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Characterization and annotation of unique EM cluster bacteriophage Kikiko

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Bacteriophages are viruses that infect and replicate within bacteria and are the most abundant biological agent on the planet. With the growing threat of antimicrobial resistance in bacteria, phages are being pursued as a possible alternative treatment to antibiotics.   
  
In the Fall of 2022 Karim Halal, a student at uOttawa, discovered a novel EM cluster phage Kikiko in a soil sample from the Rideau River walkway. Kikiko infects Arthrobacter globiformis and has a Podoviridae morphology which have a characteristic short non-contractile tail. Out the 251 phages found by students at the University of Ottawa, Kikiko is one of only 4 phages found belonging to this morphotype.   
  
Kikiko’s DNA was isolated and sent for sequencing at the University of Pittsburgh by Illumina Sequencing. The GC content of its genes is 59.8% of 53980 base pairs and its genome is circularly permuted. Of the 50 putative genes, 20 are ORPHAMs. ORPHAM are predicted to encode proteins but share little homology to any other genes in the Phagesdb pham database. The large number of ORPHAMs has made Kikiko annotation particularly interesting and challenging. Functional annotations were completed using various applications including PECAAN, GeneMark, Phamerator, HHpred, NCBI databases, PhagesDB, and Starterator. Annotations are currently in the process of being reviewed and finalized prior to submission.   
  
A unique characteristic of Kikiko is that it is the only Arthrobacter phage in the EM cluster among the other Microbacterium phages which infect Microbacterium paraoxydans and Microbacterium foliorum. The overall aim of this project is to characterize Kikiko and compare it to other EM cluster phages. Comparative genomic data has shown that Kikiko is dissimilar to other EM cluster phages with gene content similarity averaging 50.81%. This lack of similarity contributes to the uniqueness of Kikiko within the EM cluster. We are currently using host range assays to test whether Kikiko can infect Microbacterium hosts as well as if Scruffy, another EM cluster phage, can infect Arthrobacter globiformis. Preliminary data shows that Kikiko can only infect A. globiformis, although additional work may be done to test if Kikiko and Scurffy can bind to multiple hosts.   
  
AlphaFold is also being used to further investigate gene functions by generating predicted protein structures based on amino acid sequences. AlphaFold has aided in the functional assignment of gene 34. This ORPHAM has strong probability on HHpred of being an SGNH glycosidic. Analysis in Alphafold confirmed an N-terminal SGNH hydrolase domain, as well as high probability structural prediction of a C-terminal carbohydrate binding domain. We hypothesize that gp34 is a bifunctional protein that is targeted to and cleaves carbohydrates during the infection cycle. Further AlphaFold analysis of additional ORPHAMs may reveal additional gene functions that may help explain Kikiko’s relationships to other EM cluster phages.