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2023 SEA Symposium Abstract

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Isolation, Characterization, and Annotation of Mycobacterium smegmatis Bacteriophages SrishMeg2525, MadKillah, Nedwong, PurduePete, AlpineSix, and UptownCabaret

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In the fall of 2022, 24 unique Mycobacterium smegmatis bacteriophages were isolated from soil samples, mainly around Purdue University in West Lafayette, IN. Many successful isolations occurred from samples in mulch beds on the Purdue campus. After direct isolation, the phages were purified and amplified. Once each of the student groups reached a high enough titer, DNA extraction was performed. To characterize the unique samples, electron microscopy imaging was utilized and a restriction enzyme digest was performed. The 24 samples displayed unique morphologies and restriction enzyme gel results. From these isolations, six high-titer samples were chosen to be sequenced and characterized further.   
In the spring of 2023, the research team is annotating six phages, including cluster C phages SrishMeg2525, Nedwong, and PurduePete, cluster O phage MadKillah, cluster F phage AlpineSix, and cluster G phage UptownCabaret. Of these six phages, the cluster C phages are of Myoviridae morphotype while all others are of Siphoviridae morphotype. These phages are currently being annotated using the programs DNA Master and PECAAN. Membrane proteins are being further investigated using the protein structure prediction program DeepTMHMM.  
 Using critical findings acquired throughout the annotation process, various case studies were conducted by students and the research team using the structure prediction program AlphaFold2. One of those case studies explored membrane proteins in phage PurduePete, specifically the relationship between structure and function in membrane proteins with two transmembrane regions. These regions were analyzed by determining their amino acid properties, with large hydrophobic tryptophans being favored near transmembrane region boundaries. Another case study explored the relationship between HIT proteins in phage SrishMeg2525 and in the organism Mycobacterium tuberculosis. While the superposition of these two structures demonstrated these proteins were related, the distance observed indicates there may be functional differences. Both case studies led to an increased understanding of potential phage use.  
With each new bacteriophage isolated and annotated, new discoveries can contribute to the overall search for advancements in biotechnology in a quickly changing research industry.