DESCRIPTION FOR THE GENERAL PUBLIC

The ability to sense and respond to environmental conditions is especially important for the survival of unicellular organisms, such as bacteria. In many microorganisms the adaptation is possible due to two-component systems (TCSs) and signaling transduction. The general mechanism of signaling transduction is as follows: in response to external stimulus (called first messenger), specific cyclic nucleotides (second messengers) are activated or degraded, thereby amplifying the signal. This initiates a cascade of reactions in which the signal is finally transmitted to the effector protein which interact with target elements to promote a variety of responses, e.g. expression of genes that are useful in a given environment. The common feature of TCSs and signaling transduction is signal transport in response to external changes, although in TCSs the transmitted molecule is a phosphate group. The classic TCSs are composed of histidine kinase (HK) and response regulator (RR). In response to an external stimuli autophosphorylation of a conserved histidine residue of HK occurs, followed by transfer of a phosphate group to the RR aspartic acid residue. Nowadays, reports are increasingly appearing that RRs can be phosphorylated at other amino acid residues, such as serine, threonine or tyrosine. This alternative phosphorylation occurs with involvement of kinases other than HKs, including a member of the recently discovered Ubiquitous bacterial Kinase family. Both canonical and alternative phosphorylation of RR can cause its activation, enabling regulation of gene expression. As a result, TCSs regulate the ability to survive under environmental stress, e.g. by regulating genes involved in biofilm or spore formation, as well as regulating genes related to pathogenicity.

The described phenomena occur in the bacterium *Tannerella forsythia*, which is one of the pathogens associated with the occurrence of periodontitis. We predict that $PorX_{Tf}$ protein, which is the main subject of the planned research, is involved in both processes in this bacterium. It is very likely that the protein functions as the RR which together with the unknown HK creates a TCS involved in regulation of Type 9 Secretion System (T9SS). *T. forsythia* uses T9SS to transport proteins from the cytoplasm, across periplasm, to the outer membrane of the cell. Among the transported proteins of the bacterium are virulence factors that help bacteria to effectively colonize gingival pockets and attack host cells. In the light of reports of alternative activation routes and close proximity of the genes, we would like to verify whether $UbK1_{Tf}$ phosphorylates $PorX_{Tf}$. Moreover, we speculate that $PorX_{Tf}$ may act as a negative regulator in signaling pathways by hydrolysis of second messengers released in response to external stimuli. For this purpose, we will check the enzymatic activity of $PorX_{Tf}$ towards comprehensive set of nucleotides available in our laboratory. The second objective is investigation if $PorX_{Tf}$ is the RR involved in regulation of T9SS of *T. forsythia*. For this purpose, we will check the phenotypes of mutants containing mutations in the sequence of $PorX_{Tf}$. Lastly, we will examine if $UbK1_{Tf}$ phosphorylates $PorX_{Tf}$ and how it affects the enzymatic activity of the protein.

Periodontitis is not only a dental problem, which in severe cases leads to bone resorption and tooth loss, but is also associated with an increased risk of disease such as cardiovascular disease, diabetes, pneumonia and Alzheimer's disease. Periodontitis affects 20-50% of the world's population, with as much as 11% of this population suffering from severe periodontitis. Due to the fact that the bacterium possesses many virulence factors and exploit complex pathogenicity mechanisms that are still not fully understood, finding an effective treatment is a challenge. Current treatments include the use of antibiotics, regular removal of plaque from the tooth surface and, in severe cases, surgery. It is estimated that the direct costs of treatment in Europe are around EUR 2.5 billion, and the total costs, including the indirect costs resulting from the decline in productivity of patients, can be more than EUR 150 billion per year. Such a huge cost and increased risk of developing other diseases make the disease a burden not only for the patient, but also for society. Therefore, the results obtained during the project will expand the knowledge about the mechanism of virulence factors secretion in *T. forsythia*, regulation of the process and the regulation of signal transduction. Since the processes are vital for adaptation of the bacterium to environmental changes, the obtained results might allow to find an effective method of treating periodontal diseases.