DO NOT CONSIDER FOR TALK

2023 SEA Symposium Abstract

Durham Technical Community College

Durham NC

Corresponding Faculty Member: Marie Fogarty (fogartym@durhamtech.edu)

Let’s Phage it: Deconvoluting Bacteriophage Genomes with DOGEMS and Annotating Morgana

Liliana Bolaños Abarca, Zoe M Cohen, Em Hayes, Diego M Jarquin, Yasmeen J Mustafa, Mariela Pacheco-Mendoza, Kathryn J Shriver, Alex C Broussard, Marie P Fogarty

Bacteriophages (phages) are viruses that infect bacteria and are the most abundant organisms in the environment. Phages are a promising tool in the arsenal against increasingly antibiotic-resistant bacterial infections. In the Fall of 2022, students at Durham Technical Community College discovered six phages using the host *Gordonia rubripertincta* – Lila22, BillDoor, PoisonIvy, Memo, Chozen, and Morgana, and one phage using the host *Gordonia westfalica* – Esphand. Morgana had interesting plaque morphology, it presented as a temperate phage, producing turbid plaques. After three days incubation at higher titer concentrations, larger clear plaques were observed growing on top of webbed plates, suggesting the prophage had switched from the lysogenic to the lytic cycle. We chose to sequence Morgana as our first phage sample, and DNA from the remaining six phages was combined and sequenced en masse as the second sample. Morgana has 171 genes and a 67.5% GC content and was revealed to belong to the DZ cluster along with three other phages. Manual inspection and annotation refinement for Morgana was carried out using DNA Master, PECAAN, Phamerator, Starterator, Phages and NCBI BLAST and HHPRED. We were able to assign functions to ~36% of the genes including annotation of tyrosine integrase, immunity repressor, excise and Cro (control of repressor's operator) protein, confirming that Morgana is a temperate phage. When comparing Morgana to the three genomes in its cluster, in a region of forward-oriented genes, we annotated an additional gene with strong coding potential in the reverse orientation. This gene is annotated as an esterase. Esterases catalyze the hydrolysis of ester bonds, and this gene addition provides a nice example of the mosaic nature of phages and how they adapt to their changing environment. For the combined sample sent for sequencing, six full-length contiguous sequences (contigs) were returned. Of note, four of the returned apparently full-length sequences belong to the CT cluster. It is rare to get assembly of complete sequences when the DNA pool includes phages from the same cluster. We used the DOGEMS approach (Deconvolution of Genomic En Masse Sequencing) to identify which phage sample belonged with which contig. Specific sets of primers were designed for each contig and used in PCR to match contig to phage. We successfully matched Lila22, Esphand, and BillDoor to their contig sequences - cluster DV, CV, and CT, respectively. The three remaining samples all belong to the CT cluster. Thus, using DOGEMS we have added an additional three full-length sequences to the phagesDB database for annotation. Our annotation of Morgana confirms Morgana as a temperate phage and contributes to ongoing efforts to understand the diversity and evolution of phages.