CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Review Article

BIOFILM Formation: A Comprehensive Review

Protima Banerjee*, Madhulika Singh, Varsha Sharma

Department of Botany & Biotechnology, Sadhu Vaswani College, Bairagarh, Bhopal-462030, India.

ARTICLE INFO

Received: 18 Apr 2015
Accepted: 30 Apr 2015

ABSTRACT

The phenomenon of bacterial adhesion is an important phenomenon for those working within the pharmaceutical and healthcare sectors to consider. This is because many processes are centered on the removal of bacteria. The adhesion of bacteria to surfaces relates to such factors as surface charge, surface energy, and the characteristics of polymers on bacteria (leading to the formation of biofilms). The way in which bacterial cells adhere to surfaces, or within communities, is of great importance to pharmaceutical microbiologists. When describing bacterial adhesion one is simply describing one or more stages of biofilm development, neglecting the fact that the population may not reach maturity. This article provides an overview of bacterial adhesion and its chemistry.

Keywords: Adsorption; Bacterial adhesion; Biofilm; EPS; Substratum

1. INTRODUCTION

Biofilms were observed as early as 1674, when *Antonie van Leuwenhoek* used his primitive but effective microscope to describe aggregates of ‘‘animalcules” that he scraped from human tooth surfaces[1]. Biofilm formation occurs when free floating microorganisms attach themselves to a surface. They secrete extracellular polymers that provide a structural matrix and facilitate adhesion[2]. Bacterial adhesion has become a significant problem in industry and in the domicile, and much research has been done for deeper

Corresponding author *
Protima Banerjee, Department of Botany & Biotechnology, Sadhu Vaswani College, Bairagarh, Bhopal-462030, India
E mail – protimabanerjee1991@gmail.com

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved

understanding of the processes involved. A generic biological model of bacterial adhesion and population growth called the bacterial biofilm growth cycle, has been described and modified many times. The biofilm growth cycle encompasses bacterial adhesion at all levels, starting with the initial physical attraction of bacteria to a substrate, and ending with the eventual liberation of cell clusters from the biofilm matrix. [1] During the initial stage of biofilm formation (designated as ''early biofilm''), the adhesive properties of a collection of bacterial cells cause irreversible attachment to a colonizable surface. Once a biofilm is established and matures (designated as ''mature biofilm''), exopolymeric substances are produced, and the viscoelastic properties of the resultant matrix determine its structural integrity, resistance to stresses, and ease of dispersion. Since the biofilm forming ability of a bacterium has often been linked to persistence and virulence, a thorough understanding of how adhesion and viscoelasticity modulate biofilm establishment may be important for the proper design of control strategies. [3]

2. BIOFILM ADHESION

A biofilm adhesion has several properties such as; Adsorption, EPS, Attachment etc.

a) Adsorption is the interphase accumulation of cells from the bulk liquid directly on the substratum (surface of the material). Once a material is exposed to water, organic molecules begin to adsorb on its surface [4] called the conditioning film mainly composed of glycoproteins (subject to high turnover rate (not static).

b) EPS (extracellular polymeric substances) is a Pre-requisite for biofilm formation and binds the bacteria together to form the biofilm. The Sticking Efficiency Equation is as follows;

$$\Psi = \frac{\alpha}{T} \quad \text{..............(1)}$$

Where; Ψ= sticking efficiency, α = number of cells adsorbed onto substratum, T = number of cells transported to substratum

c) Attachment is the acquisition of cells from the bulk liquid by an existing biofilm.

In order to understand the process of attachment one must first examine the properties of both the substratum and the cell surface:

a) Substratum can be either very hydrophobic (Teflon) or hydrophilic (glass). Rougher and more hydrophobic materials will develop biofilms faster.

b) Cell surface should be flagella, pili, fimbriae, or glycocalyx may impact rate of microbial attachment[4].

These are important parameters in biofilm formation described above. Because a cell, once drawn to the substratum must combat the repulsive forces common for all materials. These appendages enable the cell to remain attached until better/more capable attachment mechanisms are set.

