DESCRIPTION FOR THE GENERAL PUBLIC

Oxygen is used for cellular respiration and the production of energy. Some of the enzymes performing this process may produce dangerous by-products – for example reactive oxygen species (ROS). To survive and prevent self-destruction, each bacterial cell needs to use advanced machinery. Antioxidants and specific enzymes serve as a first-line defence system. Apart from that, bacteria need response regulators that will detect ROS and then promote the production of enzymes responsible for the neutralization of ROS. If those mechanisms fail, bacteria are able to activate proteins responsible for the repair of ROS-induced damage to proteins and genetic material.

Despite the fact that most of bacteria have advanced systems for ROS neutralization and repair of ROS-induced damage, many antimicrobial therapies act through the generation of ROS. Application of ROS in concentrations higher than concentrations present in the environment leads to severe damage to bacterial cells and finally to its death. This is why ROS are produced by immune system cells when pathogenic bacteria attack the human body, by some of antibiotics, by cold atmospheric plasma and also by photodynamic inactivation.

Antimicrobial Photodynamic Inactivation (aPDI) is one of the proposed therapies which provide an alternative solution for antibiotic resistance in bacteria. It requires the simultaneous presence of compounds sensitive to light, visible light and oxygen. Because we can control a source of light, ROS are produced only at the site of infection, which is one of aPDI advantages. Another advantage is that resistance to aPDI has not been observed yet. However, recently, my research group has described the development of tolerance to aPDI in *Streptococcus agalactiae*. Tolerance can be defined as an intermediate state between susceptibility and resistance.

Streptococcus agalactiae is a leading source of invasive infections in newborns, but recently also in pregnant women, the elderly and immune-compromised patients. While our team was testing if aPDI could be an alternative to antibiotics, we observed that with the treatment of successive cycles of small doses of aPDI, the aPDI tolerance development occurred. Tolerant bacteria can still be killed when more severe conditions are applied. But because we observed tolerance, we must be aware, that it finally may lead to the development of resistance.

In this project, I want to investigate which genes are necessary for the development of tolerance to ROS produced by aPDI.

First, with the high-throughput method (RNASeq) I will examine which genes are stimulated or inhibited in aPDI process. Then I will choose 60 genes, which stimulation or inhibition I will examine in more experimental conditions with a more specific method (qPCR). This way I will narrow down the pule of genes to 10-20. Then I will cut out the genes that were affected the most. In each bacterial strain, I will cut out one gene. Then I will treat strains constructed by me with successive cycles of small doses of aPDI and check if some of the strains will develop tolerance to ROS produced by aPDI. If some strains will not develop tolerance to ROS it will mean that genes, which I cut out, were of high importance for bacteria to deal with damage caused by ROS. Moreover, I plan to characterize cut genes i.e. examine if these genes take part in biofilm formation.

I will also use an alternative approach for the selection of genes of interest. I will use the already-prepared library of mutants with mutations at random places. I will treat them with aPDI and look for those, which have higher sensitivity to aPDI. Then with the use of sequencing, I will be able to identify the gene that was mutated in each of the sensitive strains. Then I will follow with successive cycles of small doses of aPDI treatment as in the previous approach. Using both approaches will allow me to identify with higher certainty the genes responsible for response to oxidative stress.

Identification of highly important genes in the process of tolerance development may lead to the discovery of some compounds, which will prevent the development of tolerance, or at least slow it down. This is the first time when someone decided to research this process in this bacterial species. Publication of obtained results will help to understand the mechanisms, which bacteria use to avoid being killed by ROS. This will be of high importance for all antibacterial therapies whose mode of action is based on oxygen properties.