Introduction

- SCSU Genomics I students isolated 7 mycobacteriophages that infect M. avium mc^2 155 at 42°C.
- Three of these genomes were sequenced which revealed their identity in the A11 (Lucivia and Flaverint, joining Snape which was sequenced at SCSCU in 2014) and K4 (Wintermute) subclusters.
- Wintermute, the sixth phage in the K4 subcluster, is a part of a cluster of phages that is capable of also infecting M. tuberculosis (Mtbc). Flaverint is Wintermate's closest relative and can infect Mtb 20.0 times more effectively than it can M. avium.
- Bioinformatic analyses of Lucivia demonstrate how subcluster A11 phages are capable of forming lysogens without the phage and bacteria anFHRII cassettes and an integrase. Analyses may also show the presence of stoperators which are unique to cluster A phages.

The Issues

- The investigation of the unique genomes of cluster K and A phages allows us to gain insight on how they can infect their hosts. Each cluster has characteristics that are unique to that cluster, and investigating them using bioinformatics may show us how different clusters are capable of infecting bacteria.
- Understanding how certain clusters of mycobacteriophages can also infect Mtb has proven to be difficult to investigate bioinformatically, but these analyses have the potential to lead to wet lab experiments that will hopefully provide definitive answers.

Results/Discussion

- **Phage Hunting at SCSCU**
  - 3 phages sequenced at SCSCU in 2015: Lucivia (A11), Flaverint (A11), Wintermate (K4)
  - Wintermute subcluster
  - 75 Members in 8 subclusters
  - Several phages shown to have Mismaged/mtbc infectivity

- **Start-Associated Sequences (SAS)**
  - 13 tip motif found in all only 1 cluster phages
  - Thus far, SASs are located in the more variable “right” side of the genomes

- **SAS** (K4): General Intergenic SAS, 692 instances

- **Hammy (K6): SAS exhibits tip overlap, 143 instances
- Zouli (K2): SAS 54 bp from ORF start; 8 instances

- **Figure 1.** Subcluster K4 is most closely related to subclusters K2 & K6. Phylogenetic tree of cluster K whole genome sequences from ClustalO.

- **The K Cluster**
  - Now includes newly sequenced Wintermute (K4)
  - 75 Members in 8 subclusters
  - Several phages shown to have Mismaged/mtbc infectivity

- **Figure 2.** Different types of SAS found in K4, K6, and K2 phages found in different regions in each genome.

- **The majority of gene phams that flank SASs have no known function**
  - Phams with unknown function
  - Phams with known function

- **Figure 3.** Site denoted number of pham genes associated with SAS. Color denotes the number of SAS associated genes in a pham divided by the total number of cluster K genes in that pham as a percentage. Generated with TreeMap by Macrotous.

- **Are there phams that correlate and predict Mtbc infectivity?**
  - There are no phams that only have membership in K, G, A2, and A3 subclusters.

- **Figure 4.** Venn plot of Pham Uniqueness in K, G cluster and A2 A3 subclusters. Generated in the program “R.”

- **Figure 5.** Distribution of shared unique phams by subcluster. Yellow denotes pham presence, and red denotes absence. Generated in the program “R.”

- **The A11 subcluster**
  - 9 A11 subcluster phages: S3 found at SCSCU
  - Lucivia (2016)
  - Flaverint (2016)
  - Snape (2014)
  - Discovery date range = 2012-2015
  - Ave. length = 52,275 bp
  - Av. GC = 63.7%.

- **Figure 6.** Phylogeny of the subcluster A11 phages. Lucivia and Flaverint are most closely related to each other. Heatmap created using ‘pyJan’.

- **Figure 7.** Function of the ParABS system (doi:10.1038/microbiol8820).

- **Figure 8.** Consensus of cluster A parABS ‘phammaries’ Created in CLC Sequence Viewer.

- **Stoperators: immunity systems in cluster A phages**
  - Short repeating genetic elements that control lysogeny by preventing transcription in genes that are not responsible for infecting the phage.
  - First found in L5 (A2) and Bxb1 (A1)
  - 20 copies in Lucivia
  - They are 13 bp in length, and are found mostly in intergenic regions

- **Figure 9.** Consensus stoperator sequences in A11 phages. S3 found at SCSCU. There are 5 stoperators in the consensus sequence.

- **Figure 10.** Predicted stoperator consensus sequence of the A11 phage Lucivia. Created using MEME Suite.

Conclusions

- We discovered and performed analyses on two new phages in subclusters K4 and A11 and found interesting features exclusive to those subclusters.
- SAS’s exhibit three general types, Intergenic, Tip overlap and 54bp from a gene start. The function of these sequences has yet to be determined and their pham association is widely varied.
- There is no clear consensus between the phams of Clusters A2/A3, G, and K who have shown the ability to infect Mtbc.
- Subcluster A11 phages contain ParABS systems and replicate extrachromosomally as opposed to integrate proteins. Cluster A phages also contain stoperators, which have not yet to be analyzed in subcluster A11 phages, but were identified in Lucivia with a short identical nucleotide match to the stoperator sequences found in A1 and A2 phages, using bioinformatics software.

Future Studies

- Analysis of the SAS function and determine their position relative to RBS sites.
- Wet lab experiments analyzing the efficiency Wintermute has to infect Mtbc (done by others).
- Analysis of the intergenic phams in the phams shared by Mtbc infective phages.
- Further analysis on stoperators in A11.

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