Study of biological functions of *Yersinia enterocolitica* bacteriophage tail proteins

Abstract

Bacteriophages are the subject of research mainly in the context of their use in therapy against bacterial infections. Despite the benefits of phage therapy, there are also limitations in using phage particles, such as horizontal gene transfer (HTG). The most serious effect of HTG may be the acquisition of virulence factors by bacteria, which why phage therapy still remains a high-risk therapy. Therefore, many scientific studies have focused on the characterizing individual elements of phage virions. These studies have mainly focused on proteins that may have enzymatic functions or may be used for diagnostic purposes. Initially, phage enzymes such as depolymerases or endolysins, which lyse bacteria from inside the cell, were studied intensively. However, scientists' attention has now been focused on tail proteins. The proteins of the bacteriophage tail, which include tubular and filamentary proteins, in addition to their structural function, can perform enzymatic functions on bacterial surface structures. Fiber proteins perform the function of recognizing and binding the receptor on bacterial cells and they are called adhesin. There is a great diversity among the tail proteins which have many adaptive processes. These proteins are largely the result of the co-evolution of phages and their hosts. Therefore, comparative analysis of the nucleotide sequences alone can not determine the predicted function of these proteins. Only experimental research can be used to learn about their properties.

The *Yersinia enterocolitica* ϕ80-18 and φYeO3-12 tail phages belonging to the *Podoviridae* family were used for the research subject this doctoral dissertation. The host for these phages is *Yersinia enterocolitica*, an enteropathogen that causes disease in humans and some animals. The main symptom of infection is watery diarrhea which is a result of inflammation in the intestines. Yersiniosis is a zoonotic disease, infection which is spread mainly by contamined pork. According to ECDC (European Center for Disease Prevention and Control) report, in 2019 there were 7,058 cases of yersiniosis in 29 European countries. Due to the nature of the disease, this number seems to be an underestimation and the actual percentage of patients with the disease is probably higher. Widespread diagnostic protocolsof this disease are carried out only when there is a larger outbreak of infection, or when the reported infections lead to hospitalization. Diagnosis of *Yersinia enterocolitica* is based mainly on culture methods, which are largely time-consuming, and additionally require confirmation in molecular methods or with mass spectrometry. *Yersinia enterocolitica* is a heterogeneous group of strains that are distinguished on the basis of biotyping and serotyping. We distinguish 6 biotypes and 57 O serotypes, respectively, this complicates the identification within the species. An approach known in the literature is the possibility of using tail proteins for diagnosing pathogens. These proteins often have regions in their sequences that specifically recognize a pathogen. This may contribute to faster diagnosis already at the time of infection.

The aim of this doctoral dissertation was to characterize phage tail proteins in terms of enzymatic and adhesive properties and to evaluate the possibility of their practical use both in the control and detection of *Yersinia enterocolitica*.

Research leading to the achievement of the above goal was included in the published cycle of four original papers. They also include research on the biological properties of the ϕ80-18 bacteriophage. In the first paper, the function and mechanism of the action of the TTPAgp11 (Tail Tubular Protein A) protein derived from phage φYeO3-12 was investigated. Gp11 is a protein from the group of TTPA proteins, which includes also gp31 from *Klebsiella pneumoniae* KP32 phage, which shows α-1,4-glucosidase activity. In order to demonstrate whether the TTPAgp11 protein also has the same enzymatic activity as the TTPAgp31 protein, the recombinant TTPAgp11 protein was produced, then starch (a commercial Red-Starch substrate) and maltose were used as substrates in the colorimetric and gas chromatographic enzymatic assays. It has been shown that this protein has a hydrolytic activity towards the tested substrates and we can classify it into enzymes from the α-1,4-glucosidase family. Due to these results, the hypothesis of dual-function proteins was confirmed also in the case of Yersinia phages.

