FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Investigating Arginine Biosynthesis in Viral Replication

Daniel Chaudry, Ryleigh Griffin, Harrison Lee, Sabrina Stecklein, and Jessica O'Hara

Relevance of Arginine Biosynthesis for Viral Infection

- Viruses use the biosynthetic pathways of the infected host cells to produce the biological molecules they need to replicate
- The amino acid **arginine** is an important component in viral replication; it has been found to play a key role in:
  - Stabilizing the structure of cowpea chlorotic mottle virus (Garmann et al., 2014) hepatitis B virus (Newman et al., 2009)
  - Allowing for viral entry of porcine circovirus 2 into host cells (Khayat et al., 2011)
- In human cytomegalovirus (HCMV), host cell consumption was found to be induced for multiple amino acids, including arginine (Rodríguez-Sánchez and Munger, 2019)

Project Objective

We aimed to explore the relationship between arginine biosynthesis in the host cell and viral replication in two different model systems: *E. coli* with T4 and T4r bacteriophage and human fibroblast cells with HCMV.

Figure 1. Arginine Biosynthesis Pathway

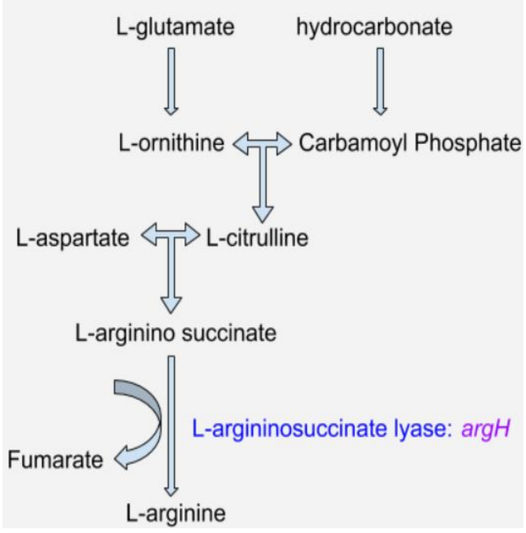


Figure 1. Simplified pathway is analogous between *E. coli* and human fibroblast cells. In addition to producing amino acids for protein synthesis, this pathway produces fumarate, a key intermediate in the ATP-producing TCA Cycle.

Viral Infection and Optical Density

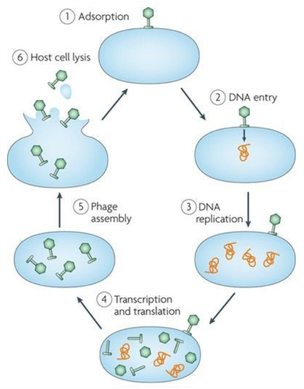


Figure 2. Lytic Bacteriophage Life Cycle. Successful bacteriophage replication causes host cells to burst open (lyse). Retrieved from Labrie et al., 2010.

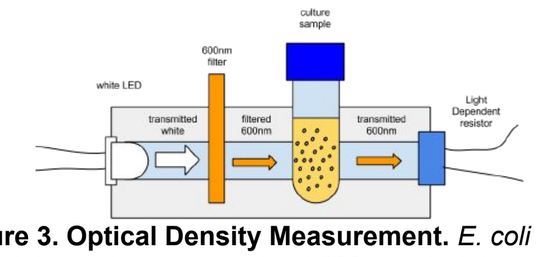


Figure 3. Optical Density Measurement. *E. coli* absorb light at a wavelength of 600 nm. A higher concentration of *E. coli* leads to higher absorbance. Lysis decreases *E. coli* concentration, lowering absorbance. Retrieved from biohackspace.org

Research Aims and Experimental Methods

- Compared Parent and *ΔargH* (knockout) strain growth in rich (LB) and minimal (M9) media conditions using plate reader
- Compared T4 and T4r bacteriophage replication in Parent and *ΔargH* strains by examining host cell lysis using plate reader and making spread plates for a plaque assay
- Examined fold change of arginine and related metabolites in HCMV infection of human fibroblast cells

Figure 4. Growth and Lysis Plate Reader Experimental Setup

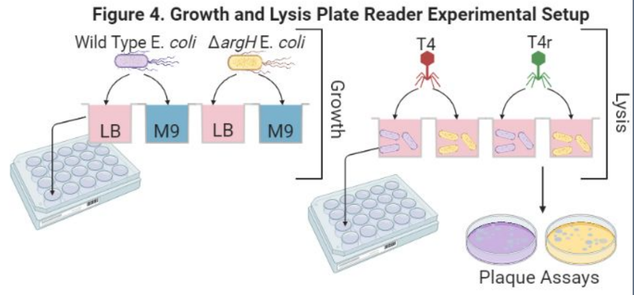


Figure 4. Experimental approach schematic. Lysis analyzed using both plate reader and plaque assay.

