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


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## Abstract

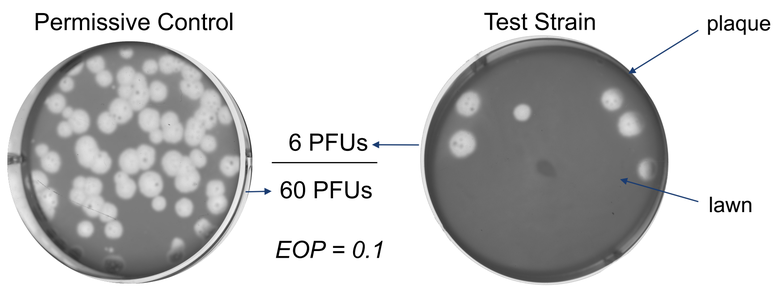
The lytic bacteriophage ICP1 is a predator of *Vibrio cholerae* and by virtue of their frequent interactions in the human gut[1], and in the environment, are in the midst of a co-evolutionary tango. One strategy *V. cholerae* has utilized to escape phage predation is the acquisition of mobile genetic elements that have anti-phage properties. Clinical isolates from 2009 revealed the Interfering Defense Island (IDI) in *V. cholerae* with a known anti-phage GajAB system[2] but without GajB. 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 promotor (p*gajA*). 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 indiscriminate effector arm of IDI's phage 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

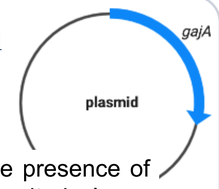
There are two ideas key to understanding the Phage Biology in this poster – sufficiency and efficiency of plaquing.

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 placing it in a clean background.

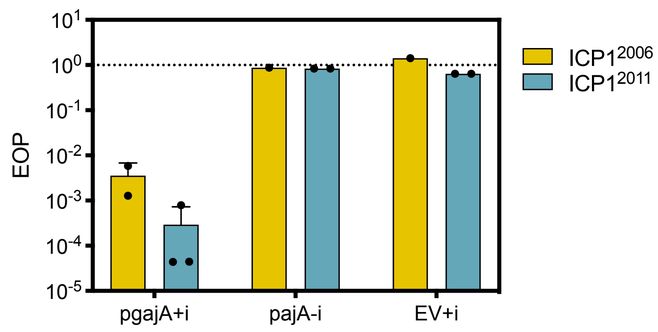
Second is the idea of efficiency of plaquing (EOP) but first you have to understand what a plaque is. Since viruses need a host to reproduce, they are grown on a 'lawn' of bacteria where individual phage infect individual cells. The virus makes copies of itself and when ready, bursts the host and infects the neighboring cells. 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.




## *gajA* is sufficient to block ICP1 infection

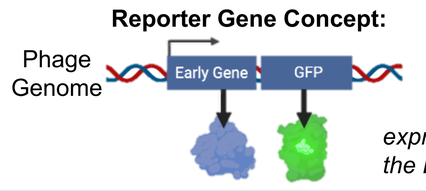



**A:** p*gajA* is sufficient for blocking ICP1 inhibition



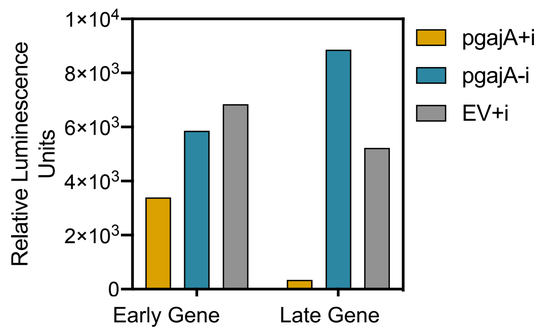

ICP1's ability to infect in the presence of *gajA* is three orders of magnitude lower compared to the empty vector control (EV). To ensure that no secondary mutation was responsible, p*gajA* was tested without inducer (-i) and had the same phenotype as the EV control. This shows that when expressed,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

