5-22-2017

Isolation and Comparative Genomic Analysis of Final Third of Satis Genome

Kelly Hartigan
Nicole Curnutt
Matthew McDermut

Follow this and additional works at: http://openscholarship.wustl.edu/undergrad_research

Part of the Bioinformatics Commons, Biology Commons, Computational Biology Commons, and the Genomics Commons

Recommended Citation
Hartigan, Kelly; Curnutt, Nicole; and McDermut, Matthew, "Isolation and Comparative Genomic Analysis of Final Third of Satis Genome" (2017). Undergraduate Research Symposium Posters. 104.
http://openscholarship.wustl.edu/undergrad_research/104

This Unrestricted is brought to you for free and open access by the Undergraduate Research at Washington University Open Scholarship. It has been accepted for inclusion in Undergraduate Research Symposium Posters by an authorized administrator of Washington University Open Scholarship. For more information, please contact digital@wumail.wustl.edu.
Isolation and Comparative Genomic Analysis of Final Third of Satis Genome

Kelly Hartigan, Nicole Curnutt, Matthew McDermut
Mentors: Christopher Shaffer and Kathleen Hafer

Abstract

A highly novel Streptomyces phage, Satis, was isolated from a direct environmental sample collected from outside Danforth House on the Washington University campus. Satis infects bacterial species Streptomyces lividans producing propionyl, closely pleated less than 1mm in diameter. Electron microscope data shows rare apical physical features. Rather than the common octahedral capsid shape, Satis has a prolate head with visible cross-linked hexagonal protein structure and average measurements of 383 nm by 47 nm with a long, flexible tail measuring 268 nm. Upon sequencing, it was found that Satis contains the longest phage genome discovered to date through the SEA-PHAGE program at 186,702 base pairs. The genome is quite novel in sequence, as its closest genetic match, bacteriophage Chymera, is similar across only 0.2% of the genome. This means that Satis belongs to no known previously characterized cluster and is considered a Singleton phage. The genome contains 325 protein coding genes, of which our group analyzed Gene 230 to the end of the genome. The vast majority of the genes in this section run 3’ to 5’ and compared to the other two sections, these genes seem to be the most unique in primary, secondary, and tertiary structure. Due to the novelty of Satis, functional evidence from comparative genomic analysis is sparse. We are currently in the process of a more thorough comparative genomic analysis between Satis and other Streptomyces phages, particularly phage JustBecause, another Streptomyces phage isolated by Washington University in St. Louis students in 2016 with similar morphology to Satis.

Characterization

Table 1: Average size of Satis with standard deviation. Calculated using a sample of five TEM photos and analyzed using ImageJ.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Start</th>
<th>Stop</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>243</td>
<td>145051</td>
<td>145454</td>
<td>Phosphatase Domain of Polyphosphate Kinase</td>
</tr>
<tr>
<td>266</td>
<td>155489</td>
<td>155981</td>
<td>Antisense DnaG</td>
</tr>
</tbody>
</table>

Table 2: Functional annotation calls of final third of Satis genome. Shows highly variable region of genome.

<table>
<thead>
<tr>
<th>Gene Order Conservation</th>
<th>Ortholog Number</th>
<th>Average Ortholog Similarity</th>
<th>Average Ortholog Identity</th>
<th>Global Alignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>97.75</td>
<td>226</td>
<td>0.9740</td>
<td>0.5898</td>
<td>.839</td>
</tr>
<tr>
<td>62.01</td>
<td>56.06</td>
<td>6.6e-40</td>
<td>6.6e-30</td>
<td>.5386</td>
</tr>
</tbody>
</table>

Table 3 & 4: Comparisons of homology of JustBecause and Chymera against Satis showing low level of gene order conservation between the two phage.

Figure 1: Phage Isolation fromOutside Danforth House on the campus of Washington University in St. Louis

Phage Functional Evidence

Figure 2: Plate photo showing plaque morphology. Satis creates pinpoint closely pleated less than 1mm in diameter as shown by dark spots circled on plate.

Figure 3: TEM photos of two Satis phages: showing prolate head and long flexible tail characteristic of the sphondinace family of phage. Taken at 25000x magnification.

Figure 4: TEM photo of JustBecause at same magnification, showing very similar size and morphology between the two phage.

Figure 5: Close up of Satis head shell shows interlocking grid of hexagonal capsid proteins.

Evolutionary Implications

- Phages display wide genetic diversity; Satis and JustBecause have genomes that are highly unique on both the DNA, amino acid sequence, and protein level.
- Multiple mechanisms for phage genomic evolution:
  - Satis, part of the sphondinace family, has conserved ORF DNA sequence, with high number of orthologs supporting the existence of a common ancestor for Satis and JustBecause, seen on SplitsTree.
  - Lack of synteny and low number of orthology between Chymera and Satis indicates a much more distant common ancestor between Satis and its other most closely related Streptomyces phage, also shown on SplitsTree.
  - Viruses with the highest mutation rates tend to have phage that are highly conserved, whereas their gene products are conserved.

Phages may be classified as 'phage tolerant' or 'phage sensitive' to mutational change; therefore their gene products are conserved.

- Possibility: Satis has a higher mutation rate than other Streptomyces phages due to its significantly larger genome.
- Fidelity may be sacrificed for speed of replication to improve Satis’ competitive fitness in out-competing other Streptomyces phages.

Acknowledgements & References

Figure 11 (Bottom): Ortholog map of entire genome of Satis vs JustBecause. Orthologs are shown with same color, unique genes are blacked out.

Figure 12 & Table 5: Satis 29 vs JustBecause 25

Figure 13 & Table 6: Satis 144 (blue) overlaid with JustBecause 144 using Phyre results left. Table below shows top HHPRED results for both genes. Results show very conserved secondary and tertiary structure.

Figure 14: SplitsTree figure shows gene similarity giving a rough evolutionary map of all Streptomyces phages published on Genbank.

Figure 15

Figure 6: The phamameter map for genes 231-325 is shown above. The majority of the genes in this section are orphans: meaning they don’t fit into any currently annotated protein families in the SEA-PHAGE program.

Figure 7: Satis gene 242 (blue) overlaid with DnaG antisense from Alcaligenes. Phyre Protein Modeling showed 7 of 8 conserved active sites in the two proteins shown in orange.

Figure 8: Satis gene 266 (blue) overlaid with DnaG antisense from Alcaligenes. Phyre Protein Modeling showed 6 out of 9 conserved active sites in the two proteins shown in orange.

Figure 9: System map of Satis and Streptomyces phage Chymena shows very low level of gene order conservation. Chymena is the closest match to Satis currently published.

Figure 10: Synteny map of Satis and Streptomyces phage JustBecause also found this year showing extremely high gene order conservation between the two phage.

Figure 11: Annotation map of genes 231-325.

Table 5: Gene Comparison of Satis vs JustBecause (Orthologs in purple for Satis). ORF851 to ORF1111.

Table 6: Ortholog Case Comparisons

Table 1: JustBecause ORFs. ORF851 to ORF1111. Gene Order: 97.75 bits vs 62.01 bits. Ortholog Identity: 0.5848 vs 6.6e-30. Global Alignment: 0.5386 vs 6.6e-40.

Figure 16: SplitsTree figure shows gene similarity giving a rough evolutionary map of all Streptomyces phages published on Genbank.

Figure 17: SplitsTree figure shows gene similarity giving a rough evolutionary map of all Streptomyces phages published on Genbank.