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Turner Lab
May 4th, 2020

Studying Bacteriophage Adsorption Rates within Co-Evolved Populations

Fatal multi-drug resistant bacterial infections are a serious and growing problem. This resistance usually occurs in patients with persistent infections, like cystic fibrosis or recurring urinary tract infections (UTIs) (Aslam, Wang et al. 2018). Over time, with the general overexposure to antibiotics that patients with persistent infections receive, it becomes more likely that these bacterial populations will become resistant to all antibiotic treatment (Ventola 2015). This can occur when a few bacterial cells mutate and become resistant to antibiotics; soon the entire surviving bacterial population will contain this mutation and the infection can no longer be effectively treated.

One potential solution to this multi-drug resistance is phage therapy. Phage therapy uses bacteriophage, viruses that selectively infect bacteria, to kill these antibiotic resistant cells. Bacteriophage often target outer membrane proteins on bacteria such as efflux pumps or ion channels in order to infect the cell. Eventually, as with the antibiotic, a resistance to the bacteriophage can develop (Lin, Koskella et al. 2017). However, the evolutionary trade-offs which occur when both bacteriophage and antibiotic treatments are used to fight bacterial infections can potentially be exploited to effectively treat persistent infections.

Bacteriophage U136B has previously been characterized as a phage which is TolC-dependent, meaning that U136B binds to the outer membrane protein TolC, which is part of *Escherichia coli* bacteria's main antibiotic efflux pump. In a previously-conducted 10 day serial passaging evolution experiment, in which *E. coli* bacteria were grown in the presence of U136B, we see the rapid evolution of phage resistance in the bacteria (Burmeister et al., accepted to PNAS). However, this project seeks to explore the evolution of phage within the experiment. In order to do this, my research mentor, Dr. Alita Burmeister, and I hope to measure the rate of adsorption of U136B to *E. coli* bacteria to rigorously confirm that phage U136B is dependent on TolC. Additionally, we would be able to track how the adsorption rate changes in evolved phage as an indicator of fitness and selection pressure. I have been unable to detect adsorption with our current methods, but I am hoping to continue these efforts.

During the spring semester of 2020, I conducted several adsorption experiments using samples of evolved phage U136B and measuring their rate of adsorption to heat-killed bacterial cells to prevent phage replication obscuring our results. However, I saw no decrease in free (unadsorbed) phage over time on the bacteria (Fig. 1). While I was unable to detect adsorption through this method, I was able to spend time during remote learning reading about alternate adsorption assay methods (Hyamn et al. 2009; Shao et al., 2008; Burmeister et al., 2016). In the future, I plan to use antibiotics to kill the bacterial cells, rather than heat; I hypothesize that the exposure to heat may denature the TolC protein, thus disrupting adsorption. I will likely start by

using an antibiotic which halts cell growth, but does not disrupt the membrane (e.g. chloramphenicol) at a concentration of 15 µg/ml of antibiotic, following the protocol in Burmeister, et al., 2016. As this specific protocol has yielded clear results in the past (although with different bacteriophages), I plan to resume this adsorption experiment for phage U136B using a similar strategy.

Moving forward in this project, I plan to adjust our methods using this new protocol to measure phage adsorption rates for the ancestral phage, as well as samples of evolved phage from each day of the previous evolution experiment. By comparing these rates, we hope to track the evolution of the phage in conjunction with the evolution of the bacteria. From previous data, we know that the bacteria evolve quickly evolved phage resistance, so we hypothesize that the phage evolved to become more effective at killing the bacteria, which would be shown by an increase in adsorption rate over time.

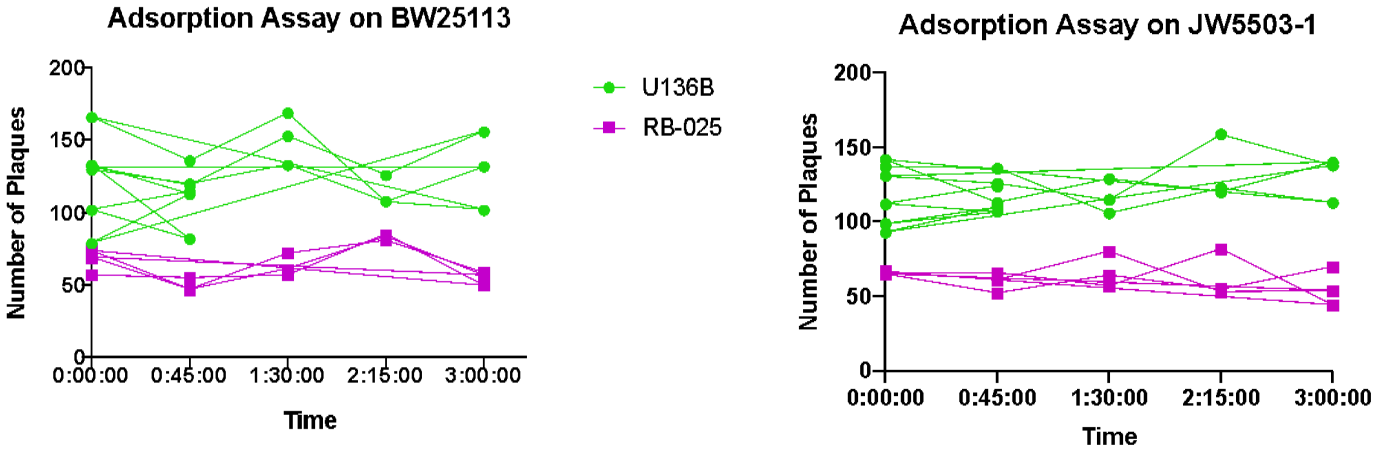

Figure 1: Preliminary results from Spring 2020 showing no decrease in free phages.

References

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Itemized Budget for Yale Science and Engineering Association Research Funding

Item	Cost	#	Total
● Media for LB:	$184 / 500 g mix	2	$184
● Petri plates 100 x 15 mm	$273/case	3	$819
● Antibiotics (CAM)	$26/25 g	1	$15
● 2.0 ml cryogenic storage vials	$257/case	1	$257
● Falcon disposable serological pipets, 10ml	$136 /200 pack	1	$136
● Pipette tips (filter, 200 µL)	$98/10-pack	3	$294
● Falcon conical tubes	$288 / 500 pack	1	$288
		Final Total:	**$1993**

These items are to be used in microbiological experiments involving the measurement of the rate of adsorption of bacteriophage samples (both ancestral and evolved) in order to track evolution of the phage in the presence of co-evolving bacteria.

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