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A Review on Microbial Biofilm

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ABSTRACT

A biofilm is a well organized cooperating community of organisms. Biofilm it is a surface based microbial cells. Biofilm are composed primarily of microbial cells and EPS (Extracellular Polymeric Substance). The category microorganisms majorly involved in cell communication and signalling and hence biofilm formation are reviewed in this paper. The major types of microorganisms majorly assisting in biofilm formation such as Pseudomonas aeruginosa, Candida albicans, and Staphylococci sp are being discussed. The features of biofilm producing organisms were also reviewed in this paper.

Keyword: - EPS, Extracellular polymeric substance, Microorganisms, Biofilm, Communication.

INTRODUCTION

A biofilm is a cluster of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix. Van Leeuwenhoek, using his simple microscopes, first observed microorganisms on tooth surfaces and can be credited with the discovery of microbial biofilms. Heukelekian and Heller (Heukelekian H, 940) observed the "bottle effect" for marine microorganisms, i.e., bacterial growth and activity were substantially enhanced by the incorporation of a surface to which these organisms could attach. Zobell (Zobel C.E,1943) observed that the number of bacteria on surfaces was dramatically higher than in the surrounding medium (in this case, seawater). However, a detailed analysis of biofilms would await the electron microscope, which allowed high-resolution photomicroscopy at much higher magnifications than did the light microscope. Jones et al. (Jones.H.C,1969) used scanning and transmission electron microscopy to examine biofilms on trickling filters in a wastewater treatment plant and showed them to be composed of a variety of organisms(based on cell morphology) (Characklis WG.1973).

BACTERIAL BIOFILM

Biofilm Formation and Bacterial Communication

How do biofilms form? The formation of a biofilm requires coordinated chemical signaling between cells. Unless an adequate number of neighboring cells are present, the costs of biofilm production to an individual bacterium outweigh the benefits. Thus, a signaling process benefits the bacteria by allowing them to sense the presence of neighboring bacteria and respond to varying conditions. The process by which a bacterium does this is called quorum sensing.

Quorum sensing uses signaling molecules, known as **autoinducers**. These are continuously produced by

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bacteria and can readily diffuse through the cell membrane. When elevated numbers of bacteria are present in an area, the concentration of autoinducers in the region will be higher.

Although the numbers and type of bacteria in a wound are critical for infection to occur, recently a new concept of bacterial biofilms has emerged as a potential way to better understand how bacteria deter healing. Therefore, a better understanding of bacterial biofilms is needed, and this may ultimately result in development of novel therapeutics for the prevention and treatment of DFU infections. The biofilm producing organisms have an inherent resistance to antibiotics and in the long run they may be very damaging because of the development of immune complex diseases (1995; Souli et al., 1998).
There are only scarce reports on biofilm formation by clinical isolates from DFU especially in North India. Keeping this in mind, the present study was undertaken to study the difference in their antibiotic resistance profile and minimum antibiotic concentration of biofilm producing and non-biofilm producing gram-negative bacilli isolated from diabetic foot ulcer in a tertiary care hospital in North India.

Dispersal
Biofilm cells may be dispersed either by shedding of daughter cells from actively growing cells, detachment as a result of nutrient levels or quorum sensing, or shearing of biofilm aggregates (continuous removal of small portions of the biofilm)because of flow effects. The mechanisms underlying the process of shedding by actively growing cells in a biofilm are not well understood. Gilbert et al. (Gilbert P,et.al.,1993) showed that surface hydrophobicity characteristics of newly divided daughter cells spontaneously dispersed from either *E. coli* or *P. aeruginosa* biofilms differ substantially from those of either chemostat-intact biofilms or resuspended biofilm cells. These researchers suggested that these differences might explain newly divided daughter cells' detachment. Hydrophobicity was lowest for the newly dispersed cells and steadily increases upon continued incubation and growth.

