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Bacterial Biofilms: Their Formation and Importance. A Review

KEYWORDS

Biofilms, matrix, autoinducer, infection.

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ABSTRACT *Biofilms are clusters of microorganisms that are form on inanimate objects or associated to host cells during infection. Biofilm structure contains an extracellular matrix excreted by the microbial cells and is a mixture of exopolysaccharide along with other components such as proteins and DNA. Intercellular communication also known as quorum sensing is critical in the development and maintenance of a biofilm. Quorum sensing is important in terms of pathogenicity and virulence of microorganisms which is often enhanced when growing as a biofilm. One of the major problem involving infections with biofilms is the drug resistance as they are highly resistant to killing by antibiotics. Biofilms are implicated in many health problems and have been found to be involved almost 80 percent of all infections.*

Introduction

Microbial diversity is an ongoing field requiring considerable research with so many microorganisms living in extreme environments. Dating back from the time of Robert Koch, the father of modern Microbiology, to the 1970s, bacteria were largely considered as single free-floating microorganisms. Through planktonic pure bacterial culture model, scientists have been able to study many deadly bacteria and developed biocides to kill such bacteria. However, emergence of drug-resistant bacteria led to a re-evaluation of the bacterial lifestyle and it is now acknowledged that the aggregation of bacteria within self-produced matrices, called biofilms, endows bacteria with mechanisms to resist biocides [1]. Biofilms are microbial communities which often live in cluster or as an organized layer of microorganisms associated with surfaces, often having complex structural and functional characteristics. Biofilm development on surfaces is a dynamic stepwise process involving adhesion, growth, motility and extracellular polysaccharide production. The nature of biofilm and the physiological state of bacterial cells within the biofilm confers high level of resistance to antimicrobial agents. Biofilms are highly important from medical point of view as they are responsible for 60 to 80 percent of the microbial infections in the body. With the emergence of biofilm associated diseases, there are considerable diagnostic problems for the clinical laboratory. These groups of bacteria carry significant medical and economic importance that includes a role in chronic diseases, antibiotic tolerance, biofouling, and waste-water treatment [2,3,4].

Structure of a biofilm

Biofilms constitute a protected mode of growth that allows survival of the microbial cells in a hostile and adverse environment. The bacteria in the biofilm are divided into different sub-populations, ranging from an almost spore-like sub-population to a more actively metabolizing microorganism population at the colony surface. The structures that form in biofilm contain channels in which nutrients can circulate and cells in different regions of the biofilm exhibit different patterns of gene expression [5,6].

There are numbers of bacterial organisms involves in biofilms formation. The main active biofilm formers are *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Bacillus subtilis.* The mechanisms that regulate biofilm formation vary greatly among different species, and even between strains. All biofilms contain an extracellular matrix that holds cells together. The matrix is actually an extracellular polymeric substances produced by the individual cells and is composed of an exopolysaccharide along with other components such as proteins and DNA. The extracellular substances are typically polymeric substances and commonly comprise of complex polysaccharides, proteinaceous substances and glycopeptides, lipids, lipopolysacharides and other materials that serve as a scaffold holding the biofilm together [7,8].

The extracellular matrix of biofilms also consists of adhesive proteins. *S. aureus* matrix contains biofilm associated proteins (Bap) that are required for biofilm formation [9]. These proteins are found anchored to the cell wall of *S. aureus* and function to hold cells together within the biofilm. Unlike *S. aureus* biofilms, *B. subtilis* expresses only a single major protein associated with the extracellular matrix known as TasA. Mutants who are deficient in TasA fail to form biofilms despite the fact that they can still produce exopolysaccharide [10]. In *E. coli,* the curli protein like TasA protein also forms amyloid filaments and is important for biofilm formation [11]. Other proteinaceous structures important for biofilm formation are pili and fimbriae. These are important for adherence of cells to each other or to different surfaces. *E. coli* produces Type I fimbriae that are required for adherence to mannose-containing receptors. These fimbriae are important for biofilm formation on plastic surfaces as well as on host cells during urinary tract infections [12,13]. *P. aeruginosa* also has many surface proteins that contribute to biofilm formation such as type IV pili and CupA fimbriae. In addition, there are other matrix-associated lectin-binding proteins that recognize and bind carbohydrate moieties. These facilitate cell-matrix or cell-cell interactions within the biofilm. In addition to the exopolysaccharides and proteins, extracellular DNA (eDNA) also provides structural integrity to the biofilm. Biofilm matrix in *P. aeruginosa* and *S. aureus* contains significant amounts of eDNA and functions to provide stability to the biofilms [14]. Due to the presence of multiple types of molecules such as polysaccharides, proteins, eDNA and several variations between matrices of different strains and species makes it impossible to find a main unifying attribute in biofilm feature or a single entity in biofilm matrix that could be targeted for biofilm control.

Formation of a biofilm

Bacterial biofilm begins to form when any bacterial cell initially binds to a surface. This depends on many factors such as temperature and pH, and on the genetic factors that encode functions, adhesions and other proteins. Biofilm formation process is also influenced by factors such as the nutritional environment [15,16,17]. Biofilm formation involves sequential stages such as initial attachment or reversible adsorption of the bacteria to the surface, an irreversible binding, a maturity phase with growth and division, the phase of exopolysaccharide production and the final development of the colony with

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dispersion of colonizing cells. The structure is permeabilized with a web of channel of water, bacterial residues, enzymes, nutrients, metabolites and oxygen [18].

