First in Phlight: Characterization of Two Novel Phages, Iridoclysis and Nidhogg, Isolated in North Carolina


Introduction

• The world’s dependence on antibiotics has led to renewed interest in the potential use of mycobacteriophages either prophylactically or therapeutically in the treatment of tuberculosis.

• To contribute to the genomic characterization of mycobacteriophages, we used Mycobacterium smegmatis as a host to isolate two novel bacteriophages, Iridoclysis and Nidhogg, by enrichment in Fall 2015.

• Both bacteriophages were annotated and characterized by bioinformatics and laboratory experiments.

Nidhogg Characteristics:

• Found in Raleigh, NC, in a grassy, semi-fertilized area, frequently watered with rainwater.

• A subcluster C1 phage with 156342 bp.

• 65% GC content displaying a Myoviridae morphotype.

• 35 tRNAs identified within the genome.

Iridoclysis Characteristics:

• Found on campus of Durham Technical Community College in a flower bed.

• A subcluster B1 phage with 68587 bp.

• 66.4% GC content displaying a Siphoviridae morphotype.

Genome Annotation

• DNA Master, PhagesDB, NCBI BLAST, Phamerator, Starterator and HHpred software programs were used to identify genes and gene functions.

• Web-based programs, Aragorn and tRNAscan-SE, were used to find tRNA genes and identify their correct ends.

An annotation of an Additional Gene Not Called by DNA Master to Nidhogg’s Genome

Annotations of tail assembly chaperone translational frameshift in Nidhogg

• Nidhogg has a -1 programmed translational frameshift.

• In many bacteriophages, a programmed translational frameshift makes two proteins with overlapping amino acid (aa) sequences essential for tail assembly.

• Frameshifts occur when there is a "slippery" sequence in the mRNA where the ribosome can occasionally change reading frame and translate an alternate protein.

• In Nidhogg, the ribosome enters the "slippery" sequence GGGAAGA in the 3rd reading frame (red) of gp213.

• The ribosome either continues in the third reading frame producing a 528 aa protein or shifts back one base pair on the mRNA into the second reading frame and continues to the end producing a 900 aa protein.

• The frameshift coordinate was identified at 72127 bp where the A (yellow) is counted twice in translation.

Effect of Temperature on Plaque Formation

• Nidhogg demonstrated ability to grow over a range of temperatures (20°C, 37°C, 42°C) with an optimal temperature of 37°C.

• Nidhogg showed a limited tolerance to the range of temperatures tested, only optimally producing plaques at 37°C.

Effect of Drying on Phage Stability

• Dried out plaques from both Iridoclysis and Nidhogg were reconstituted in 15 μl after a range of drying times (4 – 14 days), 10 μl was mixed with M. smegmatis and the phage titer was calculated after 24 – 48 hours of incubation.

• A decrease in plaque stability proportional to the drying time was observed.

• Notably, both phages were still producing plaques after two weeks of drying, demonstrating resilience to less than ideal conditions.

Bioinformatics Experiments

• Mycobacteriophage genomes use ATG, GTG as start codons.

• We calculated that Nidhogg has a preference for ATG as a start codon: 78.2% of genes used ATG; 21.3% GTG and 1.5% used TTG.

• Iridoclysis also has a preference for ATG: 68.2% of genes used ATG, 24.0% used GTG, and 7.8% used TTG.

Potential Transmembrane Protein Identification in Nidhogg and Iridoclysis

• Identification of the two transmembrane helices in Holin, a protein characterized by presence of transmembrane domains.

• Holin aids in release of phage particles from an infected host cell.

• Holins are extremely diverse and have not been identified for all phages including Iridoclysis.

Conclusions

Nidhogg

• Nidhogg is a 156 kb subcluster C1 phage with 65 % GC content.

• Annotation was manually refined for a total of 272 open reading frames - 266 genes are transcribed in forward orientation and 6 genes in reverse orientation.

• Consistent with other C1 phages, a -1 programmed translational frameshift was annotated in the tail assembly chaperone genes and 35 tRNA genes were annotated.

• Growth of Nidhogg is temperature sensitive with an optimal temperature of 37°C. Growth was severely disrupted at the other temperatures tested.

• Nidhogg is capable of producing lysogens and further work is required to determine the immunity status and whether the lysogens produced are stable.

• Stability of Nidhogg and Iridoclysis decreases with drying time, but plaques could still be produced for up to two weeks.

Iridoclysis

• Iridoclysis is a 69 kb subcluster B1 phage with 66.4 % GC content.

• Annotation was manually refined for a total of 101 open reading frames - 49 genes are transcribed in forward orientation and 52 in reverse orientation.

• Consistent with other B1 phages, no programmed translational frameshift was annotated and no tRNA genes were identified.

• Iridoclysis is less temperature sensitive than Nidhogg growing at all tested temperatures except 4°C. The optimal temperature was 37°C. Lack of growth at 4°C is explained because M. smegmatis did not grow at 4°C.

• Iridoclysis grew optimally when 0.5 mM CaCl2 was added to the top agar. The phage titer decreased with increasing calcium concentration.

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1. Hatfield, PLOS Pathogens 2014, 10

2. Cataldo, J, Bacteriol 2011, 193