Bioinformatics of Local Bacteriophage Orcanus

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Abstract

Bacteriophages are viruses that infect a bacterial host to replicate. There are more bacteriophages than any bacteria, animal, or plant in the global ecosystem. There are an estimated 10^{31} population of bacteriophages in the world (Hatfull, 2011). One reason that bacteriophages should be studied is that they have an undiscovered amount of genetic diversity because of their massive population. Studying this untapped potential will help understand more about genetics and specific genes. A soil sample was collected, and a bacteriophage was filtered out of it. After the bacteriophage was isolated, DNA was extracted and sent in for sequencing. Once the sequenced genome was sent back it was compared to the sequences of other phage genomes. The location of genes in the sequence were determined by using computer programs such as DNA master, Phamerator, PECAAN, Starterator, Glimmer and GeneMark. These web-based programs were also used to determine the function of the genes in the Orcanus genome. Orcanus is a siphoviridae, temperate phage in the AS cluster and AS1 sub cluster. For the phage Orcanus, the gene that codes for tyrosine integrase was present. This supports the idea that Orcanus was a temperate phage, meaning that the phage can integrate with the host's genome. Through the analysis of the genetic sequence, new genes with unknown functions were observed as well as previously documented genes. The research on the specific genes of Orcanus broadened the database and understanding for bacteriophage genetics that could be used for future research.

Methods

- Soil samples were collected, and enrichment cultures were set up to test for bacteriophages in four different bacteria samples
- Using a spot test, it was found that there was a phage that is active in the bacterium *Arthrobacter globiformus*
- A plaque assay was used to purify the bacteriophage. It was repeated 7 times to ensure homogeneous morphology of the bacteriophage (purity) See Figure 1.A
- The seventh plaque assay resulted in a web plate that was used for collecting the plate lysate. A spot titer was used to calculate the concentration of the phage. See Figure 1.B
- The phage was viewed using a transmission electron microscope See Figure 1.C
- DNA of the phage was isolated using phenol/chloroform extraction
- The phage genome was annotated using the web-based programs of phamerator, starterator, PECAAN, DNA master, Glimmer, and GeneMark

Results

- There were 3.3 * 10^{12} pfu/mL in the spot titer
- The tail measured 109 nm and the head measured 61 nm
- The concentration of DNA measured 133.7 ng/uL

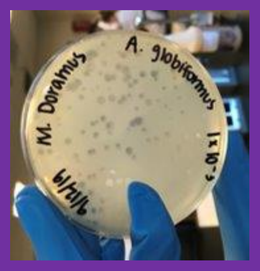

Figure 1.A

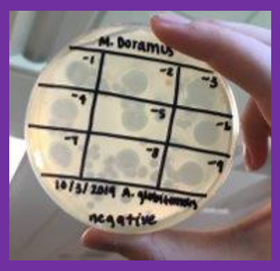

Figure 1.B

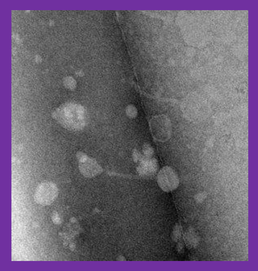

Figure 1.C

attP and attB Sites

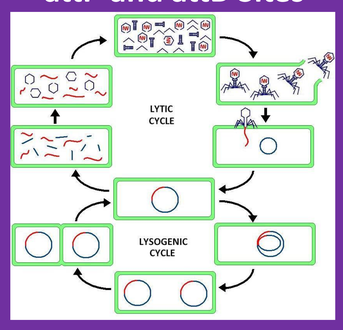

Figure 2

- Figure 2 displays the life cycle of a temperate phage. A phage inserts genetic material into the bacterial cell. If the phage is temperate like Orcanus, the genetic material can integrate into the bacterial genetic material and reproduce with the bacterial cell. This is known as the lysogenic cycle. The phage can switch to the lytic cycle where it uses the bacteria genome to produce the phage components into new phages until the bacterial cell bursts.

