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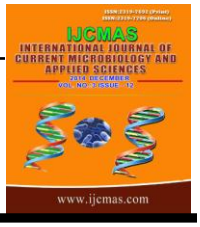

Review Article

Formation and Significance of Bacterial Biofilms

M. Zubair[1]*, M. Ashraf[1], M. Arshad[1], A. Raza[2], B. Mustafa[1]and A. Ahsan[1]

[1]Institute of Microbiology, University of Agriculture, Faisalabad-Pakistan
[2]Department of Physiology and Pharmacology, University of Agriculture, Faisalabad-Pakistan

**Corresponding author*

A B S T R A C T

Biofilms are aggregation of bacteria which are organized into structural communities and produce exopolysaccharide matrix as a major component for their stability. Basic structure of biofilm consists of micro colonies framed in extracellular polymeric substance. Formation of biofilm is multi step process which involves attachment, growth and expansion. Certain environmental factors such as nutrient content, temperature, pH, iron and oxygen act as signals for the development of biofilm. Within biofilm, bacteria communicate with each other through a process called quorum sensing. Biofilms play an important role in diseases, maintenance of infections and bio fouling in different industrial units. Biofilms of some bacteria also help in bioremediation and also play protective role in humans. It is compulsory to control biofilm because it acts as basic element for several persistent bacterial infections. Different techniques such as chemical, mechanical and biological (use of bacteriophages) are used to control and eradicate biofilms. This article provides an overview of formation, structure, importance and control strategies of biofilms.

Keywords

Biofilm,
Bacteria,
Infections,
Bioremediation,
Bacteriophages

Introduction

Biofilms are bacterial communities which are considered critical for the existence of bacteria in unfavorable conditions (Johnson, 2007). Major portion of biofilm consists of exopolysaccharide and small amount of other organic compounds. This slime layer provides safe environment for the growth of microorganisms (Rao *et al.*, 2005). Inside the biofilm bacteria become expansive and are capable to interact with each other via intracellular communication as result can tolerate the fluctuating environment (Clutterbuck *et al.*, 2007). Formation of biofilm is a complicated phenomenon in which genetic mechanisms and various other factors are involved. Among these factors characteristics of substratum and surface of bacterial cells are most important (Shi and Zhu, 2009). A number of bacterial infections are the result of biofilm formation and are problematic because bacteria in biofilm can tolerate immune defense mechanisms of host and show resistance to various

antibiotics (Hancock *et al.,* 2011). Formation of biofilm by bacteria during infection is beneficial to bacteria as it provides: (1) shielding against detrimental conditions in host (defense), (2) segregation towards nutrient rich area (colonization), (3) usage of different cooperative benefits (community), (4) biofilms normally grow as biofilms and planktonic cultures are an in vitro defects (biofilm as default mode of growth) (Jefferson, 2004). Different types of new strategies have been developed to control biofilm formation (Simo˜es *et al.,* 2010).

Biofilm

The concept of biofilm originates when in 1947, Antonie van Leuwenhoek, using his primitive but effective microscope found aggregates of 'animalcules' that he had scraped from human tooth surfaces (Costerton, 1999). Biofilm is defined as self-regulating group of bacteria which have colonized a surface (Hood and Zottola, 1995). Bacterial population is organized into a structural community, composed of self-created polymeric matrix. For the enhancement of their growth in the environment these are adhesive to inert and free living surfaces (Raza *et al.,* 2013). Complex communities of microorganisms developed on surfaces in different environments. The contamination of industrial pipelines, dental unit water lines, catheters, ventilators and medical implants by biofilm can cause disease in humans, animals and plants (Stoodley *et al.,* 2002). Microorganisms adhere to the surfaces and form an organized community which is fixed in exopolysaccharide matrix (Suntharalingam *et al.,* 2005). Surface attached factions of microorganisms growing embedded in a self-produced matrix of extracellular polymeric substances (O'Toole *et al.,* 2000).

Biofilm Formation

Biofilm formation occurs in a series of steps, such as formation of conditioning layer, bacterial adhesion, bacterial growth and biofilm maturation (kokare *et al.,* 2009). Production of extracellular microbial structures is the result of different steps of microbial interactions with surfaces. These structures help for the initial attachment, maintenance of biofilm structure and biofilm dispersal (Stoodley *et al.,* 2002). Biofilm formation is considered to start when bacteria sense environmental conditions that initiate the transition to life on a surface. Environmental signals that can affect the biofilm formation involve nutrient content, temperature, moisture, pH, iron and oxygen (O'Toole *et al.,* 2000). Cells that have dedicated to adhesion up regulate specific adhesion genes within a few minutes of their attachment to the surface. The attached bacterial cells synthesize new exopolysaccharide material in order to strengthen their adhesion to surface, and to other bacterial cells in the spreading biofilm, hence they progress from the reversible 'attachment' state to the irreversible 'adhesion' stepof biofilm formation (Costerton, 1999). It was proposed that Bap (Biofilm associated proteins) play a crucial role in the formation of biofilm. Primary attachment, intercellular aggregation and biofilm formation studies indicate that Bap aggrandizes both primary attachments to nonliving surfaces as well as intercellular adhesion. Such strains which have inadequate Bap are powerless for the formation of biofilm (Lassa and penades, 2006). There are two ways by which the Co-aggregation interactions contribute to the development of biofilms. (1) Single cells in suspension particularly concede and adhere to those cells in maturing biofilm which are genetically definite. (2) Prior Co-aggregation in suspension of secondary