Reporter 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



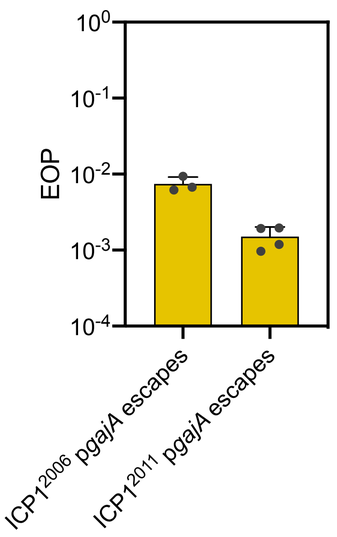

*GFP is expressed with the Early Gene*

**B:** Reporter phage shows gajA prevents late gene expression



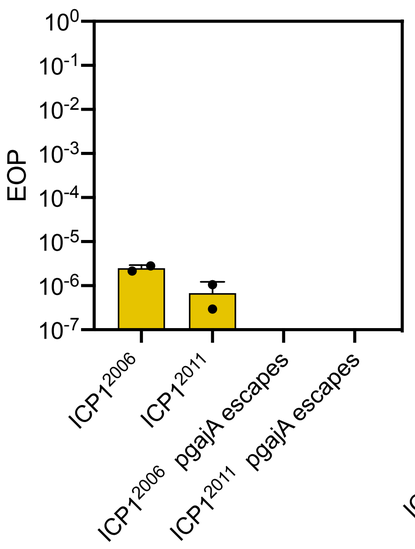

## Phenotypes of escape phage

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



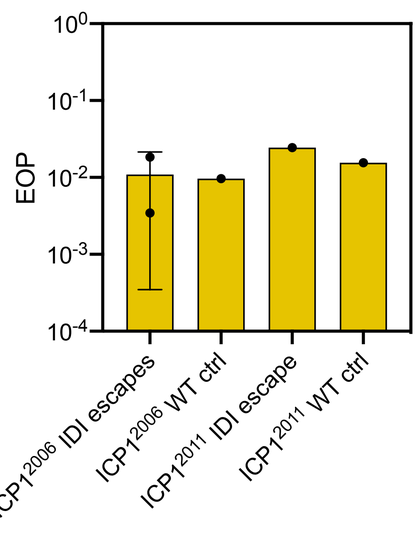

**A.** A true mutant can infect the test strain to the same degree as the permissive control (EOP=1). p*gajA* escape phage are not true mutants.

**B:** pgajA escapes cannot plaque on IDI



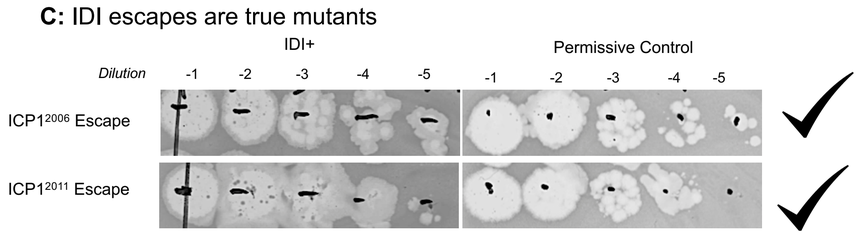

**B.** p*gajA* escape phage cannot infect IDI (EOP=0) while wild type (WT) ICP1 can.

**D:** IDI mutants have no advantage on pgajA



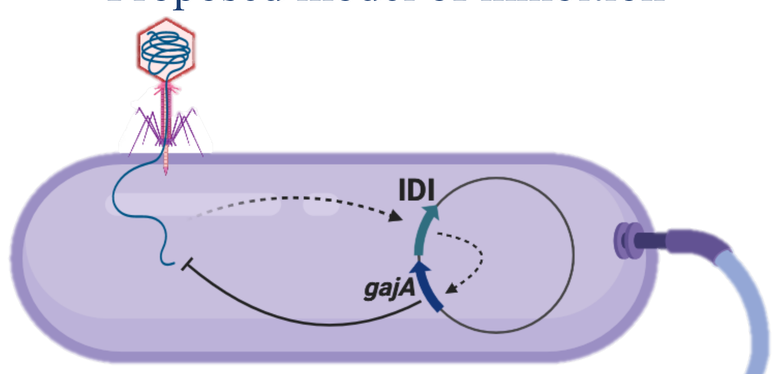

**D.** IDI escape phage have no advantage infecting p*gajA* relative to phage that have never been exposed to IDI or *gajA* (WT ctrl)

**C:** IDI escapes are true mutants




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

## Proposed model of inhibition




Upon phage infection, an unknown trigger activates an IDI component. This trigger occurs during the middle of infection as evident with reporter phage data. This activated IDI component induces expression of the effector arm of IDI- *gajA*. By an unknown mechanism gajA inhibits phage infection.

## GajA predicted tertiary structure

https://tinyurl.com/CalDay-HoffPoster

GajA's predicted tertiary structure[3,4,5] and electrostatics (c=-0.84). There is predicted similarity with OLD family nucleases but nothing to reconcile this *in vivo*.


## Acknowledgments:

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## References:


1: Seed, Kimberley D., et al. "Evidence of a Dominant Lineage of Vibrio Cholerae-Specific Lytic Bacteriophages Shed by Cholera Patients over a 10-Year Period in Dhaka, Bangladesh."
2: Doron, Shany, et al. "Systematic Discovery of Antiphage Defense Systems in the Microbial Pangenome." *Science*, vol. 359, no. 6379, 2018, doi:10.1126/science.aar4120.
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