DO NOT CONSIDER FOR TALK

2023 SEA Symposium Abstract

Lehigh University

Bethlehem PA

Corresponding Faculty Member: Michael Kuchka (mrk5@lehigh.edu)



Sara Aden

Year Two of SEA-GENES Analysis of Mycobacteriophage Taptic Gene Functions at Lehigh University

Sara Aden, Nathanael Borger, Matthew Diaz, Brooke Dubyna, Colette Gordon, Rachael Harrower, Hannah Lord, Lilly Matz, Mia Miranda, Jeremy Oblon, Max Pace, Lake Palmeri, Nader Rifai, Matt Rivera, Jamila Shah, Lauren Shebby, Iris Shin, Cal Shutack, John Siani, Aidan Singer, Will Yaeger, Renee Roberts, Vassie Ware, Michael Kuchka

The SEA-GENES Research Project focusing on the elucidation of Taptic gene functions was initiated at Lehigh University in spring 2022 and is continuing in this current spring 2023 semester. Last year, we successfully amplified over half of protein-coding genes of the Taptic genome. This spring, students first worked on amplifying and cloning the remaining somewhat more challenging genes. We have just reached the halfway point of the semester and students have managed to amplify all Taptic genes. Besides simply changing standard PCR parameters for individual genes, successful amplification was achieved by altering Mg++ ion concentration in the PCR reaction, utilizing the Q5 polymerase’s High GC Enhancer solution, and redesigning primers to make them longer and more gene-specific. Most of the Taptic amplicons have been cloned into the pExTra vector and have been transformed into the host, *Mycobacterium smegmatis*. Phenotypic assays are underway to determine if over-expression of individual Taptic genes is cytotoxic to the host or if it provides defense against infection by Taptic and other mycobacteriophages. To date, we have identified six genes (Taptic genes *41*, *48*, *52*, *56*, *58* and *86*) that are either cytotoxic or dramatically reduce growth when over-expressed in *M. smegmatis*. Taptic gp52 is predicted to be an HNH endonuclease. All of the other genes encode small proteins with no known function. To identify host proteins that interact with Taptic gp58 and gp86 and determine the mechanism by which over-expression of these proteins kills the host cell, we have initiated a bacterial two-hybrid analysis, the first step of which is cloning the genes encoding these proteins into the vector p2Hα. These experiments are being carried out by students in an independent research course whose projects stem from SEA-PHAGES and SEA-GENES courses (see abstract “SEA Explorations in an Advanced Phage Genetics Course at Lehigh University”). If time permits, we will initiate bacterial two-hybrid analyses on other interesting Taptic genes within the SEA-GENES course.