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Characterization and annotation of a novel FM-cluster bacteriophage Ottawa

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Bacteriophages are viruses that infect bacteria. Phages that infect actinobacterial species are grouped into over 140 clusters with syntenic similarity above 50% between the genomes within the same cluster. This implies great functional similarity between phages of the same cluster, but significant variability across multiple clusters. Based on the remarkable abundance and diversity of bacteriophages, sequencing genomes can uncover novel phage clusters that do not meet the >50% similarity threshold.

We are investigating the novel FM cluster which includes the Ottawa and Kharcho phages, both discovered by the uOttawa phagehunters class. Found on the bank of the Ottawa river, Ottawa is a *siphoviridae*-family bacteriophage with infective specificity against *Arthrobacter globiformis*. Ottawa’s genome includes 111 putative genes across 63522 base-pairs, of which 65.2% are GC pairs. Plaques generated on an *A. globiformis* lawn are generally small, with variable cloudiness suggesting mixed lysogenic and lytic character. Ottawa’s genome contains 60 potential orphan genes.

Our team is identifying essential genes to further elucidate the infective abilities of Ottawa. Early investigations show that the putative tape measure protein (TMP) gene in Ottawa spans 7.3% of the entire genome, compared to <2% in phages with the greatest synteny for this gene. Although this gene function is conserved in most phages, this observation may represent a potential marker for FM phages. The long TMP gene is reflected by Ottawa’s long tail (270nm).

Upon digestion by restriction enzyme, only two of eight assayed enzymes were able to partially cleave Ottawa’s genome, which is similar to the behaviour of the Kharcho genome. Based on preliminary studies in Kharcho, it is apparent that the six enzyme recognition sites were not present within the genome. Kharcho was incompletely digested by the remaining two enzymes, possibly indicating the presence of 2-aminoadenine or DNA methylation modifications. This observation is hypothesized in Ottawa, but future experiments with restriction enzymes will allow to make definite conclusions. These properties may confer major advantages to phages in protecting against bacterial nucleases.

We are also investigating a potential translational frameshift located at gene 44 in Ottawa, which encodes a tail assembly chaperone (TAC) gene. A frameshift may exist with gp45, but the frame-shifted protein would have a C-terminus without homology to a TAC gene. We are creating an E. coli expression plasmid that will tag gp44/45 with an N-terminal GST and allow us to examine if this frameshift can occur in E. coli. If E. coli does support the frameshift, we will purify the frame-shifted protein and identify the slippery sequence by sequencing the protein using mass spectrometry.