1. Aim of research: Genes encoding peptidases, enzymes hydrolysing peptide bonds, by average constitute approximately 3% of all genes in prokaryotes. Therefore, it is not surprising that bacterial peptidases play a key role in processes such as degradation of unwanted proteins, nutrients acquisition, proteolytic modification of proteins and their precursors and regulation of gene expression or they are virulence factors of human pathogens. One example of disease, in which aetiology bacterial proteases play a crucial role, is periodontitis. Periodontitis is arguably the most prevalent infection-driven chronic inflammatory disease affecting, in its severe forms, even 15% of adults. If untreated, periodontitis may not only lead to teeth loss but also contribute to progression and/or development of systemic diseases such as rheumatoid arthritis, diabetes, neurodegenerative diseases, and cardiovascular diseases. Although the aetiology of periodontitis is multifactorial, three bacterial species: *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, forming so-called "red complex", are thought to be crucial periodontopathogens. A characteristic feature of "red complex" bacteria is a very strong extracellular proteolytic activity, which is thought to play a central role in the virulence of these pathogens. The role of *P. gingivalis* and *T. denticola* secretory proteases in the pathogenesis of periodontitis is characterized in details. Despite the importance of proteases in virulence of "red complex" bacteria, until recently our knowledge regarding secretory proteases from *T. forsythia* have been still very limited. The situation has changed dramatically with the discovery by our research team of a novel family of six secretory proteases of *T. forsythia*, referred to as KLIKK proteases. However, despite a growing body of evidence, both published and preliminary, clearly indicating unique biochemical and structural properties and a possible role of KLIKK proteases in the virulence of *T. forsythia* through manipulation of innate immunity, they are still grossly under-investigated in comparison to proteases of other periodontopathogens. *Therefore, in the proposed project, we plan not only to provide a molecular explanation of unique features KLIKK proteases but also evaluate their role as possible virulence factors of* T. forsythia.

2. Description of research: To achieve it, KLIKK proteases will be obtained employing a bacterial expression system. First, the substrate specificity of the enzymes will be characterized in details. In the next step, we will solve the crystal structures of KLIKK proteases. Briefly, crystals of investigated proteins will be obtained and then the crystal structures will be solved based on results from diffraction of X-ray by the crystals. The main aim of the crystallographic analysis is to provide a molecular explanation for KLIKK protease unique substrate specificity and mechanism of latency (how the enzymes are kept in the inactive form before secretion outside the cell). We will also check if the KLIKK proteases are involved in the manipulation of innate immunity not only by *T. forsythia*. To fulfil it we will use single KLIKK protease deletion mutants of *T. forsythia* and then we will compare resistance to killing by innate immunity of the mutants with wild-type *T. forsythia*. Based on these results, we will try to find a crucial KLIKK protease for protection of *T. forsythia* against innate immunity. We will also check if KLIKK proteases could protect other bacteria, including other periodontopathogens: both in a planktonic form (in suspension) and embedded in the laboratory-grown multispecies biofilm from killing by innate immunity. Finally, we check if through activation of receptors on the surface of human neutrophils and gingival epithelial cells KLIKK protease could contribute to the development of the periodontitis.

3. Reason for doing research: Despite numerous efforts, there are still no drugs, which could be used not only in treatment but especially in prevention of periodontitis. Such therapy is now not available mainly due to involvement of more than one bacterial species in aetiology of the disease. Moreover, in many cases, more than one secretory protease, which are crucial virulence factors of "red complex" bacteria, are responsible for the same function such as evasion of innate immunity. To solve this problem, it is necessary to describe as many proteases of periodontopathogens as possible, which should shed more light on the molecular mechanism of development and progression of periodontitis. Then it could be possible to find a protease(s), which could be used as a target for drug development. Therefore, in this project, we will characterize in details KLIKK proteases of *T. forsythia*, especially their role in *T. forsythia* virulence. Despite the putative role of KLIKK proteases in periodontitis aetiology, there is also a second strong reason to conduct proposed research: the enzymes possess unique features.

4. Outcome of research: Thus, detailed biochemical and structural characterization of KLIKK proteases should result, among others, in the description of completely novel mechanisms of activation of proteolytic enzymes. In addition to the pure scientific value, the obtained results may be used in the future to develop drugs designed based on solved crystal structures: inhibitors not only of the KLIKK proteases but also other homologous proteases like human pappalysin.