CONSIDER FOR TALK

2022 SEA Symposium Abstract

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The Power of DOGEMS: Analysis of Several Mycobacteriophage Genomes

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Lehigh’s 2021 cohort discovered 11 new phages that infect *Mycobacterium* *smegmatis* and analyzed two sets of DOGEMS data: 2020 and 2021. Contig-specific primers and PCR (based on DOGEMS data) identified Clusters for Tiri (K1; 2020 DOGEMS), Norz (A1; 2021 DOGEMS) and Chomp Phage (C1; 2021 DOGEMs). Chomp was hypothesized to be a C1 cluster based on a myoviridae morphotype revealed by transmission EM. For Norz, students performed PCR to bridge gaps between contigs. Four additional Sanger sequencing finishing reactions were required to connect separate contigs for Norz. An additional phage, Perplexer (A4) was chosen for sequencing based on unusual immunity relationships. Annotations of phages Norz, Perplexer and Chomp are in progress by a subgroup of the 2021 cohort, who are currently enrolled in the Advanced Phage Genetics course (included in another abstract). Comparative genome analysis and functional annotation of Tiri revealed a truncated immunity repressor (gp44) due to a nonsense mutation that correlates with a previous finding that Tiri is unable to form a lysogen. Additionally, infectivity of all 2021 isolated *M*. *smegmatis* phages was tested on Cluster N Xeno lysogen lawns. No new Cluster N phages or heterotypic phages subjected to Xeno prophage mediated defense, were identified. Phages hypothesized to be temperate based on plaque characteristics were identified and putative lysogens were isolated from those phages using halo assays, supernatant release assays, and efficiency of lysogeny measurements. Immunity relationships were investigated using the 2021 purified phage lysates and lysogens, to uncover putative cluster relationships between phages and/or to identify phages impacted by prophage-mediated defenses.