The second paper was concerned with the general characteristics of the ϕ80-18 bacteriophage. In the begining there were difficulties with phage multiplication, and thus we were unable to extract the DNA template necessary for the synthesis of tail protein genes. It turned out that there is a lack of literature data on its biology and general characteristics. The bacteriophage was isolated in the 1990s, but it only was described in 2020 during one of the studies presented in this this doctoral dissertation. For ϕ80-18 phage, a one-step growth curve was determined, its stability in a wide range of pH and temperature was determined, phylogenetic affiliation was analyzed, but also genome and proteome analysis was performed and the hosts range was determined. Phylogenetic analysis confirmed that the phage belonged to the *Podoviridae* family and the *Autographivirinae* subfamilies. The host for this phage is the pathogenic strain of *Y. enterocolitica* O: 8 biotype 1B, but also the serotypes: O:4, O:4.32, O:20 and O:21. The obtained results indicate the possibility of using ϕ80-18 in the biocontrol of the American biotype 1B *Y. enterocolitica*.

The third paper presented was concerned with the characteristics of the TTPBgp12 (Tail Tubular Protein B) and TFPgp17 (Tail Fiber Protein) proteins, which are derived from the phage φYeO3-12. These proteins belong to the appropriate groups of proteins described earlier for *Klebsiella pneumoniae* bacteriophages. Therefore, the TTPBgp12 protein, in addition to its structural function, has been considered to have some enzymatic function. The substrate specificity for the TTPBgp12 protein could not be determined. In the course of the research, it was found that this protein inhibits the growth of bacteria and slows down the development of *Y. enterocolitica* bacterial biofilm. The paper presents an extensive comparative in silico analysis for both proteins. An interesting result was obtained from the analysis for the TFPgp17 protein, which was similar to the RsaA protein present in the S layer in gram-negative and gram-positive bacteria. The structural similarity of both phage and bacterial proteins may be the result of the evolutionary adaptation of the virus to effectively infect its host. The paper also presents the results of tests on the stability of the tail proteins in

the presence of selected sugars. The stabilizing effect of N-acetylgalactosamine on the TFPgp17 protein turned out to be the most important. GalNac is the main source of carbon for *Yersinia* bacteria in the digestive tract of humans and pigs. It is therefore, not surprising that this amino sugar can stabilize the entire phage particles present there. These tests partially confirm this hypothesis.

The latest paper was about the TFPgp17 protein and its use as a specific adhesin to detect the pathogenic *Yersinia enterocolitica* serotype O:3. The TFPgp17 protein was produced in a complex with MBP (maltose binding protein) with a histidine tag, which was necessary to enable detection. It did not disturb the adhesion properties of the phage protein and additionally allowed for a significant shortening of the purification procedure. In addition, the presence of a tag makes it possible to immobilize the protein (with a tag) to a carrier in a strictly targeted way and use it to develop sensors, e.g. optical sensors. In the conducted research, an ELISA test was performed, which analyzed not only *Y. enterocolitica* strains of various serotypes, but also other strains, e.g. *K. pneumoniae*, *S. aureus*, *P. aeruginosa*. The test was designed to assess the specificity of the TFPgp17 protein. It was shown to be highly specific and able to detect only *Y. enterocolitica*, serotype O:3. To confirm the obtained results, the TEM microscopy analysis was performed, where the bacteria were immobilized to a mesh and then incubated with TFPgp17. We detected the creation of a connection between the protein and the bacteria. This was possible thanks to the presence of the applied tag, which was detected with anti-tag antibodies modified with colloidal gold. TEM imaging showed the attachment of the TFPgp17-MBP / his tag complex on the surface of the *Y. enterocolitica* O:3 bacterial cell, which confirmed the results obtained in the ELISA test.

In conclusion, the goal of this dissertation has been achieved. In the course of the research, it was possible to obtain and characterize 3 proteins of the bacteriophage tail. For two of them, apart from the structural function, it was possible to demonstrate their biological properties. For the TTPAgp11 protein the enzymatic function of α-1,4-glucosidase was determined.For the TTPBgp12 protein it was shown a significant inhibitory effect on the growth of pathogenic bacteria and the formation of bacterial biofilm. The indication of the antiseptic potential of the TTPBgp12 protein can be used for the development of biologically active preparations in the future. In the case of TFPgp17 protein, its adhesin function was confirmed. Its specificity for the bacteria was determined. In addition, an efficient and rapid method of obtaining this protein in a modified form was developed for wide use in the development of biosensors for the rapid detection of *Yersinia enterocolitica* O:3 contamination.