FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Viral Implications of the *glyA* gene in *E. coli*

Nicole Sigalovsky, Gwen Moronell, Allison Artemenko, Jamie Izara, Olivia Ekbatani

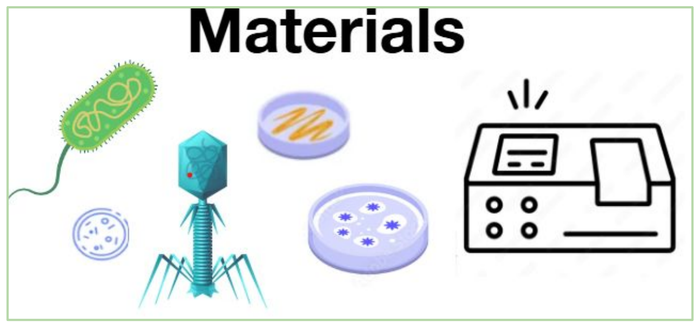
Introduction

- Viruses invade cells, altering their cellular metabolism through amino acids
- The *glyA* gene leads to the assembly of the amino acid glycine.
- The amino acid glycine acts as a nitrogen source which is crucial for cell growth and development, particularly in bacterial and viral cells (Newman et al., 1976)

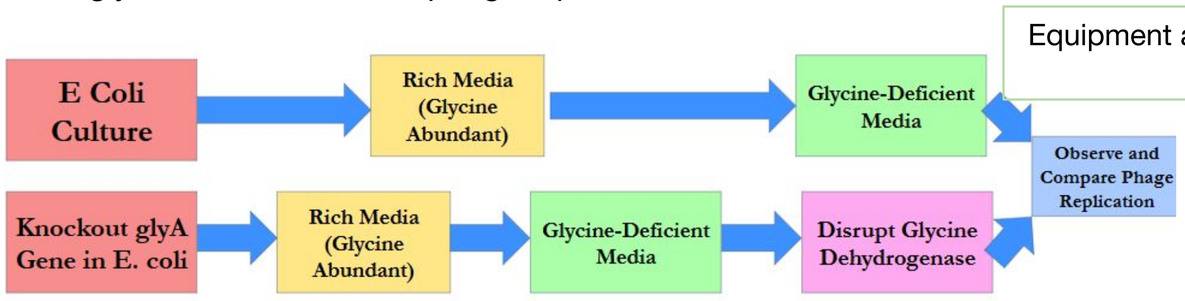
-If the *glyA* gene is removed, what could happen to bacteriophages ability to lyse the knockout cells? This answer can lend itself to other fields of study when trying to target cell death.

Objectives

1. Identify how a knockout strain grows compared to parent strain.
2. Discover how well bacteriophage responds to *E.coli* without *glyA* gene and determine how the presence of glycine affects bacteriophage replication.



Equipment and biological agents used in lab



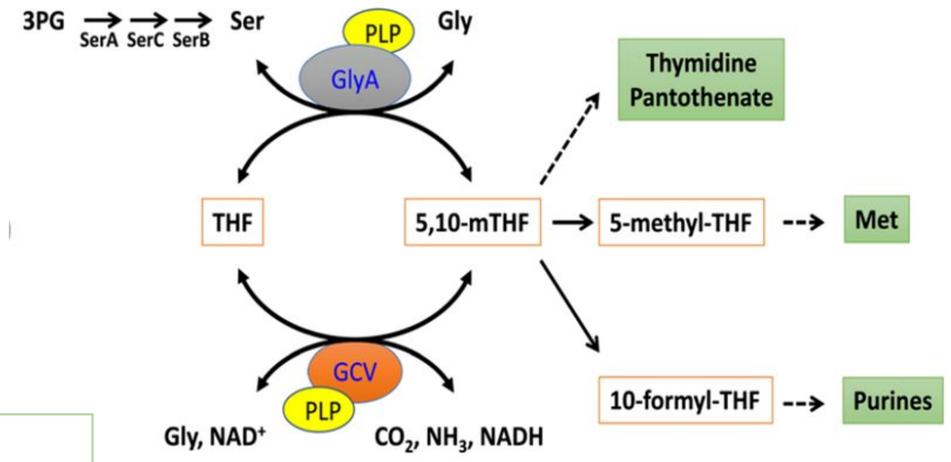


Figure 1

Methods

- Our control group will be LB media with an abundance of the *glyA* gene obtained from the Keio knockout collection
- Our experimental group will be LB media that is glycine deficient
- We will also completely knockout the *glyA* gene
- We will prepare overnight cultures with both of the strains
- Then we will perform serial dilutions, phage quantification, and plating to see how limiting the *glyA* gene affects replication

