Bacterial biofilms formation and quorum sensing

Food & Diet

Biofilms is a community of microorganisms irreversibly attached to a surface, producing extracellular polymeric substances (EPS), exhibiting an altered phenotype compared with corresponding planctonic cells, especially with reagard to gene transcription, and interacting with each other' (D. Lindsay, December 2006)

2. 1 Biofilm consist of three components:

Surface of attachment is a core factor for bacterial biofilm formation. It is conditioned by adsorption of organic and inorganic nutrients that influence subsequent bacterial attachment. (D. Lindsay, December 2006)

Extracellular polymeric substances (EPS) may account for 50 % to 90% of the total organic carbon of biofilms. It is also considered as the primary matrix material of the biofilm that serves as a storage facility for nutrients. (D. Lindsay, December 2006)

The bacteria are the best studied microorganism with respect to colonization of surfaces and subsequent biofilm formation. (D. Lindsay, December 2006)

The biofilm formation occurs in the following sequential phases:

Figure 1: The sequential phases of biofilm formation (Paul Stoodley, 2003)

2. 2 Attachment- during the attachment free-swimming bacterial cells settles on surface, arrange in clusters and attach.

Reversible Attachment – the weakest link in the formation of biofilms. Reversible attachment of bacterial cells to a surface can occur by sedimentation and Browian Motion (the random motion of micro particles

suspended in liquid) of microbial cells; movement by motile bacteria, or electrostatic and physical interactions between the bacterial cell surface and microorganism itself. (D. Lindsay, December 2006)

Irreversible attachment – after reversible attachment to the surface bacterial cells produce EPS ‘ due to stimulation of membrane bound sensory proteins of the bacterial cell, which allows for the development of cell-to-cell bridges that in turn, cement the cells to the surface’ (Donlan, 2002)

2. 3 Colonization (growth) and Quorum Sensing

The surface colonization is the final phase in the formation of biofilms. ‘ Attached bacteria grow and divide, forming microcolonies that are considered to b the basic organizational units of a biofim.’ (Costerton et al., 1994)

2. 4 Quorum Sensing

The process of the biofilm formation becomes interesting during the phase of colonization. Microorganisms within the biofilm can communicate through the phenomenon called quorum sensing.

‘ Quorum sensing- is a mechanism in which bacteria coordinate the expression of certain genes in response to their population density by producing, releasing and detecting small signal molecules. This mechanism was 1st discovered in the marine bacterium Vibrio fisher and was thought to be restricted to only a limited series of species. Later on, similar system was found to be present in many other bacteria. Biofilm formation has been recognised to be a quorum-sensing-regulated phenomenon for an ever-

increasing number of bacteria.’ (Defoirdt et al., 27 October 2004) This phenomenon allows recognizing that there is dense population of other bacterial cells ‘ it is more inclined to join them and contribute to the formation of a biofilm’. (A. Proal, 26 may 2008)

According to Deborah A. Hogan quorum sensing in bacteria is mediated by small diffusible signalling molecules that accumulate in the extracellular environment. (D. A. Hogan, Apr. 2006) Signal export involve: ‘ Passive diffusion across the membrane, the action of efflux pump and specific transporters’. (J. P et al., 1999). When a signal is sufficiently high the cognate regulator is activated within the local population of cells, leading to coordinated gene expression. (D. A. Hogan, Apr. 2006)

AHL-mediated quorum sensing ïƒ the I protein is the AHL synthase enzyme. , The AHL molecules diffuse through the plasma membrane. As bacterial cells density increases, the AHL concentration increases as well and once a critical concentration has been reached, AHL binds to the R protein, a response regulator. The AHL-R protein complex activates or inactivates transcription of the target genes.’ (Defoirdt et al., 27 October 2004)

Peptide-mediated quorum sensing in Gram-positive bacteria ïƒ ‘ a peptide signal (PS) precursor protein is cleaved releasing the signal molecule. The peptide signal is transported out of the cell by an ATP binding cassette (ABC) transporter. Once a critical extracellular peptide signal concentration is reached a sensor kinase (SK) protein is activated to phosphorylate the response regulator (RR). The phosphorylated response regulator activates transcription of the target genes’ (Defoirdt et al., 27 October 2004)

Some bacteria (e. g. marine bacterium Vibrio harveyi) possess two quorum sensing systems. (Miller et al., 2002)

Quorum sensing in Vibrio harveyi ïƒ there is two types of signal molecules. ‘ AI-1 is and AHL and its biosynthesis is catalysed by the luxLM enzyme. AI-2 is a furanosyl borate diester; its biosynthesis is catalysed by the LuxS enzyme. AI-1 and AI-2 are detected at the cell surface by the LuxN and LuxP-LuxQ receptor proteins, respectively. At low cell density, LuxN and LuxQ autophosphorylate and transfer phosphate to LuxO via LuxU. The phosphorulated LuxO is an active repressor for the target genes. At high cell density, LuxN and LuxQ interact with their autoinducers and change from kinases to posphatases that drain phosphate away from LuxO via LuxU. The dephosphorylated LuxO is inactive. Subsequently, transcription of the target genes is activated by LuxR’ (Defoirdt et al., 27 October 2004)

2. 5 Detachment

D, Lindsay and A. Von Holy studies suggested that detachment is an active process that is regulated by the attached cell population. There are several strategies of how biofilm bacteria disseminate into other areas for further surface colonization. (D. Lindsay, December 2006):

Cells located at the periphery of the biofilm are released into the surrounding environment and return to the planctonic state. , This strategy is adopted by P. aeruginosa, which produces and alginate enzyme that dissolves the alginate matrix, releasing cells into surrounding environment, (D. Lindsay, December 2006)

‘ Bacteria may alter various surface components, such as glycolipids, peptidolipids, lipopolysaccharides, which may alter cell surface hydrophobicity, facilitating reliase of a surface bound cell e. g. E. coli and P. aeruginosa cells de-adhere from biofilms by increasing their cell surface hydrophobicity.’ (D. Lindsay, December 2006)

Biofilm can be made up of single or multiple species. According to K. D. Xu it is estimated that dental biofilms contain more than 500 different bacterial species; conversely , in latter stages of the disease, the primary bacterium in the lungs of Cyctic Fibrosis patients is P. aeruginosa. (Xu, 2000) While mixed-species biofilms predominate in nature, single species biofilms can be found in clinical settings, including medical implants. (Yi et al., Oct 2004)

Antibiotic Resistance and changes in biofilms mode of growth

The resistance of bacterial biofilms to antibiotics is higher compared with planctonic cells. Biofilms existing cells become 10-100 times more resistant to the antimicrobial agents. (Xu, 2000)

According to Philip S. Stewart and J. William Costerton the mechanism of antimicrobial resistance, such as efflux pumps, modifying enzymes, and target mutations, do not responsible for the protection of bacterial biofilms. (Stewart & Costerton, 14 July 2001)