Abstract

The objectives of this thesis are the application of Attenuated Total Reflectance Fourier Transform Infrared (ATR FT-IR) spectroscopy for the analysis of *Pseudomonas syringae* biofilms grown in a flow cell and its subsequent interaction with antibacterial agents. Based on the combination of the recorded ATR spectra with the biofilm morphology analysis via light and fluorescent microscopy, I suggested a model for the development of *Pseudomonas syringae* pathovar *morsprunorum* CCM 2534 biofilm in a flow cell with 2% Luria-Bertani (LB) complex medium. This model consists of the initial colonization of the ZnSe prism (adhesion), subsequent washing of weakly attached cells due to the replacement of the inoculation suspension with the fresh 2% LB medium, which is followed by a brief substrate recolonization phase. Subsequently, the biofilm undergoes a restructuration with a motile subpopulation of *Pseudomonas syringae* migrating on top of already existing early microcolonies, where the availability of nutrients including oxygen is higher. The last developmental stage, a maturation of *Pseudomonas syringae* biofilms, is occurring predominantly outside the ATR FT-IR detection range and leads to a formation of large mushroom-shaped microcolonies. I also investigated the changes to the development of *Pseudomonas syringae* biofilm during model conditions (inoculation with dead bacteria, bottom-up inoculation, and altered initial biomass) in this work. Finally, I investigated the interaction of *Pseudomonas syringae* biofilm with antibacterial agents (LEGO-lipophosphonoxins, copper nanoparticles, and $CuSO_4$). For example, even the subinhibitory concentration of $CuSO_4$ (15 μM) was revealed to affect the development of *Pseudomonas syringae* biofilm, while the high $CuSO_4$ concentration (200 μM) kills *Pseudomonas syringae* biofilm, but does not mediate fast removal of its remains from the substrate. Overall, the ATR FT-IR technique was confirmed to be great for the investigation of the initial stages of *Pseudomonas syringae* biofilm development, however its limited detection range fails to capture the changes within the highly structured biofilm of this bacterium. I also explored the application of the impedance measurements for a simultaneous dual-sensing investigation of *Pseudomonas syringae* biofilm with ATR FT-IR and impedance spectroscopy to compensate for the limitations of ATR FT-IR. The preliminary results seemed promising for the future investigation of *Pseudomonas syringae* biofilm with interdigital electrodes and/or with integrated dual-sensing systems.

Key words: Biofilm, *Pseudomonas syringae*, FT-IR spectroscopy, antibacterial agents