

Recombinant gene expression technologies are currently in the mainstream of both basic and applied research in molecular biology, molecular genetics, biotechnology and medicine. Cloning and expression of genes in heterologous organisms, followed by recombinant proteins isolation, analysis and application, have become indispensable scientific and biotechnology industry tools. Currently these technologies enter a new era of synthetic biology and evolution *in vitro*, creating new genomes, genetic regulatory circuits, metabolic pathways, biological parts, devices, and novel or redesigned systems, based on those found in Nature. However, though such highly advanced biomolecular technologies have been developed, some important areas still suffer from major deficiencies, visible in frequent problems with biosynthesis of proteins from recombinant genes subjected to cloning and expression, which are often toxic to a recombinant host. As opposed to relatively uniform DNA in its chemistry and structure, every protein poses a different challenge, as they have different chemical and biological properties, due to their composition of 20 amino acid building blocks, giving unlimited number of combinations and internal interactions of amino acids residues within formed polypeptide, which is further complicated by posttranslational modifications. The proposed project addresses and potentially solves this fundamental problem of expression of recombinant, toxic genes. Scientists worldwide are encountering difficulties in expression of toxic genes on a daily basis. The project proposes to use the genetic engineering, synthetic biology and evolution *in vitro* approaches to modify the extremely toxic – lethal biological system already existing in Nature: a lytic bacteriophage-bacterial host system for biosynthesis of toxic or otherwise problematic proteins. In this system, in spite of the ultimate death of the hosts, a bacteriophage genome manages to express own genes, coding for a number of toxic proteins – both as components forming the bacteriophage which can be described as a lethal macromolecular, nucleoprotein complex, and as own toxic proteins converting the hosts' metabolism to produce bacteriophages instead of cellular components and, ultimately, lysing the host cell. Key aspect of the project is the selection of an adequate bacteriophage for construction of such 'lethal' expression system. We plan to explore biodiversity to find the most suitable system or its parts. However, thus far, there is no better alternative, than the mesophilic-thermophilic bacteriophage TP-84, infecting several thermophilic *Geobacillus* sp. strains. The bacteriophage TP-84 / *Geobacillus* biological system operates through extraordinary wide temperature range of 30-80°C, thus being suitable for mesophilic and thermophilic protein biosynthesis and even some psychrophilic, if they survive 30°C. We have initially tested TP-84 / *Geobacillus* by developing a method for introduction of isolated TP-84 genomes, subjected to genetic engineering, into *Geobacillus* cells and managed to produce recombinant Green Fluorescent Protein from jellyfish *Aequorea victoria* in TP-84 / *Geobacillus*. Further work will include introduction of various 'biological parts' to the system, including transcription promoters, regulatory circuits, genomic fragments from different bacteriophages and bacterial genomes to create 'mosaic' bacteriophage, which will form a novel type of gene expression system. Besides such specialized constructions, our long term goal is to establish TP-84 as a model thermophilic bacteriophage for scientific research.