## INVESTIGATION OF A NOVEL TRICOMPONENT BACTERIAL ANTIPHAGE DEFENCE SYSTEM

<u>Lukas Volodka</u><sup>1</sup>, Inga Songailiene<sup>1</sup>

<sup>1</sup>Vilnius University, Life Sciences Center, Institute of Biotechnology, Department of Protein - DNA Interactions lukas.volodka@chgf.stud.vu.lt

During the course of evolution bacterium have acquired numerous of antiviral defense systems which help them to evade bacteriophage infections. This research is focused on a newly identified tricomponent antiviral defense system TerY-P<sup>1</sup> organized in an operon. It has been demonstrated that that the system contributes to a significant immunity of bacteria against common E. coli phages T3, T7 and  $\phi$ V-1<sup>2</sup>. Cloning of the individual components fused with histidine affinity tags and cloning of full operon has been successfully performed, further followed by the expression of the defense system in uninfected E. coli. In the absence of phage infection, the heterologous expression so far has been observed only for one of the proteins TerY. We hypothesize that the system may work as an inducible toxic cascade, thus the other components may appear upon phage infection. Therefore, we checked if all components can be synthesized in cell-free expression system, which resulted in the expression of all the components. *In vivo* protein expression yielded 2 of the 3 components (TerY and TerP). Other information about the components comes from bioinformatical analysis which shed some light on their possible functional and structural aspects. In the ongoing experiments, we are planning to identify the target of phosphate modification, or the presence of the mRNA of the corresponding genes under different conditions.

<sup>[1]</sup> Anantharaman, V., Iyer, L. M., Aravind, L. (2012b). Ter-dependent stress response systems: Novel pathways related to metal sensing, production of a nucleoside-like metabolite, and DNA-processing. Molecular BioSystems, 8(12), 3142. https://doi.org/10.1039/c2mb25239b

<sup>[2]</sup> Gao, L., Altae-Tran, H., Böhning, F., Makarova, K. S., Segel, M., Schmid-Burgk, J. L., Koob, J., Wolf, Y. I., Koonin, E. V., Zhang, F. (2020). Diverse enzymatic activities mediate antiviral immunity in prokaryotes. Science, 369(6507), 1077–1084. https://doi.org/10.1126/science.aba0372