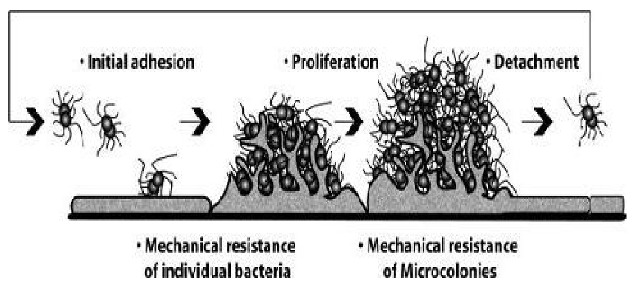

Fig 1: Showing Stages of Biofilm Formation

Mechanism of Bacterial Adhesion and Development

Biofilm growth is governed by a number of physical, chemical and biological processes. Attachment of a cell to a substrate is termed adhesion, and cell-to-cell attachment is termed cohesion. It is the mechanisms behind these forms of attachment, which ultimately determine the adhesive and cohesive properties a biofilm will exhibit.

The process of biofilm formation is complex, but generally recognized as consisting of five stages:

1. Development of a surface conditioning film.

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved

2. Movement of microorganisms into close proximity with the surface.
3. Adhesion (reversible and irreversible adhesion of microbes to the conditioned surface).
4. Growth and division of the organisms with the colonization of the surface, micro colony formation and biofilm formation; phenotype and genotype changes.
5. Biofilm cell detachment/dispersal.

The Conditioning Film

The conditioning layer is the foundation on which a biofilm grows, and can be composed of many particles, organic or inorganic. Anything that may be present within the bulk fluid can through gravitational force or movement of flow settle onto a substrate and become part of a conditioning layer. This layer modifies substrata facilitating accessibility to bacteria. Surface charge, potential and tensions can be altered favorably by the interactions between the conditioning layer and substrate. The substrate provides anchorage and nutrients augmenting growth of the bacterial community.

Reversible Adhesion

Initially, planktonic microbial cells are transported from bulk liquid to the conditioned surface either by physical forces or by bacterial appendages such as flagella. A fraction of the cells reaching the surface reversibly adsorbs. Factors such as available energy, surface functionality, bacterial orientation, temperature and pressure conditions, are local environmental variables which contribute to bacterial adhesion. If repulsive forces are greater than the attractive forces, the bacteria will detach from the surface. This is more likely to occur before conditioning of a substrate.

Irreversible Adhesion

In real time, a number of the reversibly adsorbed cells remain immobilised and become irreversibly adsorbed. It has been argued that the physical appendages of bacteria (flagella, fimbriae and pili) overcome the physical repulsive forces of the electrical double layer[6]. Subsequently, the appendages make contact with the bulk lattice of the conditioning layer stimulating chemical reactions such as oxidation and hydration[7] and consolidating the bacteria– surface bond. Some evidence has shown that microbial adhesion strongly depends on the hydrophobic–hydrophilic properties of interacting surfaces. [8]

Population Growth:

As the stationary cells divide (binary division), daughter cells spread outward and upward from the attachment point to form clusters. [9] Typically, such interactions and growth within the developing biofilm form into a mushroom-like structure. The mushroom structure is believed to allow the passage of nutrients to bacteria deep within a biofilm.

After an initial lag phase, a rapid increase in population is observed, otherwise described as the exponential growth phase. This depends on the nature of the environment, both physically and chemically. The rapid growth occurs at the expense of the surrounding nutrients from the bulk fluid and the substrate. At this stage the physical and chemical contribution to the initial attachment ends and the biological processes begin to dominate. Excretion of polysaccharide intercellular adhesion (PIA) polymers and the presence of divalent cations interact to form stronger bonding between cells. [10]

Final Stages of Biofilm Development:

The stationary phase of growth describes a phase where the rate of cell division equals the rate of cell death. At high cell concentration, a series of cell signalling mechanisms are employed by the biofilm, and this is collectively termed quorum sensing[11]. Quorum sensing describes a process where a number of auto inducers (chemical and peptide signals in high concentrations, e.g. homoserine lactones) are used to

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved

stimulate genetic expression of both mechanical and enzymatic processors of alginates, which form a fundamental part of the extracellular matrix. The death phase sees the breakdown of the biofilm. Enzymes are produced by the community itself which breakdown polysaccharides holding the biofilm together, actively releasing surface bacteria for colonisation of fresh substrates.

