CONSIDER FOR TALK

2024 SEA Faculty Meeting Abstract

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The “N” Game in Lehigh University’s SEA Program

Vassie C Ware, LEHIGH SEA PROGRAM RESEARCHERS

Lehigh celebrates 15 years in the SEA this year, having expanded from two undergraduate course offerings in phage discovery and genome annotation in 2009 to four courses that now include investigations of phage gene functions in SEA-GENES and several phage biology questions in an Advanced Phage Research Laboratory. Research findings from two projects will be discussed. One of our long-standing research interests among undergraduate and graduate students interested in phage biology is the investigation of host defense mechanisms attributed to cluster N prophages. Interestingly, of the 43 sequenced cluster N phages found on the Actinobacteriophage Database at phagesdb.org, eleven (11) have been isolated by Lehigh students, starting with Butters in 2011. Our research has focused on understanding molecular mechanisms that govern defense. Using PurpleHaze (subcluster A3) and Island3 (subcluster I1) as model heterotypic phages to study defense mechanisms in a Butters lysogen, we have learned through forward and reverse genetics approaches that at least two separate prophage-mediated antiphage systems involving Butters genes *30/31* and reverse gene *57* (*57r*) operate in a Butters lysogen. Additionally, advanced phage research students are investigating the functions of cluster N phage Kevin1 genes *30* (a functionally annotated AAA-ATPase) and *31* (an orpham), both predicted to be expressed by the prophage. Gene *30* is cytotoxic when overexpressed from atc-inducible plasmid pExTra in *Mycobacterium smegmatis*, whereas a gene *30* mutant lacking the AAA-ATPase domain is not cytotoxic. This finding suggests that gene *30* cytotoxicity is mediated through the AAA-ATPase domain. Notably, both Kevin1 wild-type and mutant gene *30* phages produce lysogens. Experiments also show that overexpression of gene *31* in *M. smegmatis* is not cytotoxic. Co-expression of genes *30* and *31* within the host abrogates gene *30* cytotoxicity, suggesting that genes *30* and *31* encode a toxin-antitoxin system expressed in the lysogenic state. AlphaFold2 structure modeling predicts an interaction between Kevin1 gp30 and gp31. Experiments are in progress to delete gene *31* in both wild-type and mutant gene *30* Kevin1 phage by BRED strategies and to test resultant mutant phages for efficiency of lysogen production. We predict that modulation of gene *31* activity will impact Kevin1 lysogen establishment and maintenance. A summary of research findings from work on Butters antiphage systems and Kevin1 gene *30/31* cytotoxicity will be presented, with highlights from contributions by undergraduates.