Results

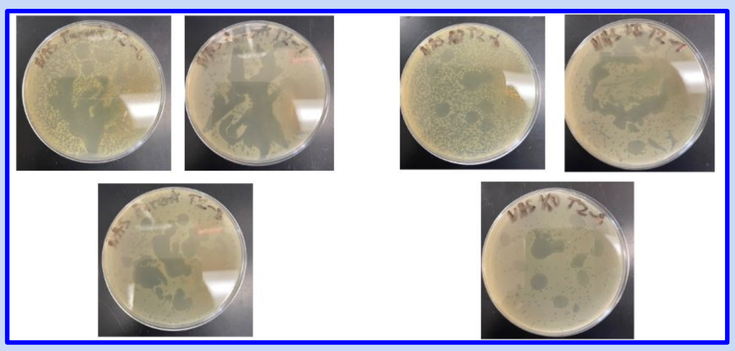
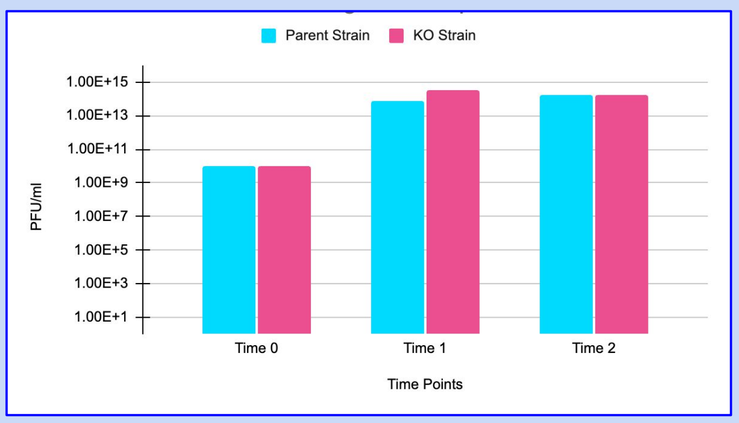


Figure 3: Plaque assays with t4r, phage comparing between the parent strain and glyA Grown at 37 degrees with continuous shaking; Parent in LB media at 10^-6, -7, and -8 dilution concentrations on the left, ΔglyA in LB media at 10^-6, -7, and -8 dilution concentrations on the right.



This graph shows the plaque forming units (PFU) in a milliliter (ml) of our parent and *glyA* knockout strain when plaque assays were performed on data of bacteriophage grown in these strains. The data was collected before it had time to grow, then after 2.5 hours had passed by, then after another 1.5 hours had passed.

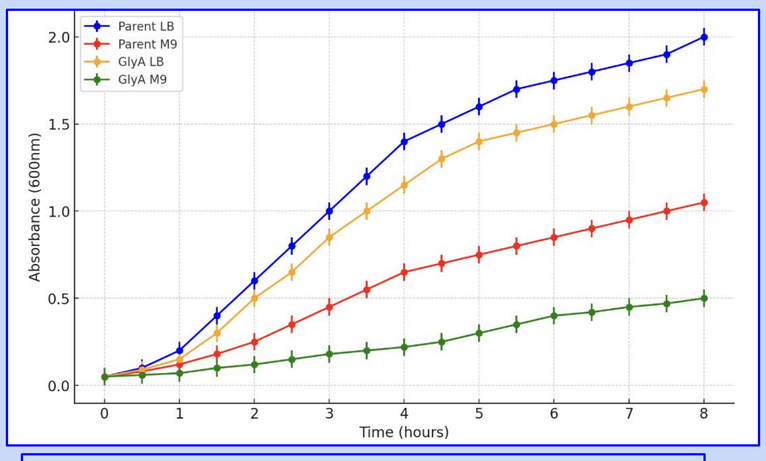


Figure 5: Comparative Growth Curves between parent strain and GlyA with T4r phage The graph shows absorbance readings at 600 nm of E.coli strains grown the various media. The LB media is is richly supplied with nutrients, while the M9 is minimal media of only the essentials for growth. This graph compares the opacity of bacteria in the media throughout 30 minute time intervals for 8 hours to gage the growth rate of the parent and knockout strain in their various media.

