Evaluation and Characterization of CyranoPS Phage Protein Interactions with *Corynebacterium glutamicum*

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Abstract

Bacteriophages are the most abundant biological entities on Earth, with an estimated population of approximately 10^{31} particles, causing around 10^{24} infections per second. Their significant role in bacterial communities and environmental processes has drawn considerable interest in biotechnological research. The ability of phages to infect specific bacteria and regulate their activity has become a powerful tool for advancing scientific and industrial applications.

The aim of the study was to characterize the function of the temperate phage CyranoPS and investigate its impact on the strain *Corynebacterium glutamicum* MB001. *C. glutamicum* is a non-pathogenic bacterium that plays an important role in biotechnology due to its use in industrial production, particularly for the synthesis of amino acids and other biological products. The wild-type strain of this bacterium (ATCC 13032) is naturally resistant to phage infections. This resistance is partly due to the regulation of cryptic prophages (CGP1, CGP2, CGP3) within its genome. A nucleoid-associated protein, CgpS, keeps these prophages inactive, which effectively "silences" the prophage regions and prevents their activation.

For this study, the strain *C. glutamicum* MB001 was selected, which is a prophage-free derivative. This means that the strain lacks the cryptic prophage regions in its genome, making it susceptible to phage infections, including infection by CyranoPS. This genetic modification is essential for exploring CyranoPS's infective potential and understanding how this phage interacts with its bacterial host.

Through Biolector-based phenotypic analyses and protein interaction studies, it was found that different proteins encoded by CyranoPS, including CyranoPS_17 (a main repressor), CyranoPS_19 (a ROK-like repressor), and CyranoPS_24 (a WhiB-like protein), exert diverse effects on bacterial growth. Particularly noteworthy was the comparison between two variants of CyranoPS_17: the full-length form (CyranoPS_17.1) and a truncated form (CyranoPS_17.2). The study revealed that CyranoPS_17.2 exhibited a stronger inhibitory effect on bacterial growth than its full-length version, potentially due to structural differences in the protein, which may enhance the phage's regulatory effect.

Although pull-down assays—a method used to identify protein-protein interactions—did not reveal direct interactions, the findings suggest that CyranoPS proteins may act through indirect regulatory mechanisms. This implies that the phage proteins might influence bacterial physiology by modulating gene expression, regulatory networks, or other cellular components.

The study confirmed that CyranoPS phage proteins have significant effects on *C. glutamicum* and their influence is highly diverse, depending not only on the type of protein but also on its structural form. Investigating the interactions between phages and their bacterial hosts provides valuable insights into natural processes and enables the development of methods for controlling bacterial mechanisms for industrial purposes. Phages can be used to eliminate specific bacteria, prevent biological contamination, or inhibit particular metabolic pathways.