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Investigating Cytotoxicity and Defense Functions of Mycobacteriophage Mercurio genes in host M. smegmatis

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Drug-resistant infections caused by pathogenic Mycobacterium species, such as M. tuberculosis, M. leprae, and M. abscessus, represent a growing public health threat. Representing an untapped source of natural bacterial lysis mechanisms, bacteriophage gene products have been investigated for their abilities to overcome antiphage responses, contribute to lytic activity, or prevent superinfection by other phages. Although thousands of bacteriophage genomes have been sequenced and annotated, approximately 70% of their gene products lack structural characterization and functional assignment. These genes often have no homologs, even in closely related phages, highlighting significant genetic diversity. Characterizing these genes could uncover species-specific bacterial lysis pathways or identify phages with enhanced lytic potential. This study, conducted as part of the HHMI-supported SEA-GENES project, describes results from overexpression-based phenotypic screening of genes encoded by cluster G4 mycobacteriophage Mercurio isolated using the non-pathogenic Mycobacterium smegmatis. The findings from this study will contribute to characterizing a broad network of mycobacteriophages across diverse clusters. Bacteriophage genes with growth-inhibitory effects on host bacteria have been identified through genome-wide overexpression screenings. In this study, plate-based cytotoxicity assays against M. smegmatis revealed that one of the eleven screened genes significantly inhibited host growth. This gene encodes a putative DNA-binding domain protein. Understanding superinfection inhibition is critical for developing effective phage cocktails for clinical use, as all included phages must contribute to bacterial lysis. Thus far, defense assays in this study identified no gene products from Mercurio capable of preventing superinfection by heterotypic phages BPs or D29, suggesting potential synergy with other phages. This study represents the first genome-wide overexpression screening of a mycobacteriophage from Mercurio's cluster. The findings establish a valuable foundation for exploring the unknown functions of diverse, novel bacteriophage gene products.