„Construction of a novel phage display system based on a thermophilic bacteriophage TP-84”
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Peptide/protein or other molecule phage display systems are based on a fusion of a foreign polypeptide sequence with the N- or C-terminal end of the bacteriophage structural protein, thus exposing the target peptides/proteins or other molecules on the surface of the virion. Phage display technology was developed more than 30 years ago and was awarded the Nobel Prize „for the phage display of peptides and antibodies”. In recent years, there has been an intense development in protein/peptide phage display techniques. However, all previously constructed systems are based on bacteriophages that infect mesophilic bacteria with optimum growth conditions from 30°C to 39°C. There are some limitations to these organisms, such as problems with the production and presentation of hydrophobic peptides on the phage surface or the recombinant protein aggregate formation. The solution to these limitations can be a thermostable phage display system based on a thermophilic bacteriophage.

The main purpose of this work was to construct a system to present protein/peptide molecules on the surface of TP-84 bacteriophage, which infects the Gram-positive bacterium *Geobacillus stearothermophilus*. The first stage of the study involved creating three sets of the TP-84 phage gene libraries. Six genes were selected for potential usage in the new phage display system, three of them encoding capsid proteins, one gene encoding the distal tail protein and two encoding other TP-84 structural proteins. Selected genes were inserted into the pUC19 plasmid in the native or fusion form, with added sequence encoding the 6xHis or 3xFLAG molecular markers, together with a 9-nucleotide linker. Initially, three versions of the libraries were obtained for five out of six TP-84 genes. The gene 8 of the TP-84 phage, encoding a minor capsid protein, was inserted into the plasmid vector only after the development of a tandem cloning method.

The second stage of the study involved whole TP-84 phage genome cloning into the copy-control vector pBAC-lacZ. Two different techniques were used to insert the TP-84 genome, divided into eight parts (in the sizes from 4,200 bp to 7,200 bp), Gibson assembly and Golden Gate cloning. As a result of the Gibson assembly technique, a clone of pBAC-lacZ with the fragment of 6,600 bp of the TP-84 genome was obtained, with 15-nucleotide insertion. However, all attempts to obtain a clone with the whole TP-84 phage genome inserted into the pBAC-lacZ vector using both Gibson and Golden Gate cloning methods have failed. This may indicate the possible toxicity of certain TP-84 genes to *E. coli*.

During the last phase of the study, the focus was on developing a method allowing the recombinant TP-84 genome to be placed into the host cells. The PEG8000-mediated transfection was carried out with a positive result of inserting the bacteriophage TP-84 genetic material, both in native and recombinant form, into the host *G. stearothermophilus* 10.

In summary, the results described in this work form the foundation for the development of a functional thermophilic peptide/protein TP-84 phage display system. Nevertheless, there is a requirement to continually optimize this phage display system and a more detailed research on the structure and biology of the TP-84 phage and its host may be necessary.