Let's Talk About Phage, Baby!

Using Growth Curves to Quantify Bacterial Resistance to Phage

Teresa Carter[1,3] Mike Blazanin[2] Paul Turner[2]
[1]Middle Tennessee State University
[2]Department of Ecology and Evolutionary Biology, Yale University
[3]Research Experience for Undergraduate Veterans

Background

Ever since the invention of antibiotics, the evolution of antibiotics resistant bacterial pathogens has posed a significant issue in both agriculture and medicine. One proposed solution is the use of bacteriophages to treat bacterial infections. Bacteriophages, or phages for short, are a type of virus that kills bacteria. In order to characterize phage effectiveness, we need to measure the ability of phage to infect and kill a bacterial strain. However, current approaches to measure bacterial resistance to phage are labor intensive and low-throughput.

Question

Can growth curves of bacterial density over time when grown with phage be a better measure of bacterial resistance to phage?

Previous Work

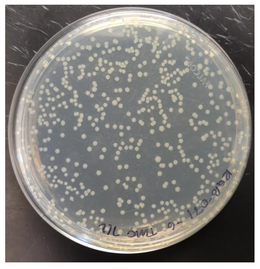

Figure 1

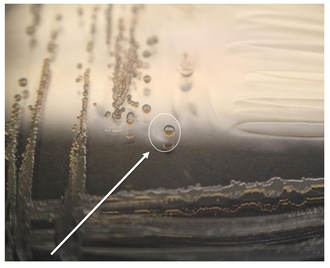

Figure 2

We acquired strains of E coli from collaborators:

- **BW25113** (a "wild type" strain)
- **JW55031** (which contains TolC, the phage receptor for U136B, knocked out),
- Three strains that evolved in the presence of phage from BW (**RGB-036, RGB-058, and RGB-071**).

These three strains were plated (Fig 1) and isolated colonies we selected (Fig 2) and then used in our experiments.

Experimental Plan

We will first measure the resistance of our five bacterial strains to phage using the conventional approach: efficiency of plaquing assays.
Then we will use the higher throughput semi-automated method of growth curves. From this, we will be able to determine if the new growth curve method agrees with the established method for measuring bacterial resistance to phage.

Results

We inoculated each bacteria, then plated them with varied dilutions of phage. After incubation, we counted the efficiency of phage at forming plaques on each bacterial strain (Efficiency of Plaquing).

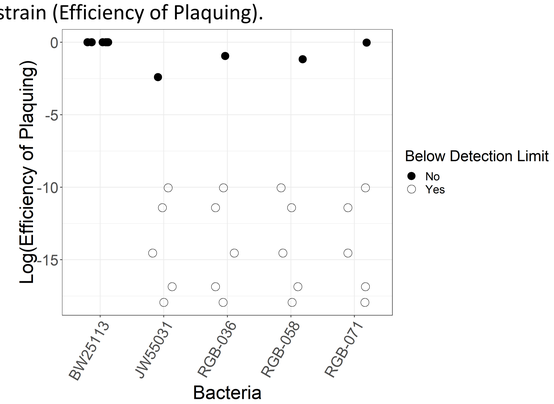

Figure 3. In comparison to strain BW25113, the other four strains were much more phage resistant. In the last replicate experiment, the four other strains had much higher EOPs than the previous replicates

The wells of a 96 well plate were filled with bacteria, phages, and media. The ratio (multiplicity of infection, MOI) and initial densities of bacteria & phages were varied across four levels. The plate was then incubated and the bacterial density was read every 30 minutes for 12 hours. Selected growth curves are shown below.

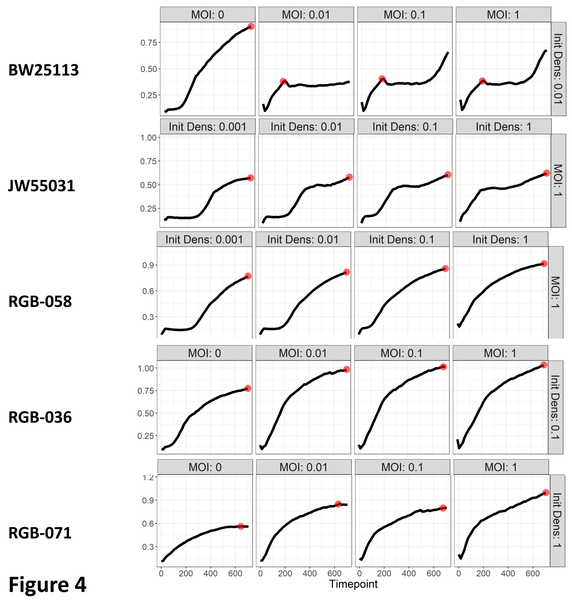

Figure 4

- Lysis of BW25113 by phage is apparent, while we only observe minimal effect of phage on the other bacteria's growth
- In JW55031 and RGB-058 the lag time decreases as initial density increases. This suggests increasing phage media decreases lag time.
- In RGB-036 and RGB-071 surprisingly, the final bacterial density increases as MOI increases. This suggests bacteria grow to higher densities on phage media than fresh media.

We then condensed the growth curve data by identifying the peaks of the curves and plotting just the height of that peak below.

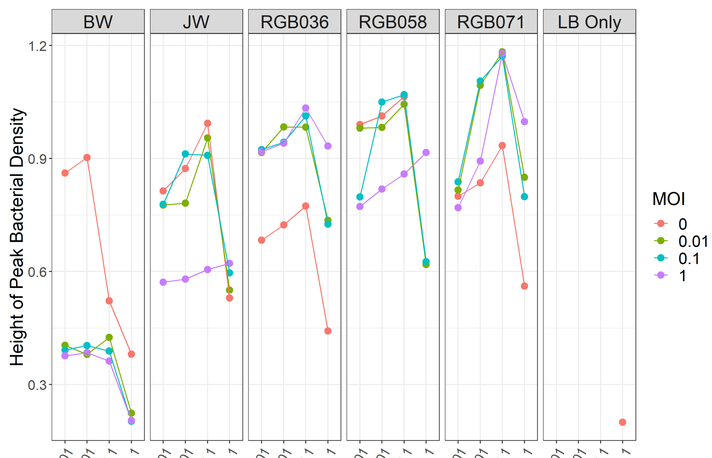

Figure 5

- One observable pattern was strain BW25113 was the most sensitive to phage U136B.
- The other strains appear less affected by the addition of phage.
- One pattern across many of the conditions is that as the initial density increases, the peaks decline in density.

Conclusion

Through our research, we were able to gain some valuable information about the efficacy of phage U136B on multiple strains of E coli. BW25113 was the strain that was the most sensitive to phage. The other strains of bacteria were relatively more phage resistant. We observed several unexpected patterns with respect to MOI and the initial density that merit further investigation.
This research will contribute to our ability to characterize the resistance of bacteria to phage, supporting the development of phage replacement or antibiotics.

Acknowledgments

Research Experience for Veteran Undergraduates
Yale
hhmi Howard Hughes Medical Institute
NIH National Institutes of Health
Turning Discovery Into Health

Dr. Paul Turner, Mike Blazanin, and The Paul Turner Lab

References

"Two Lytic Bacteriophages That Depend on the Escherichia Coli Multi-Drug Efflux Gene TolC and Differentially Affect Bacterial Growth and Selection." Alita Burmeister, Rose Bender, Abigail Fortier, Adam Lessing, Ben Chan, Paul Turner. *bioRxiv*. 2018.