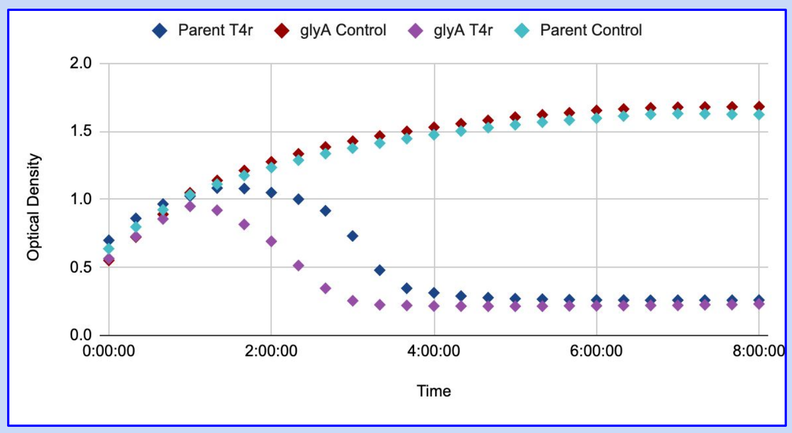
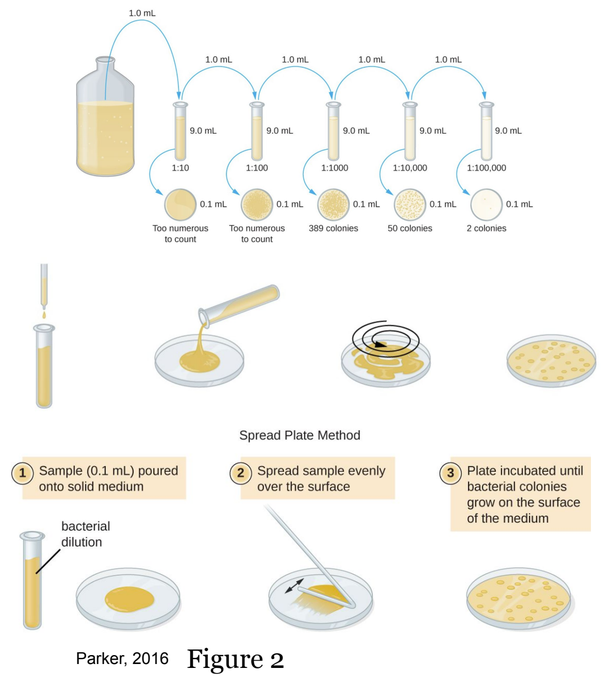


Figure 6: Comparative lysis curves between parent strain and *glyA* in M9 media and LB media. This graph shows the optical density for the parent and knockout *E.coli* strains on their own as the control, and when T4r bacteriophage is added to them. The data was taken over 8 hours. Lysis means the breakdown of a cell, and a lower optical density indicates less cells, or more lysis. Since the controls do not have the bacteriophage, there is nothing to induce lysis.



Parker, 2016 Figure 2

Discussion

- The phage replicated effectively despite the absence of the *glyA* gene.
- Both the parent and KO strain grew less in the M9 media compared to LB. This was expected since LB has more nutrients.
- There was lysis in the bacteriophage
- Both strains had similar amounts of plaque units formed which was unexpected since one had less bacteriophage

Future Directions

- In the future, it would be beneficial to repeat our experiment to confirm that our results are accurate.
- We would also test other strands to see how they affect bacteriophage replication

References and Acknowledgments

Financial support for this project was provided by the First-Year Innovation and Research Experience (FIRE) at the University of Maryland, College Park.

Newman, E. B., Klein, S. M., Lowry, O. H., Taylor, R. T., Moore, S., Prival, M. J., Kaneda, T., Bird, I. F., Pizer, L. I., Miller, B. A., Buchanan, J. M., & Roberts, R. B. (1976, January 14). The use of glycine as nitrogen source by escherichia coli K12. Biochimica et Biophysica Acta (BBA) - General Subjects. https://www.sciencedirect.com/science/article/pii/0304416576901732

Ito, Tomokazu & Hori, Ran & Hemmi, Hisashi & Downs, Diana & Yoshimura, Tohru. (2019). Inhibition of glycine cleavage system by pyridoxine 5'-phosphate causes synthetic lethality in glyA yggS and serA yggS in Escherichia coli. Molecular Microbiology. 113. 10.1111/mmi.14415.

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., & Mori, H. (2006). Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: The Keio collection. *Molecular Systems Biology*, *2*, 2006.0008. https://doi.org/10.1038/msb4100051

Figure 1: Ito et al. 2020

Figure 2: Host Pathogen Interactions Module 3 Protocol Instruction Manual

Parker, Nina. "9.1 How Microbes Grow." Essay. In *OpenStax*. Houston, Texas, 2016. https://openstax.org/books/microbiology/pages/9-1-how-microbes-grow