CHARACTERIZATION OF TOXIN-ANTITOXIN SYSTEM IN THE VICINITY OF CRISPR-CAS OPERON

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Bacteriophages (viruses of bacteria) represent a lethal threat to bacteria. In the course of evolution, microbes developed many tools to fight viral infections. CRISPR-Cas system in prokaryotes functions as an adaptive immune system that provides acquired resistance against invasive genetic elements like viruses or plasmids. CRISPR-Cas systems are very diverse and differ by gene arrangement and composition of the effector complex [1]. During recent years molecular mechanisms of CRISPR-Cas were established [2–6].

Auxiliary genes are found quite often in the vicinity of CRISPR-Cas systems; however, their role in the CRISPR-provided bacterial immunity remains unclear [1, 7].

The study aims to characterize a toxin-antitoxin (TA) module, located in the same operon with a Type I CRISPR-Cas system in cyanobacteria. The link between CRISPR-Cas and particular TA system is unknown, while biochemical and structural data on the TA system is also lacking.

Genes of interest were cloned in heterologous host *E. coli. In vivo* assays showed that the two-gene module forms an active toxin-antitoxin system. Protein expression conditions were optimized and proteins were purified from *E. coli*. Amino acids, critical for the function of the TA system, were determined both *in vivo* and *in vitro*. Finally, protein crystals, suitable for X-ray crystallography, were obtained. Collected data was sufficient to determine the ternary structures of the toxin and toxin-antitoxin complex.

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