ΔargH Growth Is Inhibited in Minimal Media

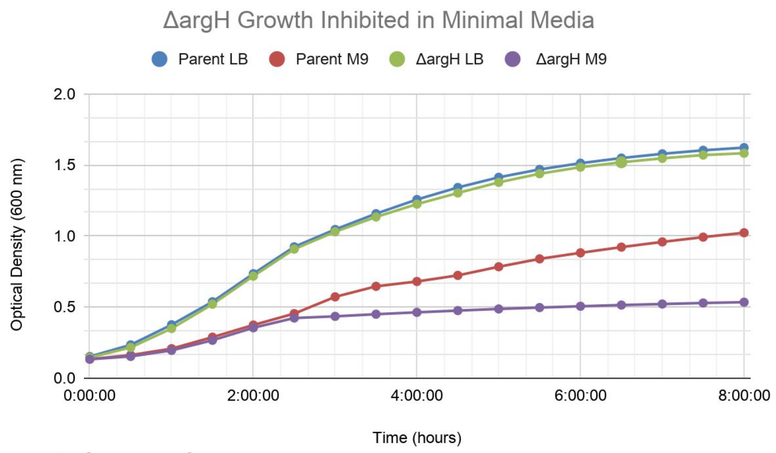


Figure 5. Growth Curves. The parent and *ΔargH* (knockout) strains were grown in LB media and M9 media. Growth was monitored using a plate reader, which performed continuous shaking at 37°C and measured optical density at 600 nm every 30 minutes for 8 hours. In the LB media, the parent and *ΔargH* strains displayed an almost identical pattern of steady growth. Both strains exhibited less growth in M9 media. The *ΔargH* strain plateaued and ceased growth after about 5 hours.

ΔargH Removal Did Not Impact Phage Replication

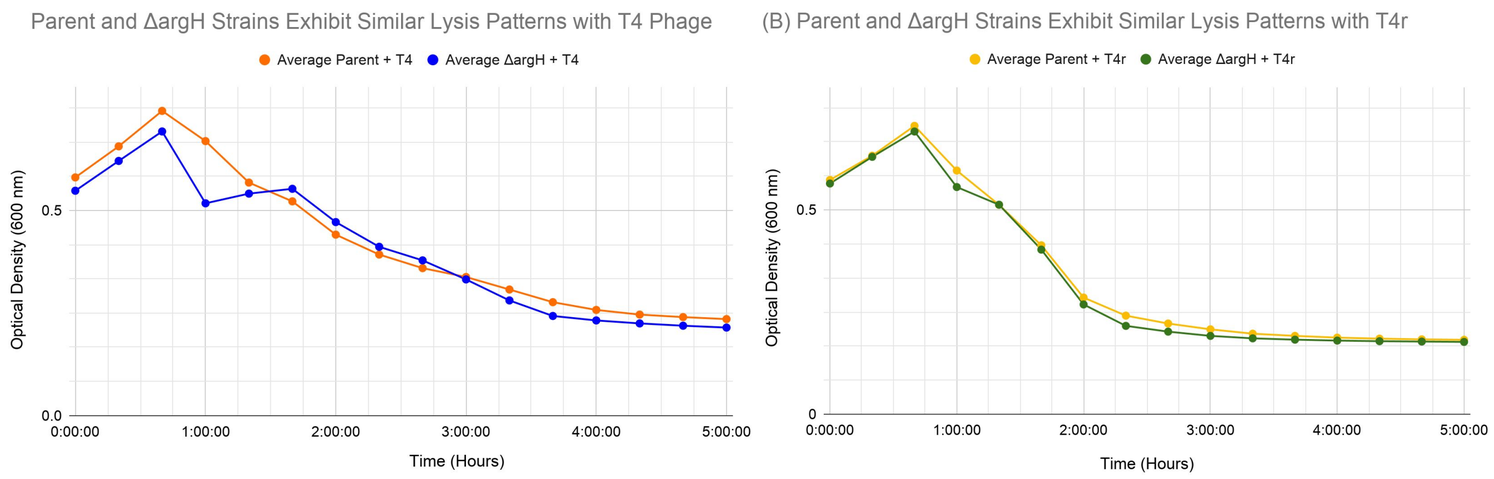
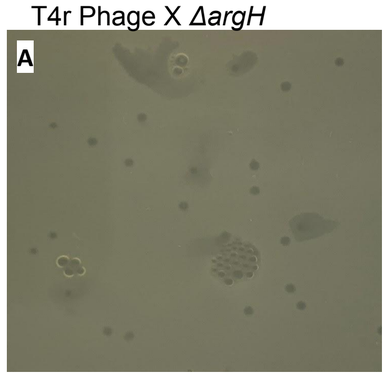


Figure 6. Lysis Curves. The parent and *ΔargH* knockout strains were cultured with **(A)** T4 bacteriophage and **(B)** T4r bacteriophage. Optical density at 600 nm was measured at 30 minute intervals for 8 hours using a plate reader. No major differences were evident between the lysis curves of the two strains.

