Introductions

- Represent team from Hope College in Holland, MI
- Undergraduate liberal arts college
- Program at Hope
  - Phage Genomics Research course
  - Honors course for 20 first-year students
  - Replaced standard intro biology lab in the fall semester

About Me...

- Hometown: Ramsey, MN
- Major: Biology
- Interest: Molecular Biology

The Hope College Team
Isolation and Purification

- All soil samples collected from Holland area
- 20 phages successfully isolated (100%)
  - Students worked independently
  - 15% isolated phage by direct plating
  - 85% used an enrichment procedure (Lorenz)
- Diverse morphological and structural characteristics

Selection of Phage

Ideal Criteria
- Purity of Phage isolate
- Evidence of Uniqueness
  - Plaque morphology
  - RE digest pattern
- Phage structure
- Quantity and Quality of DNA

Our Phage Olympics
- 20 isolates (100%)
- ~15 unique phages isolated
- Diverse plaque morphology
- 19 successful RE digests confirmed diversity within class
- No EM in time for consideration
- Only 3 had good quantity
Our Selection—Pumpkin

- Pure phage stock
- EcoRI digest indicated relationship to Cluster E
- ~100 μg high quality DNA

Caitlin Peirce isolated Pumpkin from a vegetable compost pit.

Pumpkin – Phenotype

**Plaque morphology**
- 1-2 mm clearing surrounded by turbid ring
- comet shape

**Phage Structure**
- 69 nm capsid
- 240 nm tail
- Siphoviridae

Pumpkin – Genomic Sequence Overview

**Genomic Statistics**
- 74,491 bp
- 63% GC
- 140 genes
  - 138 protein encoding genes (PEGs)
  - 2 tRNA genes (Arginine & Glycine)

**Genome Structure**
- Linear genome, cohesive ends
Pumpkin – Comparative Analysis

- Validated relationship to cluster E
- No orphan genes
- Matched 4 genes previously orphaned in 2 cluster E phages

Characterization of Pumpkin Genes Matching Other Myxoviruses/Phage Genes

Pumpkin – Annotations

Putative functions assigned to 26 PEGs
- 19% of genome

Polynucleotide Kinase/Phosphatase (Pnkp) and RNA ligase
- Demonstrated function in RNA repair and ligation
  - E. coli phage T4
  - Pnkp enzyme activities shown in Cjw1 and Omega
- Pnkp and RNA ligase genes in all cluster E phages + Omega
- Hypothesis: Pnkp and RNA ligase used to evade bacterial response to viral infection?
Pumpkin – Cluster Analysis

- Relationship between cluster E and non-clustered Omega

![Diagram showing relationships between clusters A, B, C, D, E, and F]

Pumpkin gene location and percent protein identity to matching Omega gene products

![Graph showing gene location and percent identity]

Conclusion

- Success isolating and characterizing
  - All 20 first-year students isolated phage
  - Diversity among class: ~15 are unique
- Selected novel phage for sequencing – Pumpkin
- Successfully analyzed complete genome of Pumpkin
  - 140 genes
  - Assigned function to 26 PEGs (19%)
  - Confirmed cluster E relationship
  - Propose relationship of Omega to cluster E
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