Cell to cell communication

In recent studies researchers discovered that bacteria can communicate with one another, in a cell-to-cell signals and behave cooperatively as a unit within the biofilm. This process is known as quorum sensing or autoinduction [19]. This process is responsible for the expression of virulence factors and controls a large number of developmental processes included those related to biofilm formation. Quorum sensing has been found among both gram-negative and gram-positive bacteria and bacteria monitor their own population density through sensing the levels of signal molecules, sometimes called autoinducers because they can stimulate the cell that releases them. The concentration of these signal molecules increases along with the bacterial population until it rises to a specific threshold and signals the bacteria that the population density has reached a critical level or quorum. The bacteria then begin expressing sets of quorum-dependent genes [20,21].

In many other gram negative bacteria including *P. aeruginosa*, quorum-sensing systems respond to a class of autoinducer termed acyl homoserine lactones (AHLs) which are important in biofilm formation but vary according to bacterial strains. AHLs molecules diffuse into the target cell, once they reach a sufficiently high level, bind to special receptor proteins and trigger a conformational change. Usually the activated complexes act as inducers-that is, they bind to target sites on the DNA and stimulate transcription of quorum-sensitive genes. The gene needed to synthesize AHLs is also produced frequently, thus amplifying the effect by the production and release of more autoinducer molecules [22].

In gram-positive organisms, the autoinducers are often peptides and these are detected outside the cell. To be detected extracellularly, autoinducer molecules are generally sensed by membrane-associated sensor kinases, which activate cognate response regulators by phosphorylation. That, in turn, activates the expression of the target genes [23]. For *B. subtilis,* the production and secretion of the quorum-sensing molecule surfactin is important for biofilm formation. Aside from its surfactant properties, surfactin causes potassium leakage from the cytoplasm which is sensed by a membrane associated sensor kinase to specifically trigger the expression of the genes involved in extracellular matrix production [24]. In addition to quorum-sensing molecules, several other signals trigger biofilm formation. These include secondary metabolites such as antibiotics, pigments and other specific molecules. At sub-inhibitory concentrations many antibiotics such as imipenem, tobramycin etc. function not to kill cells, but rather as signals that trigger changes in gene expression [25]. Within biofilms, the pigment phenazine pyocyanin functions in extracellular electron transfer to generate energy for growth of the community in the biofilm [26].

Significant of biofilms

Drug resistance is becoming a major problem involving infections with biofilm. Biofilms are highly resistant to killing by antibiotics, it will require 10-1000 times greater than the concentrations needed to kill free-living or planktonic cells [27]. Biofilms and their associated complications have been found to be involved in up to 80 percent of all infections. They are implicated in many medical problems including urinary tract infections, catheter infections and middle-ear infections. Biofilms are present on the teeth of most human and animals as dental plaque, where they may cause tooth decay and gingivitis [28]. Biofilms are critical in ocular diseases because *Chlamydia, Staphylococcus* and other pathogens survive in ocular devices such contact lenses and in cleaning solutions causing many eye complications. There are also less common but more lethal biofilm associated infections such as endocarditis, cystic fibrosis infection and infections of permanent indwelling or implanted devices such as joint prostheses and heart valves [29,30]. Some orthopaedic devices may have *S. aureus* and *S. epidermidis* infections and once these devices are infected with the biofilm, it is very difficult to eliminate the biofilm merely by using antibiotics but often the device must be replaced. Bacterial biofilms are one of the main reasons for incipient cutaneous wound healing failure and reduce topical antibacterial efficiency in healing or treating infected skin wounds [31]. Microbial cells of biofilms are regularly sloughed off and this can have many consequences, for example, biofilms in a city's water distribution pipes can serve as a source of contamination after the water leaves a water treatment facility. Microbial biofilms are also a major problem in industry. They can cause fouling of equipments, contamination of products, long-term damage to water distribution facilities and other public utilities [32].

The main strategies used today in controlling biofilm formation are based on nutrient control of biofilm, pH control, antimicrobial agents and chemical agents. The strategy that combines a broad spectrum microbial repellent agent with a surface coating that impairs bacterial growth has been investigated. This could be obtained through the modification of the surface by antibacterial compound which reduce or prevent the biofilm formation by either inhibiting bacterial adhesion or killing bacterial cells which have adhered [33]. Furanones, a product of marine algae has shown promises as biofilm preventatives in tests on abiotic surfaces. New antimicrobial agents that can penetrate biofilms, as well as drugs that prevent biofilm formation by interfering with intercellular communication, are being developed [34].

Majority of microorganisms are able to form multicellular biofilm communities which are further composed of subpopulations of different microbial cells. This posed a great challenge for microbial ecologists and microbiologists alike in thorough understanding of their complex structure and various induction mechanisms. It will take several years of research to fully understand the total nature of biofilm, with many discrepancies arise among the mechanisms that induce biofilm formation in different species and many unexplainable mechanisms regarding formation and their relation to infectious processes.

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