstract: Microorganisms play critical roles in the various aquatic systems forming microbial mats. The microbial mats are held together and bound to their substrates by slimy extracellular polymeric substances (EPS) having various applications which they secrete. They use all types of metabolism and feeding strategy that have evolved on Earth, and have shown immense use in various industrial applications. An attempt to study the role of EPS and their general characteristics in microbial mats has been made by the authors.
Keywords: Extra polymeric substances, lipopolysaccharides, matrix, metabolites, glucans, uronic acid, quorum sensing

INTRODUCTION

Microorganisms occur in natural habitats in the interfaces of air–seawater or freshwater, seawater–sediment, hard bottoms or growing suspended in the water column in the form of microbial cellular aggregates (Sanjay *et al.*, 2011). The investigation of microbial aggregates on tooth surfaces by Antonie van Leeuwenhoek resulted in the identification of microbial biofilms (PenXio *et al.*, 2007). These forms of cellular aggregates in aquatic systems are called as microbial mats or biofilm (commonly referred as slime). These microbial mats play a crucial role in a variety of disciplines, including biotechnology, immunology, biofouling and biodeterioration (Yousuf *et al., 2015*).

The microorganisms in these biofilm are embedded in a matrix of extracellular polymeric substances (EPS) which provide the mechanical stability for the biofilms (Itzel Galaviz *et al.*, 2016). Importantly, bacteria in biofilms exhibit a set of 'emergent properties' that differ substantially from free-living bacterial cells (Figure 1). EPS are produced by all prokaryotic and eukaryotic organisms in the system. The Extracellular polymeric substances (EPS) produced by these microorganisms are a complex mixture of biopolymers primarily consisting of polysaccharides, as well as proteins, nucleic acids, alginates, lipids, acetyl

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groups, Sucrose derived glucans, fructans, cellulose, alginate, xanthan, colonic acid, uronic acid and humic substances (Tsuneda *et al*., 2003).

Majority of these compounds (EPS) are in the form of polysaccarides. About 99% of the microorganisms live within these biopolymers. These are also called ‘the dark matter of biofilms’ because of the large range of matrix biopolymers and the difficulty in analysing them (Michael *et al*., 2014). However, EPS are very complex, the knowledge regarding EPS is far from complete and much work is still required to fully understand their precise roles in nature (Youssef *et al*., 2015). The microbial cells respond to environment by means of gene expression leading to formation of secondary metabolites paving way for formation of biofilms.

EPS AND BIOFILM ARCHITECTURE

EPS are responsible for the three dimensional architecture of the biofilm and is responsible for adhesion to surfaces and for cohesion in the biofilm. The formation and maintenance of structured multicellular microbial communities crucially depend on the production and quantity of EPS. The architecture of biofilms is influenced by many factors such as the hydrodynamic condition, concentration of nutrients, bacterial motility and intercellular communication. It is also been influenced by interaction of anionic EPS containing carboxylic groups, with multivalent cations. For example, Ca^{2+} can form a bridge between polyanionic aginated molecules, stimulating the development of thick and compact biofilms with increased mechanical stability (Michael *et al*., 2014). EPS structures have been studied on different model systems and by applying different spectroscopic techniques. Larger network structures with regions of unequal densities were visualized by electron microscopy *Streptococcus equi* (Jean *et al*., 2004)

Fibrous structures were seen by scanning electron microscopy in EPS from *Bacillus* sp (Judith and Peter, 2004). Double helices were observed in rhamsan after deacetylation by optical rotation spectroscopy and differential scanning calorimetry (Kate *et al*., 2013). Even though biofilms are commonly referred as slime which states that they are not rigid structure, their mechanical stability is important and references show that it is mainly because of the presence of these wonder macromolecules called EPS. They are located at or outside the cell surface independent of their origin and are distinguished as bound EPS and soluble EPS based on their physical state composed of a number of monosaccharides and non-carbohydrate substituents, EPS are distinguished as homo- and heteropolysaccharides. While the homopolysaccharides are neutral, majority of the heteropolysaccharides are polyanionic due to the presence of either uronic acids (glucuronic acid, galacturonic acid and mannuronic acid) or ketal-linked pyruvate (O'Niel *et al*., 1999).

Bacterial secretions, shedding of cell surface materials, cell lysates and adsorption of organic constituents from the environment result in EPS formation. Other factors that also contribute for EPS production by bacteria are the species combination, phases of growth, nutritional status and the environmental conditions. Chemically, EPS are rich in high molecular weight polysaccharides (10 to 30 kDa) and generally have heteropolymeric composition (Figure 2). The polysaccharide chain might be unbranched or branched with side chains of other compounds attached to the polymeric chain (Ronald *et al*., 2012).Cell-to-cell communication also called quorum sensing is also an important factor in shaping the biofilm (Bhaskar and Narayan, 2005). The composition of EPS synthesized varies from microbes to microbes.

