



## BIOFILMS

Janus A. J. Haagensen and Søren Molin

BioCentrum-DTU, Centre for Biomedical Microbiology, Building 301, Technical University of Denmark, DK-2800 Lyngby, Denmark, JAJH@BioCentrum.dtu.dk

Surface associated microbial biofilms represent the most wide-spread mode of appearance of bacteria and is an important way of persisting in the environment. Bacteria are able to colonize all kinds of different surfaces and are often found on plants and higher living organisms but also in industrial settings and on medical devices.

The architecture of biofilms and the structural relationship between different parts of the biofilm in relation to the functionality of the microbial consortium are important parameters to investigate. But also biofilm reactions and persistence when treated with anti-microbial agents are addressed and of high interest. The biofilm mode of growth facilitates proliferation of the attached cells because of nutrient availability compared to many aqueous environments. It makes the cells able to enclose in a protected matrix of polymers and the surface growth also seems to enable the cells to adapt to still changing conditions like nutrient and oxygen gradients, competition from other species, predators and invaders.

Studies of biofilms living as heterogeneous assemblies on surfaces have been facilitated by employing molecular tools in combination with advanced fluorescence microscopy. But also techniques like Atomic force microscopy, micro arrays and cell sorting using FACS are today important parts of biofilm investigations.

Determinations of the identity and activity of individual cells are achievable by use of FISH and molecular tagged reporter systems. Inspections by scanning confocal laser microscopy followed by image analysis generate spatial visualizations, providing important information about community heterogeneity and functional domains.

Interactions between *Pseudomonas* and *Acinetobacter* growing in flow chambers were investigated and resulted in classification of consortium performance and development. In parallel investigations the *Acinetobacter* biofilm structural development was revealed by time lapse recording and quantification.

Bacteria living as surface attached cells in biofilms can be more tolerant to various anti-microbial agents than their planktonic counterparts. This apparent change in phenotype leads to severe treatment problems in many cases of persistent or chronic infections, and it is therefore important to understand the cause(s) of this.

This issue was addressed by initiating an approach, aiming at identifying specific resistant subpopulations in microbial consortia with *Pseudomonas aeruginosa* and *Escherichia coli*, two important pathogens with potential

for persistent biofilm conditioned infections. But also samples from different cystic fibrosis patients have been introduced in these studies.

The major strategy has been to localize live and dead cells in the biofilms through the use of live/dead staining and monitoring by confocal microscopy after treatment of the biofilms with antimicrobial agents.

A slightly different part of the biofilm investigations have shown that induced biofilm development result in improved conditions for plasmid transfer, a relationship between plasmid conjugation and biofilms. The presence of actively conjugating plasmids in *E. coli* cells induce biofilm development, which is coupled to the synthesis of conjugation pili attached to the bacterial cell surface. *E. coli* development and maturation was investigated with respect to cell to cell adhesion factors resulting in differently structured biofilms depending on the adhesive properties on the cell surface.