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Now that’s the Tea on Oksu

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Bacteriophages are viruses that infect bacteria and they are among the most diverse organisms on the planet. Bacteriophages have emerged as a topic of interest due to their unique biology and therapeutic potential. During the 2022 fall semester, students from the Translational and Molecular Medicine program at the University of Ottawa isolated and characterized bacteriophages specific for various host bacteria. Through this initiative, the novel bacteriophage Oksu was identified, using *Mycobacterium smegmatis* as a host. Since *Mycobacterium smegmatis* is a new host strain used at the University of Ottawa, Oksu was an ideal candidate for further investigation and characterization.

Oksu was isolated from a soil sample collected from a garden bed near Roger Guindon Hall in Ottawa, Ontario. The unique characteristics of this bacteriophage were discovered using transmission electron microscopy (TEM), restriction digest and Illumina sequencing. Oksu was named after a Korean corn tea that the student enjoyed drinking during her early childhood years. Oksu is a temperate bacteriophage with siphoviridae morphology that produces plaques with a clear center and cloudy outer ring. It is one of the 205 members of the F1 sub-clusters of mycobacteriophages. Illumina sequencing of Oksu uncovered a genome length of 59317 bases, including 108 genes, of which 4 are orphams.

Using bioinformatic software including PECAAN, BLASTp, Starterator, HHPred, DeepTMHMM and Phamerator, we annotated Oksu’s genome by identifying and characterizing each gene. One of Oksu’s unique properties is its moderate gene content similarity to other comparator bacteriophages, which was only approximately 60%. Due to this low similarity with other comparator bacteriophages, Oksu did not exhibit strong synteny with other bacteriophages, particularly near the end of the genome. Using a phylogenetic tree, we found low genetic homology between Oksu and bacteriophages that infect different species and strains. Moderate similarity was identified only within *Mycobacterium smegmatis* mc2155 bacteriophage strains, the same strain that Oksu infects, indicating a narrow host range.

Alongside annotations, additional wet lab experiments and comparative genomics were carried out to learn more about Oksu. We have isolated lysogens of Oksu and are in the process of assessing super-infection immunity and whether the other mycobacteriophages found this Autumn are capable of infecting the Oksu lysogen. We are also using AlphaFold predictions and Foldseek searches to see if novel functions can be identified for orphams and NKFs in Oksu.