

DETERMINANTS OF PHAGE SENSITIVITY TO THE OLOKUN ANTIPHAGE DEFENSE SYSTEM

Vincentas Raudys¹, Edvinas Jurgelaitis¹, Mindaugas Zaremba¹

¹Vilnius University, Life Sciences Center, Institute of Biotechnology, Department of Protein - DNA Interactions, Vilnius, Lithuania
vincentas.raudys@gmc.vu.lt

Bacterial and archaeal populations are constantly infected by bacteriophages, the most abundant biological entities on Earth [1]. Under this intense evolutionary pressure, prokaryotes have developed antiphage systems, while phages have developed countermeasures to overcome them. This coevolutionary “arms race” has led to a vast expansion in the diversity of antiphage systems, many of which possess unique and largely uncharacterised mechanisms of action. Several of these systems have been exploited for applications in science and medicine. For example, CRISPR-Cas systems have revolutionized genome editing, and restriction-modification systems have been used for DNA manipulation for more than half a century [2].

In this study, we lay the groundwork for elucidating a novel prokaryotic antiviral defence system – Olokun, discovered in the *E. coli* UMB0934 strain [3]. A phage-escaper workflow was applied to identify bacteriophage mutants capable of overcoming Olokun-mediated defence. Candidate mutant bacteriophages were isolated and validated. Following purification of phage genomic DNA, sequences were determined using next-generation sequencing. Raw sequencing reads were analyzed using bioinformatic approaches, leading to the identification of potential determinants of phage sensitivity to Olokun.

This work advances understanding of prokaryotic antiphage defence systems and their potential biotechnological applications.

[1] M. Zuppi, H. L. Hendrickson, J. M. O'Sullivan, and T. Vatanen, “Phages in the gut ecosystem,” *Frontiers in Cellular and Infection Microbiology*, vol. 11, p. 822562, Jan. 2022, doi: 10.3389/fcimb.2021.822562.

[2] H. Georjon and A. Bernheim, “The highly diverse antiphage defence systems of bacteria,” *Nature Reviews Microbiology*, vol. 21, no. 10, pp. 686–700, Jul. 2023, doi: 10.1038/s41579-023-00934-x.

[3] A. Millman et al., “An expanded arsenal of immune systems that protect bacteria from phages,” *Cell Host & Microbe*, vol. 30, no. 11, pp. 1556-1569.e5, Oct. 2022, doi: 10.1016/j.chom.2022.09.017.