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2025 SEA Faculty Meeting Abstract

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Functional Characterization and Protein Interaction Analysis of Mycobacteriophage Amelie's Toxic Gene 47

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A genome-wide overexpression screen of individual mycobacteriophage Amelie genes identified 26 genes that inhibit the growth of *Mycobacterium smegmatis*. Among these, 10 genes were highly toxic, completely halting host growth upon overexpression. Notably, over one-third of the identified genes have no known function, highlighting the potential for discovering novel mechanisms of phage-host interactions. As phage proteins often interact with host proteins during infection, characterizing these interactions can provide valuable insights into their biological roles. To investigate this, Amelie genes 44, 47, 50, 65, and 73 were amplified and cloned into the p2Hα plasmid for use in a two-hybrid assay. Amelie gp47 was found to interact with *M. smegmatis* proteins NusA and Chain A Polyribonucleotide Nucleotidyltransferase. Interestingly, gp47 belongs to a family of genes found in cluster K1, K3, K5, and K6 phages; however, homologous proteins in these phages only interact with NusA. To further explore the mechanism underlying gp47's cytotoxicity and its interactions with host proteins, truncated and mutated versions of gp47 were constructed using pExTra plasmids. Cytotoxicity assays revealed that mutations disrupting the CxxC motifs in gp47 abolished its toxic effects on bacterial growth, implicating this motif as essential for its cytotoxicity. Further analysis revealed that the cysteine-rich motif of gp47 interacts with the KH1 and KH2 domains of NusA. These findings emphasize the utility of genome-wide overexpression screens in uncovering novel phage-host interactions and provide new insights into the functional roles of phage proteins during infection.