

Description for the general public

The first assumption and objective of the project will be selection of 4-6 bacteriophages (bacteria infecting viruses) from our laboratory collection, lytic against *Bacillus anthracis* bacteria, the causative agent of anthrax. Genetic diversity of these phages (restriction patterns) and their host range will be main criteria. In the next step we will attempt to clone endolysins encoded by these phages – the enzymes able to degrade bacterial peptidoglycan. The main goal of the project will be a comparative analysis of amino acid sequences of the newly obtained proteins and the study of their bacteriolytic activity against vegetative anthrax cells and other bacteria from a *B. cereus* group, but primarily against the endospores. The phages encoding new lysins will subsequently undergo a detailed characteristics (morphology, burst size, effectiveness and dynamics of infection), including their ability to bind to the spores.

Bioinformatic analysis following the whole genome sequencing of the selected phages will enable not only indication of the endolysin-encoding genes, being the target objects of the studies but also identification of other proteins of those viruses with their annotated functions. Online databases and computer programs will be employed for this purpose. Molecular cloning technique will be used to obtain enzymatic proteins derived from the phages of interest which will be afterwards purified in an affinity chromatography. For the cloning purpose plasmid molecular vectors and bacterial *Escherichia coli* competent cells, appropriate for protein production in heterologous systems will be used. In the final phase of the studies the purified protein solutions will be tested for their biological activity, i.e. their host range and the endospores and the living anthrax bacteria killing effectiveness. After obtaining and studying a few endolysins encoded by various anthrax-infecting bacteriophages we expect to find differences between their sequences which may result in their different biological activity. We will assess their diversity, also in reference to a PlyG lysin. Moreover, the proteins' activity will be tested in different temperature and pH conditions to evaluate their stability in the environment.

Tackling the problem of anthrax has a multiple argumentation. The anthrax bacillus is a leading pathogen of bioterrorists' interest and the inhalational form of infection is extremely hard to cure, almost always lethal when not treated with antibiotics immediately. Ability to form the endospores makes it more likely to be used as an aerosol. Due to an extreme spore resistance to environmental conditions they gain predominance over other pathogens that require complicated procedures to protect them from degradation in biological clouds. Additionally, looking for new methods of treatment as well as decontamination is especially important since antibiotic resistance among bacteria remarkably increases; hence the phage-derived endolysins seem very promising. The usual way of a soil decontamination is to use formaldehyde or per acetic acid; these chemicals are used successfully but pollute the environment. Evaluation of the proper production and application protocols could make phages or their lysins eligible to become natural and eco-friendly disinfectants.

Endolysins discovered so far as well as their hosts are lytic only against vegetative anthrax cells. The best known endolysin derived from the anthrax-infecting phage is the PlyG encoded by a Gamma phage. PlyG is highly specific and shows good killing capabilities against the anthrax bacilli, however is only moderately successful in degrading the spores, even in the presence of the germination inducer. Isolating of the new phages and production of their lytic enzymes demonstrating proven lytic activity against the endospores could be an effective measure to combat this hazardous pathogen.