Biofilm Structure
Extracellular Polymeric Substances
Biofilms are composed primarily of microbial cells and EPS. EPS may account for 50% to 90% of the total organic carbon of biofilms (Hussain.et.a.,1993) and can be considered the primary matrix material of the biofilm. EPS may vary in chemical and physical properties, but it is primarily composed of polysaccharides. Some of these polysaccharides are neutral or polyanionic, as is the case for the EPS of gram-negative bacteria. The presence of uronic acids (such as D-glucuronic, D-galacturonic, and mannuronic acids) or ketal-linked pryruvates confers the anionic property (Sutherland.I.W.,2001)This property is important because it allows association of divalent cations such as calcium and magnesium, which have been shown to cross-link with the polymer strands and provide greater binding force in a developed biofilm (Fleming.H.et.al.,2000). In the case of some gram-positive bacteria, such as the *staphylococci*, the chemical composition of EPS may be quite different and may be primarily cationic. Hussain et al. (Fleming.H.et.al.,2000) found that the slime of coagulase-negative bacteria consists of a teichoic acid mixed with small quantities of proteins. EPS is also highly hydrated because it can incorporate large amounts of water into its structure by hydrogen bonding. EPS may be hydrophobic, although most types of EPS are both hydrophilic and hydrophobic (Sutherland.I.W.,2001). EPS may also vary in its solubility. Sutherland (Sutherland.I.W.,2001) noted two important properties of EPS that may have a marked effect on the biofilm. First, the composition and structure of the polysaccharides determine their primary conformation. For example, many bacterial EPS possess backbone structures that contain 1,3- or 1,4-□-linked hexose residues and tend to be more rigid, less deformable, and in certain cases poorly soluble or insoluble. Other EPS molecules may be readily soluble in water. Second, the EPS of biofilms is not generally uniform but may vary spatially and temporally. Leriche et al. (Leriche.V.et.al. 2001) used the binding specificity of lectins to simple sugars to evaluate bacterial biofilm development by different organisms. These researchers' results showed that different organisms produce differing amounts of EPS and that the amount of EPS increases with age of the biofilm.
EPS may associate with metal ions, divalent cations, other macromolecules (such as proteins, DNA, lipids, and even humic substances) (Hussain.et.al. 1993). EPS production is known to be affected by nutrient status of the growth medium; excess available carbon and limitation of nitrogen, potassium, or phosphate promote EPS synthesis (Leriche.V.et.al.,200)1. Slow bacterial growth will also enhance EPS production (Leriche.V.et.al.,2001). Because EPS is highly hydrated, it prevents desiccation in some natural biofilms. EPS may also contribute to the antimicrobial resistance properties of biofilms by impeding the mass transport of antibiotics through the biofilm, probably by binding directly to these agents (Fleming.H.et.al. 2000).

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ALGAL BIOFILM

In industry, biofilms are often considered a nuisance as they reduce heat transfer in heat exchangers and cooling towers, foul membranes, and contaminate food processing equipment (Qureshi et al., 2005). In the field of wastewater treatment, however, biofilms play a beneficial role.Most research in using algae to reduce nutrient levels in wastewater or to produce biofuel feedstock has focused on suspended microalgae. Because of the harvesting challenges associated with algae grown in this form, there has recently been an increased interest in the use of immobilized or attached algal communities (Hoffmann, 1998). When algae are grown as surface attached biofilms, the biomass is naturally concentrated and more easily harvested, leading to lower downstream processing costs. By producing algae in the form of a biofilm, costly concentration operations can be avoided, and an easily harvestable source of biofuel feedstock can be provided (Roeselers et al., 2007). Notwithstanding these potential benefits, there is no consensus on the best method of growing and harvesting algal biofilms. Therefore, there is a need for further investigation to address the engineering design of algal biofilm systems. Algae are capable of reducing nitrogen and phosphorus concentrations in wastewater through biomass assimilation, and if harvested, offer the added benefit as a source of feedstock for the production of biofuels and bioproducts. The integration of microalgae-based biofuel and bioproducts production with wastewater treatment has major advantages for both industries. However, major challenges to the implementation of an integrated system include the large-scale production of algae and the harvesting of microalgae in a way that allows for downstream processing to produce biofuels and other bioproducts of value. Difficulties in harvesting, concentrating, and dewatering the algae have limited the development of an economically feasible treatment and production process. When algae are grown as surface attached biofilms, the biomass is naturally concentrated and more easily harvested, leading to less expensive removal from wastewater, and less expensive downstream processing in the production of biofuels and bioproducts. A novel rotating algal biofilm reactor (RABR) was designed, built, and tested. The RABR achieved effective nutrient uptake from wastewater and algae biomass production (31 g m-2 day-1) at pilot scale. An efficient spool harvesting technique was also developed in order to obtain a concentrated biosolids product (12-16% solids) suitable for further processing in the production of biofuels and bioproducts. The algal biofilms grown on the RABR(Rotating algal biological reactor) were able to reduce phosphorus and nitrogen concentrations in the wastewater at pilot scale, with P and N removal rates of 4.1 Results of this study indicate that the RABR with spool harvester represents a promising approach to the production and harvesting of algae in wastewater.