colonizers followed by the ensuing adhesion of this co-aggregate to the growing biofilm. Co adhesion is the process in which bacteria in suspension (planktonic) form biofilm community (Rickard *et al.,* 2003). In response to population density, bacteria communicate with each other with in community by using different chemicals known as signal molecules; this process is called as quorum sensing QS (Heilmann and Gotz, 2010). Presence of macro-colonies with water channels embedded in it indicates the maturation of biofilm (Dufour *et al.,* 2012). Recent studies have shown that detachment, also called as 'dispersion' or 'dissolution' is a mechanism that is controlled by attached cell populations. *P. aeruginosa* produces an enzyme called alginate lyase, which dissolves the alginate matrix releasing cells in the surrounding environment (Lindsay *et al.,* 2006). There are different environmental factors such as temperature, Ph. and rheological and adhesive properties of biofilm have marked effect on biofilm development (Garrett *et al.,* 2008).

Biofilm Structure

The confocal scanning laser microscope (CSLM) has been effectively used to observe the biofilm development. It has revealed that biofilm is composed of primarily of micro colonies of different species of microbial cells (+15% by volume) and matrix material (+85%), (kokare *et al.,* 2009). These micro colonies form the basic structure of the biofilm (Costerton, 1999). Bacterial cells in biofilm micro colonies are held together by a slime-like matrix termed 'extracellular polymeric substances' or 'extracellular polysaccharides' which both abbreviate to EPS (Stoodley *et al.,* 2002). Genetic analysis showed that EPS plays a significant part in determining the architecture of biofilm (Branda, *et al.,* 2005). Much of the biofilm matrix (97%) consists of water; microbial cells are 2-5%, polysaccharides (1-2%), proteins (< 1-2%), DNA and RNA (1-2%) and some unknown amount of ions is also part of matrix. Water binding and mobility within the biofilm matrix are fundamental to the diffusion processes that occur within the biofilm that is why water is arranged within the healthy structure of biofilm (Sutherland, 2001). The water can be restricted within the capsules of microbial cells or can exist as a solvent and the solutes dissolved in it determine its physical properties such as viscosity. Bacterial cells present in the matrix are described distinctively by their absence of Brownian movement (Costerton, 1999). The number of cells in a biofilm may reach as high as $1.0 \_ 10^9$ cells per clump which can comprise an infectious dose of a pathogen (Anwar *et al.,* 2008).

Importance of bacterial biofilm

It has been stated that biofilms play an extensive role in the transmission and persistence of human disease. In humans, *V. cholera* biofilms in the GIT of patients suffering from cholera may be protected from acid pH and/or antibiotic, hence serving as a carrier for fecal-oral mode of transmission of the disease (Anwar *et al.,* 2008). Opportunistic bacteria become pathogenic when they organized into biofilm (Hansch, 2012). There are many biofilm related infections i.e. necrotizing fasciitis, osteomyelitis, biliary tract infections, native valve endocarditis and cystic fibrosis pneumonia (Costerton *et al.,* 1999). Medical devices also get contaminated due to biofilm formation. For example urinary catheters, central venous catheters, prosthetic heart valve and intra uterine devices (kokare *et al.,* 2009). Formation of biofilm on medical appliances has resulted to the characterization of a new infectious disease

known as chronic polymer-associated infection (Stoodley *et al.,* 2005). Biofilms act as foci for persistent infection (Stephens, 2002). Microorganisms associated with biofilm on indwelling devices include coagulase-negative *staphylococci, klebsiella pneumonia, pseudomonas aeruginosa, staphylococcus aureus and enterococcus spp*. (kokare *et al.,* 2008). Wound infections are also considered to be caused by biofilms of bacteria which develop over the surface and producing EPS that acts as a protective covering from host immune defenses and antibiotics (Lindsay and Holy 2006). Pathogens, such as *Salmonella* and *Escherichia coli*, are part of natural aquatic biofilms and become source of infection in immunocompetant humans (Costerton *et al.,* 2003). In the food industry biofilms cause solemn engineering problems such as blocking the flow of heat across a surface, increase in fluid frictional resistance of surfaces and increase in the corrosion rate of surfaces which leads to energy and production losses. Pathogenic micro floras that grow on food surfaces and in processing environments can cross-contaminate and cause post-processing contamination (Cheng *et al.,* 2007). Biofilms are also cause of bio fouling in different industrial units such as heat exchangers which increase the operational and maintenance costs (Eguia *et al.,* 2008).

