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Analysis of Mycobacteriophage Kumao Gene Functions with the SEA-GENES Research Project at Lehigh University

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Kumao is a Singleton mycobacteriophage discovered from a soil sample collected by a Lehigh undergraduate student in 2015. Its genome was sequenced at the Pittsburgh Bacteriophage Institute and annotated by Lehigh University students (SEA-PHAGES) and a University of Pittsburgh Phage Hunters Integrating Research & Education (PHIRE) student in 2017. The Kumao genome is 70,373 base pairs in length with 115 protein-coding genes and one tmRNA-encoding gene. Notably, among these genes, 35 are orphams that lack identifiable homologues in other phages. The fact that Kumao remains clustered as a singleton and has orphams that comprise over 30% of the genome provided the rationale for choosing this phage for functional analysis in our SEA-GENES research project. Additionally, the Kumao genome contains an integrase (gene *96*), but no immunity repressor was functionally annotated in the original annotation. Interestingly, preliminary evidence shows that Kumao may form a lysogen (currently being investigated by a student in a SEA-PHAGES Advanced Phage Research course – see abstract from Advanced Phage Research Course at Lehigh University), suggesting that an annotated gene product may function as an immunity repressor in the Kumao system. Annotation updates using bioinformatics tools are also currently in progress (in Advanced Phage Research) to determine if functional annotation revisions are required. In our SEA-GENES Research Project thus far, our efforts have yielded successful PCR amplification of over two-thirds of Kumao's protein-coding genes. Subsequently, a subset of these genes has been cloned into the pExTra vector, allowing for controlled gene expression by the tet-inducible pTet promoter. Throughout the spring 2024, our objectives include conducting cytotoxicity assessments on all cloned genes by semester's end. Additionally, we plan to execute defense assays to determine whether the overexpression of Kumao genes confers protection to *M. smegmatis* against Kumao and other bacteriophage infections. Genes that may confer defense against Kumao infections may also provide critical insights into questions about lysogeny establishment.