BACTERIAL BIOFILMS ON FUNGAL SURFACES

Pseudomonas aeruginosa* biofilm formation on *Candida albicans

Biofilm interactions between the Gram-negative bacterium *P. aeruginosa* and the fungus *C.albicans* may have relevance to the study of infections associated with cystic fibrosis (CF). Individuals with CF, a genetic disease that results from mutations in the CFTR transmembrane conductance regulator, are highly susceptible to chronic, progressive pulmonary infections that severely damage lung tissues and most often lead to respiratory failure in early adulthood (Rajan and Saiman, 2002). Several lines of evidence suggest that the microorganisms in CF sputum are in a biofilm-like state (Costerton *et al*., 1999; Hoiby *et al*., 2001; Singh *et al*., 2000). While the predominant colonizer of the CF lung is *P. aeruginosa*, *C. albicans*, a dimorphic fungus, and *Aspergillus fumigatus*, another opportunistic fungal pathogen, are also commonly observed (Bakare *et al*., 2003; Bauernfeind *et al*., 1987; Bhargava *et al*., 1989; Burns *et al*., 1999; Cheng *et al*., 1990; Haase *et al*., 1991; Hughes and Kim, 1973; Navarro *et al*., 2001). The effects of mixed bacterial-fungal infections on thehost lung are not yet known.

In vitro analysis of the relationship between *P. aeruginosa* and *C. albicans* has shown that *P. aeruginosa* attaches to and forms biofilms on the surface of *C. albicans* (Hogan and Kolter, 2002). Within the *P. aeruginosa* biofilms, the fungal hyphae are killed. Studies using different *P. aeruginosa* and *C. albicans* strains have yielded data that support the hypothesis that *P. aeruginosa* biofilm formation is necessary, but not sufficient, for killing (Hogan and Kolter, 2002). Thus, the *P. aeruginosa–C. albicans* biofilm interaction may enable us to study some of the links between biofilm formation and virulence.

The bacterial factors that participate in fungal attachment are under regulatory control. *P. aeruginosa* attachment is enhanced by unknown factors that are regulated by cell density-dependent acylhomoserine lactone signals and nutrient availability (Hogan and Kolter, 2002). Interestingly, some *P. aeruginosa* strains, including strain PAO1, do not readily attach to *C. albicans* hyphae when grown under the conditions that promote *P. aeruginosa* strain PA14 attachment (Hogan and Kolter, 2002). These data illustrate that *P. aeruginosa* strains have different bacterial surface characteristics or structures that will impact their ability to physically interact with different cell types. As has been described for bacterial attachment to abiotic surfaces such as glass or plastic (Marshall *et al*., 1971) and to plant cells (Hendrickson *et al*., 2001). *P. aeruginosa* PA14 attachment to the fungus occurs by

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one bacterial pole (Hogan and Kolter, 2002). Time-lapse microscopy shows that bacterial attachment to fungal hyphae is at first reversible with *P. aeruginosa* cells rapidly attaching to and detaching from the hyphal surface (data not shown). At some frequency, cells remain attached to the hyphal cell and initiate the formation of biofilms. *P. aeruginosa* antagonism towards *C. albicans.* Using both vital staining techniques and viable counts, it has been shown that the fungal hyphae within *P. aeruginosa* biofilms are killed within 24–48 hours of biofilm formation (Hogan and Kolter, 2002). To identify those factors that participate in this antagonistic interaction, the rate of fungal killing by different *P. aeruginosa* mutants were compared to the wild type using a quantitative, plate-count assay to monitor fungal viability of a constitutively filamentous *C. albicans tup1*/*tup1* mutant (Braun and Johnson, 1997; Hogan and Kolter, 2002). Under the nutrient-limiting conditions of the assay, biofilm formation is necessary for fungal killing. *P. aeruginosa* mutants that lack a functional flagellum, such as the *flgK* mutant, are delayed in biofilm formation on the fungal surface and are delayed in fungal killing. Mutants lacking certain global regulators, such as RpoN, or quorum sensingrelated transcription factors, such as LasR and RhlR, were both defective in biofilm formation (see Atkinson *et al*., this volume) and decreased in their ability to kill *C. albicans* hyphae (Hogan and Kolter, 2002). Though these mutations in global regulators are pleiotropic, the correlation between the ability to form biofilms and fungal killing towards *C. albicans* is consistent with other data that link biofilm formation and virulence. Virulence factors that have been implicated in human disease, such as the secreted phospholipase C, which degrades eukaryotic membrane lipids, also participate in biofilm related fungal killing (Hogan and Kolter, 2002; Hollsing *et al*., 1987; Lanotte *et al*., 2003; Woods *et al*., 1997). The fact that biofilm formation enhances, and may be required for, killing suggests that these genes may be differentially regulated within biofilms on biotic surfaces, or that these secreted factors are more efficacious when they are produced in close proximity to the target cells. Because *P. aeruginosa* biofilm formation on *C. albicans* occurs much more readily under nutrient limiting conditions, it has been speculated that the bacteria are using the fungal hyphae as a source of nutrients and that biofilm formation allows for the synergistic action of degradative enzymes and for the capture of nutrients released upon fungal lysis.

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