Reemergence of Specialized Φ6 Bacteriophages

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Objective

To determine if specialism can be easily reversed in Φ6 through the use of experimental evolution.

Φ6

- Bacteriophage in the *Cystoviridae* family
- Infects pathovars of plant pathogenic bacteria *Pseudomonas syringae.*
- Unique due to lipid membrane and segmented, double stranded RNA genome
- Shares similarities to animal viruses, allowing it to be a safe alternative to gain of function research.

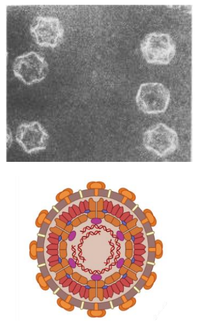

Figure 1. Electron Transmission microscope image and model of Φ6– cross section highlighting 3 dsRNA segments in genome.

Experimental evolution can help us learn how dsRNA viruses expand or narrow host range.

- Φ6 can **specialize** to become better at infecting a host at the cost of a narrower host range.
- Can Φ6 revert this change and **generalize** its host range?

Preparation

- LC media (1L Millipore filtered water, 10g NaCl, 10g tryptone, 5g yeast), pH balanced to 7.5) was prepared in lab to grow bacteria and phage. We used various concentrations of agar to produce softer top agar and regular agar.
- Selected three viral mutants from previous specialization experiments (3eN, 1eNd20, and 1eNd30).
- *Pseudomonas syringae* pathovars were used as host and were provided: the novel host ERA (*P. s. pv East River isolate A*), and challenge hosts TOM (*P. s. pv tomato*), PP (*P. s. pv phaseolicola*), and ATRO (*P. s. pv atrofaciens*).
- Various lab equipment was used to generate data such as a centrifuge, colony counter, heat block, Bunsen burner, vortex mixer, and micropipettes.

Figure 2. Various pictures of lab equipment and processes used to procure data for the experiment. Includes agar plates, LC media being prepared, and the colony counter used to count plaque forming units.

Research

- Exposed specialized phage to other hosts to promote a host shift.
- Fresh lysate is extracted from phage infected ERA.
- Dilutions of starting lysate are tittered to ensure countable, clear plaques between 30-300 units.
- Top agar is inoculated with bacteria, vortexed, then inoculated with phage, vortexed, and poured onto agar plate.
- The number of clearing forming plaque units after incubation were recorded on an Excel sheet. Counts above 300, or too many to count (TMTC), were excluded.

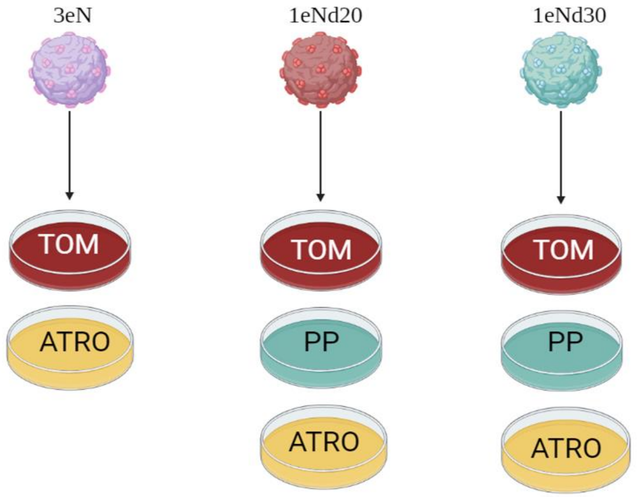

Figure 3. Diagram showing the mutant-host trials, excluding the tests done on the novel host ERA. Each trial was completed in replicates of three. PP was excluded from 3eN trials as it was a permissible host.

Figure 4. 1ed30 on PP at varying dilutions.

Analysis

- Below is the scatter plot generated from the data. The data points represent the average PFU/mL of the various 11 mutant-host trials. Vertical bars represent standard deviation.
- We calculated the PFU/mL (measure of infectious units per mL of fluid) by taking plates with countable plaques and multiplying the number plaques by the reciprocal of the dilution it was observed at.
- While 3eN and 1eNd30 had the easiest time infecting TOM out of the challenge hosts, 1eNd20 had the easiest time infecting ATRO. **It may be easier for a virus to infect a host it has never been exposed to.**

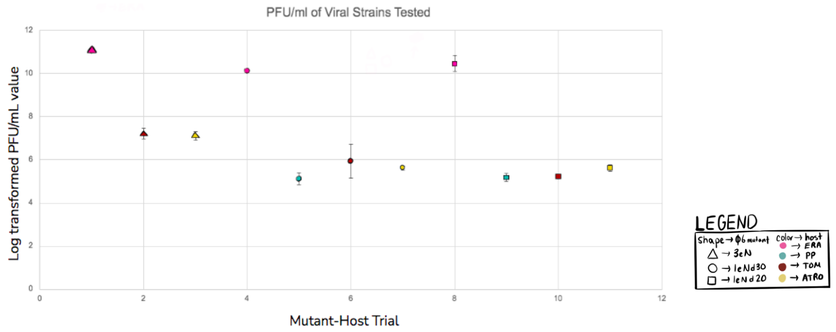

Figure 5. Log-transformed average PFU/mL of each trial graphed.

Limitations

- Triplicate plates had to be redone twice.
- Issues, with procedures involving gel-electrophoresis and re-extraction.
- Poor ng/µL concentration of extracted RNA further delayed sequencing.

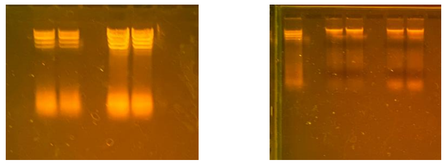

Figure 6. Pictures of failed gels.

Results & Future Work

Results

- Specialism is reversible in Φ6.
- Preliminary T-tests shows 1eNd20 has a harder time reinfecting TOM than 3eN and 1eNd30.

Future Work

- Receiving sequenced RNA to analyze host-range expansion mutations.
- Procure more plate count data to confirm previous findings.
- Evolve Φ6 to withstand various environmental factors and measure virulence.

Acknowledgements

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