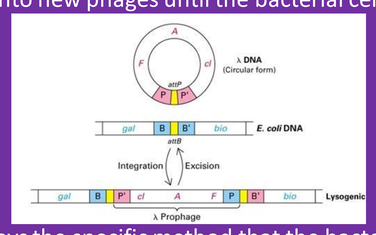

Figure 3

- Figure 3 displays the specific method that the bacteriophage uses to integrate with the bacterial DNA. The attP site of the bacteriophage aligns with the attB site of the bacteria then integration occurs. Using BLASTn, the genome of Orcanus was compared to Arthrobacter sp. 21022. There was a 24 base pair similarity that had a 100% match between base pairs 21,285 to 21,307 in Orcanus and 3,374,505 to 3,574,527 in Arthrobacter sp. The site corresponds within the bacterial genome where the gene codes for the tRNA for proline.

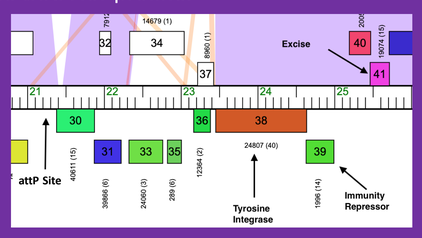

Figure 4

- Figure 4 displays an annotated phamerator photo of Orcanus from base pair 21,000 to 26,000 which includes the genes that code for tyrosine integrase, immunity repressor, and the excise proteins as well as the attP site. The integrase catalyzes the integration of genetic material into the lysogenic cycle. The immunity repressor inhibits lytic genes, so no lysing of the cell occurs. The excise protein catalyzes the excision back into the lytic cycle. Locating and determining the function of these genes in Orcanus's genome support the categorization of the phage as a temperate phage.

Conclusion

- Orcanus is in the subcluster AS1 with three other discovered members.
- Orcanus contains 63 genes within the genome.
- There are 38,737 base pairs within the genome.
- The bacteriophage was discovered within the College of Idaho Campus.
- The plaque formations displayed temperate characteristics.
- Gene 34 was tyrosine integrase; the function of this gene is to create enzymes which embed the viral genome into the bacterial host.
- 40 genes had predicted functions by analyzing the genomes of other phages.
- **23 genes did not have predicted functions because the genes had unique sequences.**
- **Lysin A was present while Lysin B was not present. Lysins are enzymes that help break down the cell walls of the bacteria.**

References

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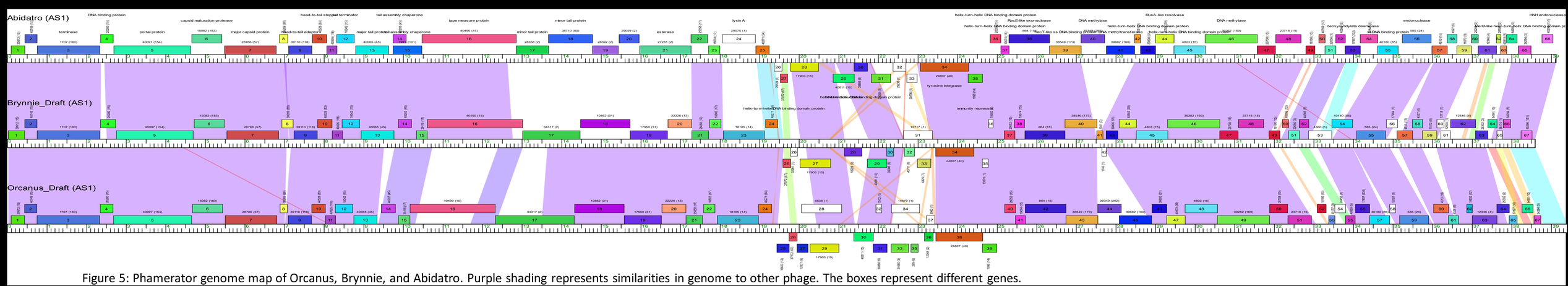

Figure 5: Phamerator genome map of Orcanus, Brynnie, and Abidatro. Purple shading represents similarities in genome to other phage. The boxes represent different genes.