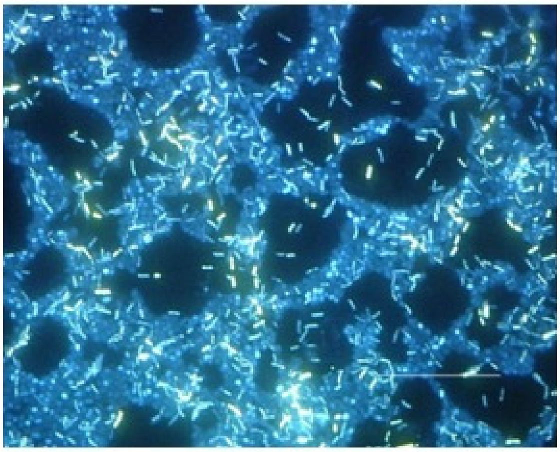

Fig.1 Microbes adhered to microbial mats

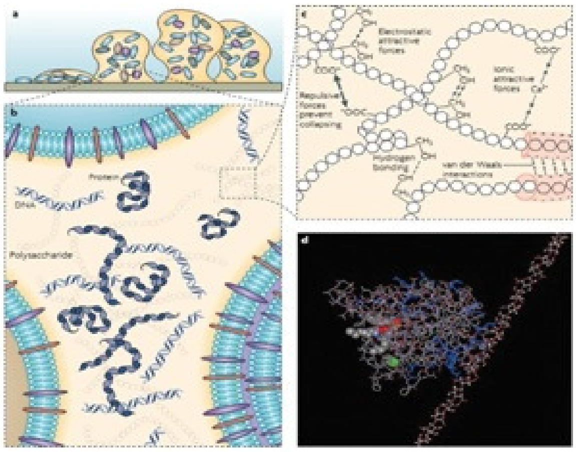

Fig.2 EPS matrix at different length scales

LIFE CYCLE OF A BIOFILM

Biofilm lifecycle can be summarized in to three important steps;

1. Attachment
2. Growth and development
3. Detachment

Process of biofilm formation

Step 1.Attachment

• Occur using weak Van Der Waal bonds. It attaches more permanently using cell adhesion structures like pili, Cell adhesion molecules such as ESP and Hydrophobicity. Hydrophobicity determines the ability of microbes to form biofilms.

• Increased hydrophobicity means less repulsion between the EPS matrix and the bacterium

• Bacterial pioneers who initially attaches to the surface starts building the EPS matrix that holds the biofilm together

• In addition to matrix substances and bacterial cells, EPS matrix may also contain materials from environment such as minerals, soil particles etc.

• This facilitate the arrival of other biofilm bacteria by providing more adhesion sites

• If there are species that are unable to attach to a surface on their own, they are often able to anchor the matrix directly to earlier colonists

• When a single bacterium joins a biofilm, expression of approximately 800 genes have reported to be altered and differentially regulated. As a result, cell undergoes a phenotypic shift in behaviour. This impart different physiological characteristics to the joined bacterium than its planktonic members. During colonization cells within the biofilm are able to communicate via Quorum Sensing

Step 2: Growth and Development

• Growth of a biofilm occurs through a combination of division of existing cells and recruitment of new cells.

Step 2: Detachment

Once the biofilm has fully formed, it contains channels in which nutrients and also, signaling molecules involved in Quorum sensing can circulate.

• Cells in different regions exhibit different patterns of gene expression .As a result of above, biofilms often develop their own metabolism.

ROLE OF EPS

S.No	**Function**	**EPS responsible**
1.	By means of cohesion it forms a hydrated biofilm matrix providing mechanical stabilization and allows cell to cell communication	Lectins, amyloid, neutral and charged polysaccarides
2.	Adhesion provides initial colonization of abiotic and biotic factors	Polysaccarides and proteins
3.	Maintains a highly hydrated microenvironment around biofilm organism thus avoiding dessication	Hydrophilic polysaccharides and proteins
4.	Confers resistance to non-specific and specific host defences during infection and confers tolerance to various antimicrobial agents	Polysaccharides and proteins
5.	Enables the digestion of exogenous macromolecules for nutrient acquisition and the degradation of structural EPS, allowing	Proteins

	the release of cells from biofilms	
6.	Act as a source of carbon, nitrogen and phosphorus containing compounds for utilization by the biofilm community	All EPS
7.	Aggregation of temporarily immobilized bacterial cells by enabling bridging between cells and cell-cell recognition	Polysaccharides, proteins and DNA
8.	Acts as a sink for excess energy by storing excess carbon under unbalanced carbon-nitrogen ratios	Polysaccharides
9.	Formation of flocs	Polysaccharides
10.	Serve as biosorbing agents by accumulating nutrients from the surrounding environment and also play a crucial role in biosorption of heavy metals.	Lipopolysaccharides and mucopolysaccharides
11.	contribute to sustainable aquaculture production, providing feed to the cultured species, removing waste and contributing to the hygiene of closed systems.	Polysaccharides, protein
12.	Act as an immunomodulator, antiviral and immunostimulant and antiadhesive agent in aquaculture	Glucan, Dextran, Levan, polyhydroxybutyrate

Conclusion

It can be simplified that there is no biofilm without an EPS matrix. EPS are essential for biofilm formation and make possible a lifestyle that is entirely different from the planktonic state. Future research avenues need to be explored to advance the current understanding of these wonder compounds. Further work needs to be carried out in order to determine the chemistry and functional aspects of naturally occurring EPS. The paper has highlighted brief implications about EPS and its role in nature and its future in various fields.

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