DESCRIPTION FOR THE GENERAL PUBLIC

Two-component regulatory systems (TCSs) serve as basic mechanisms that allow microorganisms to sense and respond to changes in many different environmental conditions. They typically consist of a histidine kinase (HK) that senses a specific environmental stimulus and a corresponding response regulator (RR) that mediates the cellular response, mostly through differential expression of target genes. Communication between the HK and RR is achieved by phosphotransfer from a highly conserved histidine residue in the sensor's transmitter domain, which is autophosphorylated in the presence of the appropriate stimulus, to an aspartic acid residue in the N-terminal receiver domain of the RR. Phosphorylation of the receiver domain then triggers a conformational change of the response regulator, which activates its C-terminal output domain. TCSs enable integration of multiple vital functions from basic metabolic regulation such as carbon or nitrogen use, phosphate assimilation and aerobic or anaerobic growth to specialized and complex developmental responses, such as formation of spores and biofilms. In stark contrast to the majority of microorganisms that have on average 52 TCSs, the genome of bacterium *Porphyromonas gingivalis* encodes only 12 proteins, which can be involved in TCS regulatory pathways. *P. gingivalis* is a late colonizer of a dental plaque and a main pathogen involved in development and progression of chronic periodontitis, which in severe cases may result in tooth loss. Periodontal infection is also associated with systemic diseases such as cardiovascular disease, osteoporosis, preterm/low birth weight babies, respiratory diseases, rheumatoid arthritis, and diabetes. Among a broad array of virulence factors secreted by *P. gingivalis*, proteases called gingipains are considered to be major virulence determinants involved in periodontal pathogenesis. Using gingipains, *P. gingivalis* is able to turn the host innate immune response to its own benefit. Gingipains provide also an important proteolytic tool for the generation of nutrients essential for growth. Taking into account that *P. gingivalis* incurs a high national burden in term of cost of treatment, it is perplexing that the molecular mechanism of pathogenicity of this bacterium is still far away from being fully understood.

Secretion of gingipains via Type 9 Secretion System (T9SS) seems to be regulated by recently discovered TCS called PorXY. To date, very little is known about this system. In order to shed more light on the regulation of gingipains secretion we want to characterize the PorX and PorY proteins apparently participating in this process. First, we will determine the interaction between these two components. Next, we will check the effect of different mutations within the catalytic domains of PorY and PorX on *P. gingivalis* growth rate and gingipains secretion. To shed more light on the mechanism of PorXY TCS action, the subcellular localization of PorX, PorY and sensor region of PorY will be determined. Finally, we will try to optimize crystallization conditions of the purified recombinant proteins and obtain preliminary diffraction data. Obtained results will allow determination of crystals quality and their usefulness in solving the protein structure in the future. Together, the impact of this project will be biochemical, functional and structural characterization of unique TCS known as PorXY. It should be stressed that the knowledge about the mechanism of virulence factors secretion in *P. gingivalis*, as well as regulation of this process, could be very useful and contribute to development of potentially therapeutic compounds attenuating virulence of *P. gingivalis.* Moreover, the project results may contribute not only to expand the knowledge about the pathogenicity of *P. gingivalis* but also about the signal transduction and virulence factors secretion in microorganisms. It is because, during the project we will characterize the unique DNA-binding domain, novel sensor domain of HK and the mechanism, that triggers the T9SS genes expression.