ABSTRACT

Persistence represents a transient state during which are bacterial cells able to survive antibiotic treatment. Only a small subpopulation of cells enter this state, these cells are then capable of causing disease recurrence. The size of a persister subpopulation is influenced by adaptive mutations formed during a chronic disease in genes related to transition of cells between a virulent form and biofilm formation. Regulation of entering and exiting persistence is also influenced by intercellular communication and by molecules produced into the cell surroundings. It is assumed that this effect is caused by PSM modulines and AIP molecules which take part in Quorum sensing mechanism.

The main aim of this thesis was to clarify how adaptive mutations of clinical isolates obtained from two patients diagnosed with cystic fibrosis influence the ability to persist. Another aim was to determine changes in membrane potential and metabolic aktivity while entering persistence and to establish the effect of extracellular molecules produced into the culture medium on growth parameters of the studied isolates.

By using techniques of TD test and establishment of persistence rate by CFU determination, it was found that the isolates obtained from patient 2 did not acquire adaptive mutations affecting persistence whereas three last obtained chronological isolates from patient 1 showed increased ability to persist. These isolates formed deeper persisters with reduced transition into a growing form. By measuring cell populations using flow cytometer, differences within membrane and redox potential between antibiotics with diverse mechanisms of action were confirmed. A method of continuous measurement of optical density was successfully optimized due to which it was possible to demonstrate the effect of extracellular molecules produced into a sterile supernatant on growth rate, the slope of killing curve and on waking up from a state of persistence.