Bacteria have created a complicated structure called biofilm, in order to protect themselves from physical and chemical factors as well as antibiotics and components of humans immunological defense. Biofilm is a group of bacteria living in microcolonies which are surrounded and attached to the surfaces by extracellular mucous substance, mainly composed of sugars, but also of proteins, nucleic acids and lipids. This extracellular matrix is essential for the biofilm structure and can exceed over 90% of biofilm biomass. Thanks to this matrix, bacteria embedded in the biofilm are unsusceptible for drugs as well as other antibacterial agents. Only by destruction of mucous structure the access to bacteria hidden inside can be gained. An useful prompt, how to destroy it, can be found in the nature, among natural bacterial enemies - bacteriophages, which are viruses able to infect only bacterial cells. Some of mentioned viruses are equipped with special enzyme (being a part of their virion) - depolymerase. Only phages possessing this enzymatic protein are able to freely penetrate and diffuse in the biofilm structure and thanks to that reach microcolonies to finally infect targeted bacteria. In presented project we are going to produce described phage encoded proteins, able to degrade bacterial capsule and biofilm matrix sugars.

The aim of this study is to prepare the recombinant proteins of novel bacteriophage depolymerases, to recognize its characteristic features and structure and to establish effects of their action. The first task to achieve this goal is to adjust conditions in every step of recombinant proteins production process using genetic engineering methods. We are planning to establish the structure of obtained proteins according to acquired crystals as well as to recognize their activity depending on different environmental conditions. Probably the most interesting task is the verification how these enzymes act against single planktonic *Klebsiella* cells, surrounded by saccharic capsule and against *Klebsiella* cells living in the complicated biofilm structure, surrounded by glycan matrix. We are going also to evaluate the enzyme influence on biofilm formation in comparison to infective phage particles (from which enzymes originate). In the topic of bacteriophages and biofilms co-existence there are many questions, which we are going to answer. It was noticed that prolonged biofilm treatment with phages, may lead sometimes to biofilm overproduction. We are wondering if such mechanism exists only when whole phage virions are used and is connected with bacterial cells destruction or enzyme action alone gives the same effect? The next question, without an answer so far, is if the presence of the enzyme inside the biofilm leads to emergence of bacteria resistant to the enzyme action, or such situation takes place only in case of infective bacteriophages. Thus we are going to evaluate the mentioned phenomenon corresponds to depolymerases and, if so, is this feature stable (present also among next generations of bacteria)? Interesting theory, which we are going to verify, is the connection between phage action effect and the stage of biofilm formation. It seems possible that well-developed biofilm, which possess in its structure many channels and pores, can be more easily detached from the surface. Bacteriophage, entering the biofilm is able to easily reach the basal part of this structure. Here, thanks to the enzymatic activity, can cause whole biofilm exfoliation, without killing particular bacteria cells living inside. We would like to examine above hypothesis in case of enzymes as well as whole phage particles. There are also trials planned of combined preparation application, consisting of enzyme/phage in combination with antibiotic, to confirm a putative effect of depolymreases action which cause biofilm structure loosening enabling better penetration of the drug to cells embedded inside. Current reports confirm the effectiveness of infection eradication by depolymerases in animal models, and also antibiotic penetration into the biofilm as a result of enzyme action. However, there is no data concerning the activity comparison of whole phage particles and depolymerases originated from them on *Klebsiella* biofilm (ubiqiutous in the environment as well as in hospitals). The project has typical basic research character, although the answers can give us the explanations about the problems with, not easy to achieve, biofilm eradication. Bacteria classified to *Klebsiella pneumoniae* are dangerous opportunistic pathogens, both living in natural and hospital environments. Because *Klebsiella* specific phages and also their depolymerases are not well known and described, we have decided to deepen the knowledge in this topic.