

The protozoan *Acanthamoeba castellanii* is a free-living amoeba that plays very important role in all ecosystems and is also causative agents of human diseases such as granulomatous amoebic encephalitis and Acanthamoeba keratitis - the disease in which amoebae invade the cornea of the eye. The serious nature of diseases caused by this protist and the problems associated with successful therapy suggest a need for better understanding of its pathogenesis to find effective therapeutic approaches. Despite of intensive searching for amoeba factors crucial for their virulence, many problems concerning of *A. castellanii* pathogenesis is still unclear. However, some recent reports suggest that amoeba can produce enzymes having the ability to digest the nucleic acids located outside of their cells. Our knowledge concerning amoeba pathogenesis suggests that such enzymes could play the role of important virulence factors. Primarily, the activity of these enzymes could explain why amoeba is resistant against an important immune defense factor named neutrophil extracellular traps (NET). Moreover, the extracellular nucleases may explain the mechanism of amoeba grazing on the bacterial biofilm – the structure that protects these microorganisms from external threats. We consider it very likely that nucleases secreted by amoeba can digest eDNA (extracellular DNA) which is an important element of the biofilm matrix. However, the genes encoding amoeba extracellular nucleases are still unknown and if we want to analyze these proteins in details, the cloning of these genes is absolutely necessary. We have already selected potential candidates and now we are going to purify them from *A. castellanii* trophozoites and introduce them into genetic vectors. These vectors will allow us to produce particular nucleases alone and fused with marker proteins (EGFP) in amoebas as well as in other cells. At this stage of study we will be able to confirm whether selected proteins are really nucleases and then we will check whether these proteins are secreted from amoeba cells. If the research is successful we will be able to answer the very important questions concerning amoeba biology and pathogenesis.