Uncovering the mechanism of a novel phage defense island in Vibrio cholerae

Jordan Hoff and Kim Seed
Department of Plant and Microbiology
University of California, Berkeley

Abstract

The lytic bacteriophage ICP1 is a predator of Vibrio cholerae and by virtue of their frequent interactions in the human gut1, and in the environment, are in the midst of a co-evolutionary tango. One strategy V. cholerae utilizes to escape phage predation is the acquisition of mobile genetic elements that have anti-phage properties. Clinical isolates from 2009 revealed the Interfering Defense Island (IDI) in V. cholerae with a known anti-phage GajA/B system2 but without GajA. The mechanism by which GajA operates is unknown and is the objective of this project by which we indirectly approach the question by not looking at the gene itself, but rather what makes phage able to overcome it. First the question of sufficiency of gajA was addressed by cloning gajA onto a plasmid under an inducible promoter (pgajA). We show that gajA is sufficient for inhibiting ICP1 infection. While not able to generate true mutants against pgajA, escapes against IDI were isolated and found to have the true mutant phenotype. Interestingly, IDI mutants did not have any advantage against pgajA. This dichotomy of being able to only generate IDI mutants that have no advantage against gajA suggests a potential model where gajA is the indiscernible effector arm of IDI’s phase inhibition but is activated by other components in IDI that are triggered by infecting phage. Further analysis will include sequencing the isolates and using comparative genomics to identify potential mutations that allow for the escape of IDI.

Background

There are two ideas key to understanding the Phage Biology in this poster – sufficiency and efficiency of plaquing.

First is the idea of sufficiency. If task x can be accomplished only with gene A, then gene A is said to be sufficient for task x. This is often tested by isolating the gene of interest on a plasmid and plating it in a clean background.

Second is the idea of efficiency of plaquing (EOP) but first you have to understand what a plaque is. Since viruses need a host to reproduce, they are grown on a lawn of bacteria where individual phage infect individual cells. The virus makes copies of itself and when ready, bursts the host and injects the infecting neighbors. Over time this cycle becomes visible to the naked eye in what is called a plaque. If you count the number of plaques on a lawn you have the number of plaque forming units (PFU) which is a metric used to determine how many phage can infect that particular host.

As scientists we can test different strains of bacteria with different genetic makeups to see how the phage react giving insight into how certain bacterial genes affect phage. We can tell how well phage infect test strains by using another metric – the efficiency of plaquing (EOP). This is calculated by taking the PFUs on the test condition and dividing it by the PFUs on a permissive host – a host that is not restrictive to phage infection. Essentially this is the fraction of phage that can infect on the test strain.

Phenotypes of escape phage

A: pgajA escapes are not true mutants (EOP=1)

B: pgajA escapes cannot plaque on IDI

C: IDI escapes are true mutants

D: IDI mutants have no advantage on pgajA

A: A true mutant can infect the test strain to the same degree as the permissive control (EOP=1). pgajA escape phage are not true mutants.

B: pgajA escape phage cannot infect IDI (EOP=0) while wild type (WT) ICP1 can.

C: IDI escape phage can plaque on IDI (test strain) to the same degree as the permissive control.

D: IDI escape phage can plaque on IDI (test strain) to the same degree as the permissive control.

Analysis:

ICP1’s ability to infect in the presence of gajA is three orders of magnitude lower compared to the empty vector control (EV). To ensure that no secondary mutation was responsible, pgajA was tested without inducer (i) and had the same phenotype as the EV control. This shows that when expressed, gajA is sufficient for ICP1 inhibition.

Reported phage for early and late genes shows that gajA does not affect early gene expression, but there is a sharp decrease in late gene expression

References:


Acknowledgments:

I am grateful for the Seed Lab and all of their support and guidance with my project. I would also like to thank the College of Natural Resources for funding my research with the SPUR grant and all other funding sources.