ABSTRACT

Microorganisms grow in planktonic form, but more often they adhere to a number of surfaces and create three-dimensional structures called biofilms. Floating biofilms, which are formed at the water-air interface, are one of the life strategies, which the bacteria can take. Non-pathogenic *Mycobacterium smegmatis* was used as a laboratory model for the study of this kind of biofilm. The understanding of mechanisms of their formation of this species may be applicable to the pathogenic species of the genus *Mycobacterium*, study of which in the laboratory brings a number of disadvantages.

This diploma thesis focuses on the morphological and proteome analysis of the *M. smegmatis* floating biofilm. Using a stereo microscope and scanning electron microscopy was observed that bacteria clump and create the „nucleation centres“ at the beginning of the biofilm development. This centers grow to the surroundings and connect afterwards. In the later stages of the development the centers fuse in compact layer, which then grows into the compact and multilayer biofilm.

The key method in this study was two-dimensional electrophoresis of proteins. The proteome analysis of floating biofilm was performed with this method. The preparation of protein samples and the method for protein concentration measurement was optimized. We focused on the study of 4 developmental stages of floating biofilm after 8, 18, 24 and 30 hours of cultivation as in the morphological analysis. As a result of 2D electrophoretic analyses there was determinated 22 proteins as important in this process. Four of them were unique for the specific developmental stage and the expression of 18 remaining was changing in time significantly. For 18 changing proteins, their expression profiles were described and grouped into sets characterized by the same expression profile and possibly the same or similar regulation of their expression. The identification of these proteins by mass spectrometry is under way.