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Identifying the blank space in Streptomyces bacteriophages Superstar and Rideau

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The genus *Streptomyces* are soil-dwelling bacteria known for their production of a variety of chemically diverse metabolites, commonly used in agricultural and clinical industries. Unlike most other Actinobacteria, *Streptomyces* produce hyphae that septate to form unigenomic spores. Phages preferentially infect early during the *Streptomyces* cell cycle, while spores are germinating into hyphae.   
  
Novel bacteriophages *Superstar* and *Rideau* were isolated on the host *Streptomyces avermitilis* in Ottawa, Canada by students in the SEA-PHAGES discovery lab at the University of Ottawa. *Superstar* has *Siphoviridae* morphology and is characterized by its long noncontractile flexible tail and icosahedral capsid. *Superstar* is a temperate phage, deduced from the presence of an immunity repressor and integrase genes. *Rideau* has *Podoviridae* morphology and is characterized by its short tail, tail fibers and icosahedral capsid. *Rideau* is a lytic phage, as there are no lysogeny genes.   
  
These phages belong to different phylogenetic clusters, indicating significant genome-level differences. *Superstar* is a cluster BD2 phage with a 50,411bp genome with 65.8% GC content. Annotation reveals *Superstar* is comprised of 85 genes, 36/85 (42%) of which were determined to be of no known function (NKF). *Rideau* is a cluster BF phage with a 46,053bp genome with 60.4% GC content. It is comprised of 68 genes, 23 of which interestingly encode tRNAs. Through functional annotation and wet-lab experimentation, we have examined interesting biology of these phages.  
  
A dot plot comparing *Superstar* against other BD2, BD3, and BD1 cluster phages and *Rideau* illustrate a high degree of nucleotide similarity between *Superstar* and other BD2 phages. Notable similarities with BD1 and BD3 phages are generally localized in the middle of the genome. Contrastingly, no similarities were identified between *Superstar* and *Rideau*, despite their overlapping host range.  
  
The GenemarkS coding potential of *Superstar* gp70 and gp70 suggested that a programmed translational frameshift could create the fused gp70/71 protein and we identified a potential slippery sequence that could mediate this frameshift. We have created an inducible *E. coli* expression plasmid of gp70/71 that will allow us to test if a translational frameshift between these two genes can occur in a heterologous system, as has been seen for tail assembly chaperones.   
  
Using AlphaFold2 software, we created structural predictions of several interesting genes in *Superstar* and *Rideau* and compared using FoldSeek against databases protein structures to elucidate their putative functions. Preliminary work suggests that *Superstar* Gp6 may encode a HEPN-RNAse toxin gene and we hypothesis that Gp4, which shares structural similarity to Gp6, may encode its antitoxin; this mechanism has not been documented in Actinobacteriophages and may be a novel prophage defense mechanism in this phage.