Adhesive Properties of Biofilms:

The matrix formed by EPS responds to stress by exhibiting,

1) Elastic tension due to a combination of polymeric entanglement, entropic, and weak hydrogen bonding forces.

2) Viscous damping due to polymeric friction and hydrogen bond breakage; and

3) Alignment of the polymers in the shear direction. [12]

Such properties change with increased temperature. Increasing the temperature of polysaccharides produces a gel-like substance which gradually increases in strength until a critical point is reached. At the critical point the gel forms a solution. [13] Such behavior affects the viscosity of the polysaccharides which can affect biofilm adherence.

3. CONCLUSION

A large amount of research work has been done and great achievements have been made in understanding the mechanisms of bacterial adhesion. However, since bacterial adhesion is a very complicated process affected by many factors, such as bacterial-material properties, environment, and, furthermore the experimental evaluation of the relative contributions of these factors is extremely difficult, more investigations are still needed to advance our understanding of the mechanisms of bacterial adhesion.

4. ACKNOWLEDGMENTS

This work was supported by the dept. of Botany and Biotechnology, Sadhu Vaswani College, Bairagarh, Bhopal (M.P.). We are thankful to Dr. Madhulika Singh and Dr. D.K. Dubey for providing facilities to carry out the above work.

5. REFERENCES

1. Garrett, TR., Bhakoo, M. and Zhang, Z. Bacterial adhesion and biofilms on surfaces. Progress in Natural Science 2008; 18(9):1049- 1056.
2. Chemistry of biofilm prevention. http://en.wikipedia.org/wiki/Chemistry_of_biofilm_prevention.
3. Lau Peter CY, Dutcher John R, Beveridge Terry J. and Lam Joseph S. Absolute quantitation of bacterial biofilm adhesion and viscoelasticity by microbead force spectroscopy. Biophysical Journal 2009; 96: 2935- 2948.
4. Donlan, Rodney M. Biofilm Formation: A clinically relevant microbiological process. Clinical Infectious Diseases. 2001; 33(8): 1387-392.
5. Hjortso Martin A, Joseph, W. Roos. Cell Adhesion: Fundamentals and Biotechnological Applications. New York: M.Dekker, 1995.
6. De Weger LA, van der Vlugt CIM, Wijfjes, AHM., et al. Flagella of a plant- growth stimulating Pseudomonas fluorescens strains are required for colonization of potato roots. J. Bacterioal. 1987; 169: 2769- 73.
7. Ganesh CK, Anand SK. Significance of microbial biofilms in food industry a review. Int. J. Food Microbiol. 1998; 42: 9-27.
8. Liu Y, Yang S and Xu H. et al., The influence of cell and substratum surface hydrophobicities on Microbial attachment. J. Biotechnol. 2004; 110:251-6.
9. Hall-Stoodley, P. Developmental regulation of microbial biofilms. Curr. Opin. Biotechnol. 2002; 13:228-33.

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved

10. Dunne WM. Bacterial Adhesion: seen any good biofilms lately? Clin Microbial Rev 2002; 15: 155-66.
11. Bassler BL. How bacteria talk to each other: Regulation of gene expression by quorem sensing. Curr Opin microbial 1999; 2:582-7.
12. Klapper I, Rupp CJ, Cargo R, et al., Viscoelastic fluid description of bacterial biofilm material properties. Biotechnol Bioeng 2002; 80: 289-96.
13. Villain-Simonnet, A., Milas, M. and Rinaudo, MA. New bacterial exopolysaccharide (YAS34) II. Influence of thermal treatments on the conformation and structure. Int J Biol Macromol 2000; 27:77-87.

IIIIIIII© International Journal of Pharma Research and Health Sciences. All rights reserved