Effect of micro-structured environments on bacterial surface motility, architecture and gene transfer in biofilms

Abstract

In nature, growth within a biofilm is a prevailing lifestyle for bacteria as it provides *many advantages* to *bacterial* cells. A biofilm is a community of microbes embedded in a matrix composed of secreted polymers. Biofilms are considered a hotspot for horizontal gene transfer and development of multidrug resistance. Proximity to neighboring cells most likely facilitates gene transfer and adaptation. Many bacterial species, including the human pathogen *Neisseria gonorrhoeae*, are able to form biofilms on abiotic and biotic surfaces. *N. gonorrhoeae*, in particular, is continuously competent for DNA uptake and its biofilm matrix has been shown to contain a large amount of extracellular DNA, an important structural component of many bacterial biofilms. However, it is not known to which extent extracellular DNA is used for gene transfer within biofilms, at which rate genes are exchanged or which factors affect the exchange rate.

In the first part of this dissertation, I investigated the spatial and temporal dynamics of multidrug resistance gene transfer in biofilms formed by the human pathogen *N. gonorrhoeae*. We designed an assay that allows direct visualization of double-drug resistance acquisition by gene transfer between two gonococcal strains each carrying a single resistance gene. We showed that gene transfer through transformation is efficient at various stages of biofilm development but the rate of gene transfer was strongly reduced after 24 h. Type IV pilus antigenic variation was shown to reduce the fraction of transformants in early biofilms. We also investigated the impact of surface topography on biofilm formation and the role of biofilm architecture on the rate of gene spreading in *N. gonorrhoeae*, using microtopographies. Micropatterning of the adhesive surface influenced the biofilm architecture, reducing biomass and increasing biofilm roughness. While the rate of gene transfer was independent of biofilm architecture, the spreading of double-resistant clones was severely affected. When selective pressure was applied to dense biofilms using antibiotics at their MIC, double-resistant bacteria had no significant growth advantage. In loosely-connected biofilms under the same antibiotic conditions, the spreading of double-resistant

clones was prominent. We conclude that multidrug resistance readily develops in gonococcal biofilms through horizontal gene transfer. However, spreading of multiresistant clones is heavily suppressed in dense biofilms.

The ability to move to more favorable environments is an essential property for bacteria. Extension and retraction of type IV pili drives a form of surface motility, named twitching motility, which is involved in biofilm formation. T4P dynamics and twitching motility are regulated by oxygen availability and cellular energy depletion. In the second part of this thesis, we designed, calibrated, and characterized a gonococcal strain expressing the ratiometric green fluorescent protein (GFP) derivative (*pHluorin*). This work was part of a study to understand the effect of proton motive force on gonococcal motility. The measurement of internal pH at varying external pH values showed that homeostasis in *N. gonorrhoeae* is remarkably poor as compared to other bacterial species.

The natural environment of bacteria is not flat and homogenous but consists of rather complex structures and heterogeneous substrates. To gain insight into control mechanisms of bacterial motility, it is useful to investigate how bacteria sense their environment and behave in confined heterogeneous geometries. T4P *driven motility is also employed* by rod-shaped, social bacterium *Myxococcus xanthus.* In the third part of the thesis, we investigated the motility of rod-like *M. xanthus* and found that it was confined to grooves, comparable to the size of bacteria. The movement of *M. xanthus* in the grooves was shown to be more efficient in comparison to the flat surface. The motile behavior of *M. xanthus* on patterned surface suggests that bacteria can sense the topography of a surface and their movements are guided by microscopic elevations.

In summary, we have shown that surface topography governs type IV pilus driven motility, biofilm architecture, and population dynamics. It will be interesting to *determine* molecular mechanisms that regulate biofilm formation and interactions within biofilm, including gene transfer, which may provide strategies to solve problems related to biofilm formation.