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2021 SEA Symposium Abstract

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A genome-wide screen of the Cluster K phage Waterfoul reveals genes that inhibit the growth of Mycobacterium smegmatis

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The SEA-PHAGES project has isolated and sequenced thousands of phages, providing a rich genetic dataset that continues to advance our understanding of the diversity and evolution of phage populations. Importantly, this work has also highlighted the need for further study, as the large majority of phage-encoded genes remain uncharacterized. Exploring the functions of these phage genes will undoubtedly provide greater insight into phage-host and phage-phage dynamics and potentially inspire novel therapeutics and molecular technologies. In this study, a genome-wide investigation of the Cluster K mycobacteriophage Waterfoul was conducted, with each of the 94 Waterfoul genes cloned into a plasmid engineered for inducible expression in the bacterial host *Mycobacterium smegmatis*. Systematic screening of this gene library in a cytotoxicity assay revealed a set of 33 genes whose expression inhibited mycobacterial growth to varying degrees; half of these genes have no known function. Gene *47,* expression of which strongly inhibited *M. smegmatis* growth, was found to be a member of a family of toxic phage genes encoding products containing a conserved domain of unknown function and two conserved CxxC motifs. Further genetic analyses, including gene truncation and mutagenesis experiments, and protein-protein interaction studies indicate that the CxxC-domain is necessary for the observed cytotoxicity, potentially inhibiting growth through an interaction with an essential host transcription factor.