Not all biofilms cause problems (Kumar and Anand, 1998). Bacterial biofilms play a protective role in humans. For example, in human gastrointestinal tract (GIT), biofilms consisting of mixed population of commensal bacteria attached to epithelial cells of gut form an impressive barrier against food borne pathogens (Lindsay and Holy, 2006). Biofilms play a major role in bioremediation of different pollutants such as chlorophenoles, azo dyes, herbicides and heavy metals such as nickel, zinc, cadmium and lead (Jain, 2006). Kurniawan and Yamamoto, 2012 studied the adsorption of lithium by biofilms.

Control of biofilm formation

It is difficult to control the biofilm development because microorganisms in biofilm evolve different mechanisms in different environment; however there are effective methods which are used to control the formation of biofilm. Anti-microbial agents (antibiotics, oxidants, and biocides), surface modifications and electro assisted methods can be used to control the bacterial adhesion (Hori and Matsumoto, 2009). Biofilm detectors such as mechatronic surface sensor check over the bacterial colonies and assist to control biofilm in early developmental stages (Simoes *et al.,* 2009). Bio fouling in industrial units such as heat exchangers is major problem and is controlled by using different chemical and mechanical methods. Ozonisation, chlorination, biocides, and synergetic chemical products are extensively used anti fouling chemical methods. Mechanical techniques include cleaning by brushes, filter systems, cleaning by abrasive balls, treatments by thermal shock (Eguı´a *et al.,* 2008). Role of quorum sensing in biofilm formation makes it a good target for the control of biofilm (Giaouris *et al.,* 2013). Chemical countermeasures such as, inhibition of AHL (N. acyl homoserine lactone, signaling molecule in quorum sensing of gram negative organisms), production, inhibition of AHL binding to receptors, inhibition of receptor auto association and inhibition of receptor- DNA interaction can be applied for inhibition of biofilm formation. Peptidic inhibitors interfere the quorum sensing and inhibit the biofilm formation in gram positive microorganisms (Musk and Hergenrother, 2006). A. K. Epstein et al describe the

control of bacterial biofilm growth on surfaces by nanostructural mechanics and geometry. Lechvalleir *et al.* (1988) showed the effect of disinfectants to inactivate the biofilm and concluded that monochloramine is better than free chlorine for the inactivation of biofilm. Kim *et al.,* (2008) compared the antimicrobial effect of chlorine, silver ion and tobramycin on biofilm and showed that antimicrobials cause different kinds of damage by their various kinds of reactivity. Use of transition metal catalysts such as cobalt phthalocyanine and copper phthalocyanine are incorporated into the material of target surface. These catalysts break peroxides and persulphates to release active oxygen species (McBain *et al.,* 2000). Antimicrobial lock technique (ALT) is applied to control bacterial biofilm on central venous catheters. In this technique antimicrobials with bactericidal are injected in catheters *in situe* at high concentrations (mg/ml) for sufficient interval of time. This technique has been tested by using many molecules including ethanol, chelating agents and taurolidine citrate and provides acceptable results for decreased incidence of biofilm on central venous catheters (Bordi and Bentzmann, 2011). Specifically targeted antimicrobial peptide (STAMP) technique can be used to eliminate disease associated organisms while bacteria associated with healthy flora are preserved. In this technique narrow spectrum molecules called as specifically targeted antimicrobial peptides (STAMPs) are used and have ability to bind with selected pathogen (Dufour *et al.,* 2011). Sambanthamoorthy *et al.* (2012) identify small molecules that antagonize Diguanylate Cyclase enzyme to inhibit biofilm formation. Use of bacteriophage (Biological control) is another strategy to control biofilm and related infections (Hanlon, 2007). Phages release enzymes which help to penetrate matrix and degrade the exopolysaccharide to destroy the biofilm (Azeredo and Sutherland, 2008). Hughes, Sutherland and Jones, (1998) revealed that phage borne depolymerase plays major role in biofilm removal.

In conclusion, studies discussed in this review have shown that bacteria colonized on the surfaces to adopt themselves in unfavorable conditions. A sequence of steps is involved in the development of biofilm. They produce exopolysaccharide as a major component of biofilm matrix which provides protection to the bacterial community. Biofilms play a major role in different types of persistent infections, food contamination and bio fouling in industrial. Strategies to control biofilm are still insufficient. Detailed understanding up to the level of genetics in the developing biofilm can help to improve the control strategies. However there is also positive aspect of biofilms in bioremediation. Application in this area is rare because it is still under-studied. Biofilms applications with respect to bioremediation can be exploited by gene transfer within the bacterial community.

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