Peptidases (proteases) are enzymes that hydrolyse the peptide bond. Genes encoding proteases represent approximately 3% of all genes in bacteria. Many bacterial proteases can be considered as virulence factors because they degrade the protein components of infected tissue, facilitate colonization of the host and the spread of bacteria within the infected organism, or protect the pathogen against immune response. An example of a disease, in which aetiology proteases play a crucial role, is periodontitis, a chronic infectious disease that leads to damage to the gingiva and tooth supporting tissues. Periodontitis, in its severe forms, affects up to 15% of people in the world, and if untreated, can lead to the loss in dentition. Besides, due to its infectious and inflammatory character, periodontitis contributes to the development and/or progression of many diseases, such as rheumatoid arthritis, osteoporosis, cardiovascular diseases or diabetes . Because the aetiology of periodontitis is multifactorial, it's impossible to implicate a single pathogen responsible for the development and progression of the disease. However, three species of bacteria are considered to be the main periodontopathogens: *Porphyromonas gingivalis Treponema denticola* and *Tannerella forsythia*, referred to as "red-complex" of the bacteria. The main virulence factors of these bacteria is extracellular proteases. In addition to characterized proteases, in the sequenced genomes of the "red complex" bacteria there are many genes encoding putative, often secretory proteases, with predicted unique properties. Examples of such proteases are two putative proteases of *Tf*: *Tf*PepO (BFO_0011), similar to the human Endothelin-converting enzyme which homologs in other bacteria species are involved in invasion of mammalian cells, and *Tf*IgAse (BFO_2042), a metalloprotease belonging to M64 family of IgA proteases (IgAses) able to hydrolyze both subclasses of immunoglobulin-A (IgA) . Therefore, the main goal of this project is not only biochemical and structural characterization of two novel proteases of *T. forsythia* but also description of their putative role in virulence of this human pathogen.

In the first part of the project, proteases *Tf*IgAse and *Tf*PepO will be obtained and purified as recombinant proteins. Then, activity of both proteases will be characterized in details. Briefly, we will determine their substrate specificity and investigate the effect of physical and chemical factors, (especially divalent cations and inhibitors), on activity of the enzymes. Then, we will check their ability to cleave physiologically important substrates, which degradation could disturb homeostasis in the human oral cavity. In the next step of research, the crystallographic analysis of *Tf*IgAse and *Tf*PepO, as well as one more protease, *Pg*PepO from another member of "red complex", *P. gingivalis*, will be performed. In short, crystals of investigated proteases will be obtained and then the crystal structures will be solved based on results from diffraction of X-ray by the crystals. The solution of the crystal structure of these enzymes may allow describing novel structures of bacterial proteases. In the last part of the project, we will check if the proteases are involved in evasion of host innate immunity and invasion of host cells. To achieve it, we will determine expression level and subcellular localization of both proteases. We will also generate the deletion mutants of proteases that will be used as negative controls in each experiment. Finally, we will also check ability of *Tf*IgAse to hydrolyse different forms of IgA and also influence of *Tf*PaepO on invasion of host cells by *T. forsythia* As a result, we want to describe a putative role of *Tf*Igase and *Tf*PepO in virulence of *T. forsythia*.

The outcome of this project will not only be a biochemical and structural characterization of novel proteases of *T. forsythia*, but also on the elucidation of a putative role of these enzymes in virulence of this pathogen. It is worth to mention, that only a few proteases from *T. forsythia* has been described to date and the current state of knowledge about the proteolytic system of the pathogen is still insufficient. Realization of the project objectives will contribute to expansion of state of knowledge in scientific fields, such as biochemistry, structural biology and microbial pathogenicity.