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2024 SEA Symposium Abstract

Lehigh University

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SEA Expeditions in an Advanced Phage Research Course at Lehigh University

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Lehigh’s SEA-PHAGES and SEA-GENES programs provides opportunities for first year and advanced undergraduates to extend their discoveries and investigations about Actinobacter phage genome structural diversity, host-phage interactions, phage gene structure/function, and phage biology into multiple courses throughout their academic career. Advanced phage research students are involved in a diverse set of projects, including mycobacteriophage genome annotations and comparative genome analyses, investigations of phage gene functions and phage-host interactions, and exploring immunity mechanisms governed by prophage-mediated gene expression within cluster N lysogens. Projects included are: **1) Functional analysis of cluster N phage Kevin1 genes 30 (a functionally annotated AAA-ATPase) and 31 (an orpham)** both predicted to be expressed in the prophage. Gene *30* is cytotoxic when overexpressed in *M. smegmatis* whereas a gene *30* mutant lacking the AAA-ATPase domain is not cytotoxic, suggesting that cytotoxicity is mediated through the AAA-ATPase domain. We also hypothesize that genes *30* and *31* constitute a toxin-antitoxin pair expressed in the lysogenic state. Experiments are underway to construct a gene *31* deletion mutant and to test cytotoxicity of gene *30* in the presence of gene *31*. **2) Confirmation of cytotoxicity of cluster W Taptic gene 73 and cluster N Butters genes 44, and 59** as a prelude to uncovering host protein interactors via bacterial two hybrid analyses. **3) Investigation of singleton Kumao lysogen establishment and genome annotation updates**. Kumao gene functions are under investigation in Lehigh’s SEA-GENES Program. **4) Annotation of newly discovered cluster N mycobacteriophage Journey.** **5) Investigation of genetic exchanges between temperate cluster AD mycobacteriophage Dori and a Butters prophage.** Collectively, these ongoing research projects highlight research undertaken by advanced phage research students at Lehigh University.