CONSIDER FOR TALK

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Characterization of Mycobacteriophage Genomes and Investigation of N Cluster Immunity Mechanisms

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Lehigh’s SEA-PHAGES program is a collaborative research enterprise for first year and advanced undergraduates who focus on isolating and characterizing Actinobacter phages to gain a better understanding of phage genome structure, gene function, and phage biology in general. In addition to uncovering new phages for comparative genome analysis, our program focuses on novel phage genes that lack family members in other mycobacteriophages (called orphams) to understand their role in phage lytic or lysogenic life cycles. We report on progress on several projects. I. We highlight genomic profiles of newly isolated Mycobacteriophages Mitti (K4) and N cluster phages Kevin1 and Nenae - the latter two phages identified following DOGEMS analyses. Conserved and divergent genomic features for these newly annotated phages with other K4 or N cluster groups will be presented. II. Of special interest to our group is the N cluster – a group of temperate phages characterized by relatively small genomes of average size 43,111bp and a highly variable region centrally positioned within the genome. We have focused on Mycobacteriophage Butters that contains 4 orphams in the variable region, and have investigated homo- and heteroimmunity patterns with a host of mycobacteriophages as well as putative roles of orphams *gp30* and *gp31*. Recently, a novel mechanism of prophage-mediated immunity was uncovered for N cluster lysogens which provides defense against attack by variable groups of heterotypic mycobacteriophages and appears dependent on genes in the variable region of N cluster genomes (Dedrick et al., 2017). Further investigation of defense mechanisms includes an approach to isolate heterotypic mutant phages (e.g., PurpleHaze, Island3) that overcome the Butters prophage-mediated defense system. Progress in characterizing “defense escape mutants” will be discussed. III. Isolation of N cluster phages Kevin1 and Nenae allows for further exploration of prophage-mediated defense systems. The Kevin1 genome is more similar to Butters (e.g., both have lysis A and B genes) while the Nenae and Redi genomes are highly conserved and contain a single lysis gene. A major difference between Kevin1 and Butters is the presence of Kevin1 orpham *gp30*, predicted to function as an AAA ATPase. Whether or not this predicted AAA ATPase is part of the defense mechanism against heterotypic viral attack is unknown. BRED experiments are in progress to delete Kevin1 *gp30* as a first step to determine its possible role in the phage life cycle or in defense against viral attack. Further, variable region differences in Butters, Kevin1, and Nenae genomes predict different patterns of defense against viral attack for each lysogen. Immunity experiments confirm this prediction. Collectively, these experiments will provide further insights into the role of novel genes in the variable region of N cluster genomes in specifying immunity patterns that protect bacterial hosts from viral attack.