T4r Phage X *ΔargH*



T4r Phage X Parent

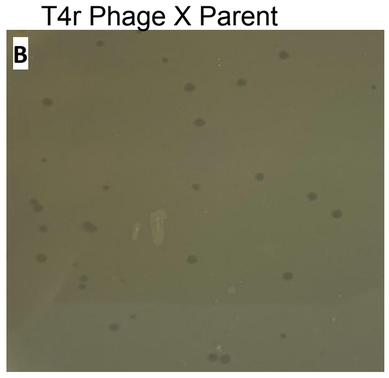


Figure 7. Comparison of Phage Plaque Assays. These phage plaque assays were produced via the double agar overlay method using a 10^{-7} dilution of parent and *ΔargH* strains. There were no major differences between the plaques, as all were small and round, with clearly defined boundaries with the surrounding *E. coli* lawn. Plaque assay comparison with T4 phage yielded similar results.

Metabolite Levels of Host Cells Change During HCMV Infection

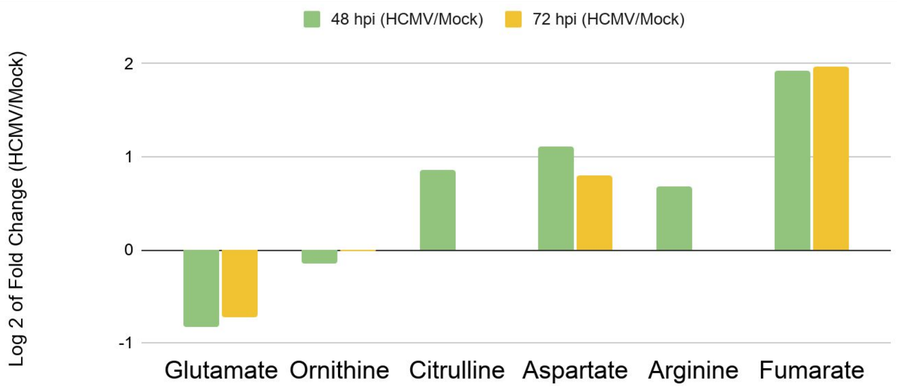


Figure 8. Human Fibroblast cells were infected with Human Cytomegalovirus (HCMV) and various metabolites of interest were measured 48 and 72 hours post infection (hpi). *Note that data 72 hpi was unavailable for citrulline and arginine*. The fold change value displayed above is a measurement of the metabolite level in the infected cell at x hpi divided by the level of the same metabolite, at x hpi in a mock infected cell. The y-axis is graphed on a log 2 scale for clarity.

Discussion of Results and Future Directions

Major Results

- *ΔargH E. coli* experienced visibly **reduced growth in M9 media** compared to the parent strain, suggesting arginine is necessary for *E. coli* growth
- Inhibiting host cell arginine biosynthesis had **no impact on lysis** with either bacteriophage in LB media
  - Most likely, LB media supplied enough arginine that the virus did not need the host cell to produce any
- HCMV infection caused increased levels of fumarate and aspartate, even more so than arginine, while the levels of glutamate and ornithine decreased, possibly because **these metabolites were consumed for use in arginine biosynthesis** (although many other pathways also use these metabolites)

Future Directions

- Conduct the growth and lysis plate reader experiments in **arginine-deficient media** in order to **analyze the effect of arginine deprivation alone on growth and viral infection**
- Determine which genes are most essential for the production of arginine by **knocking out other genes in the pathway** such as *argI* and *argF* which together catalyze the production of citrulline
- Repeat the experiment using **other types of bacteriophage**, such as T2, to compare lysis patterns and see if there are similar lysis results of T4 and T4r bacteriophage
- Investigate the effects of **inhibiting arginine transport** into the cell by knocking out genes such as *artJ*

References and Acknowledgements

- Garmann, R.F., Comas-Garcia, M., Gopal, A., Knobler, C.M., and Gelbart, W.M. (2014). The Assembly Pathway of an Icosahedral Single-Stranded RNA Virus Depends on the Strength of Inter-Subunit Attractions. Journal of Molecular Biology *426*, 1050–1060.
- Khayat, R., Brunn, N., Speir, J.A., Hardham, J.M., Ankenbauer, R.G., Schneemann, A., and Johnson, J.E. (2011). The 2.3-Angstrom Structure of Porcine Circovirus 2. Journal of Virology *85*, 7856–7862.
- Labrie, S.J., Samson, J.E., and Moineau, S. (2010). Bacteriophage resistance mechanisms. Nature Reviews Microbiology *8*, 317–327.
- Newman, M., Chua, P.K., Tang, F.-M., Su, P.-Y., and Shih, C. (2009). Testing an Electrostatic Interaction Hypothesis of Hepatitis B Virus Capsid Stability by Using an In Vitro Capsid Disassembly/Reassembly System. Journal of Virology *83*, 10616–10626.
- Rodríguez-Sánchez, I., and Munger, J. (2019). Meal for Two: Human Cytomegalovirus-Induced Activation of Cellular Metabolism. Viruses *11*, 273.
- https://biohackspace.org/projects/od600-spectrophotometer/

Financial support for this project was provided by the First-Year Innovation and Research Experience at the University of Maryland, College Park. Special thanks to Dr. Jessica O'Hara, Dr. John Purdy, Lisa Wise, and Daniel Kang.