FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Investigating the Role of Glutamine Metabolism on Bacteriophage Replication

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INTRODUCTION

Introduction:

- The Tricarboxylic Acid (TCA) cycle is a vital metabolic pathway in *E. coli* that contributes to most of its energy production. Energy generated by the TCA cycle is essential for bacterial growth and synthesizing biomolecules. Another metabolic pathway, glycolysis, uses glucose to create molecules that feed into the TCA cycle.
- Bacteriophages are obligate intracellular parasites that target bacteria. Like most viruses, they hijack host cell metabolism and resources to promote its replication and synthesis of other viral molecules.
- Human cytomegalovirus (HCMV) utilized glutamine metabolism to replenish the TCA cycle. HCMV altered glucose metabolism to promote fatty acid and nucleotide synthesis which are essential for viral replication (Sanchez and Lagunoff).
- In recent years, increasing numbers of bacteria have acquired antibiotic resistance. Research into phage therapy has seemed promising as a potential alternative to antibiotics. The goal of this research is to broaden understanding on how bacteriophage can be used as an alternative to antibiotics in an effort to combat the antibiotic resistance crisis

Hypothesis: The removal of the *ΔglnA*, *ΔglsA*, and *ΔglsB* genes in *E. coli* will inhibit glutamine metabolism resulting in decreased bacterial growth and viral replication. The addition of glutamine will boost and recover bacterial growth and enhance viral replication.

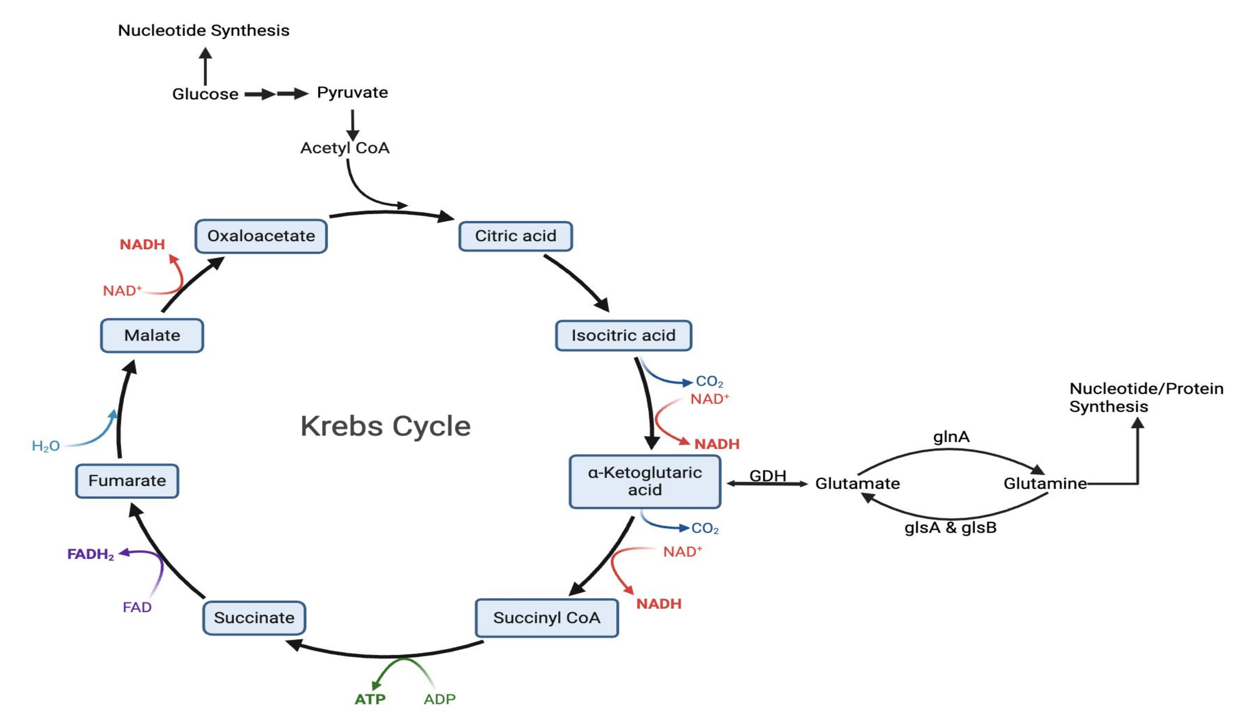

Figure 1: Diagram of the TCA Cycle and Glutamine Metabolism
Figure was made with BioRender.

METHODS

Methods:

- **Comparative Growth Curves:** Enables for comparison and analysis of bacterial growth between the wild type and three knockout strains. *E. coli* strains were grown in media on a 96-well plate and absorbance data was collected at 600 nm every 30 minutes for 8 hours. The bacteria were grown at 37 °C for optimal growth conditions.
- **Comparative Lysis Curves:** *E. coli* strains were grown in media on a 96-well plate at 37°C for 8 hours, and T4 phage was added when the strains reached log phase (average absorbance of the plate was 0.5) . Absorbance data was collected every 20 minutes at 37 °C for optimal growth conditions. Rate of lysis was determined by absorbance values and compared between the strains.
- **Two Time Point Phage Titer:** E. *coli* strains were grown in separate flasks at 37 °C until an optical density of 0.540 was reached. When reached, T4 phage was added to each strain and incubated for 90 minutes. Samples were taken at 45 and 90 minutes after phage addition. Samples were used to create plaque assays for phage quantification.

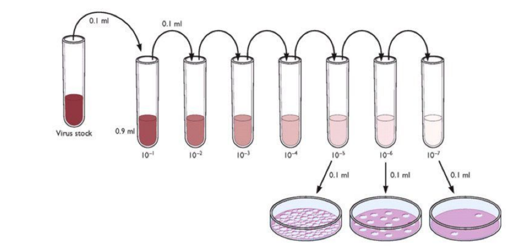

Figure 2: Model of Serial Dilution and Plaque Assay Technique
Figure adapted from Racaniello, 2009.

Characterization of Growth

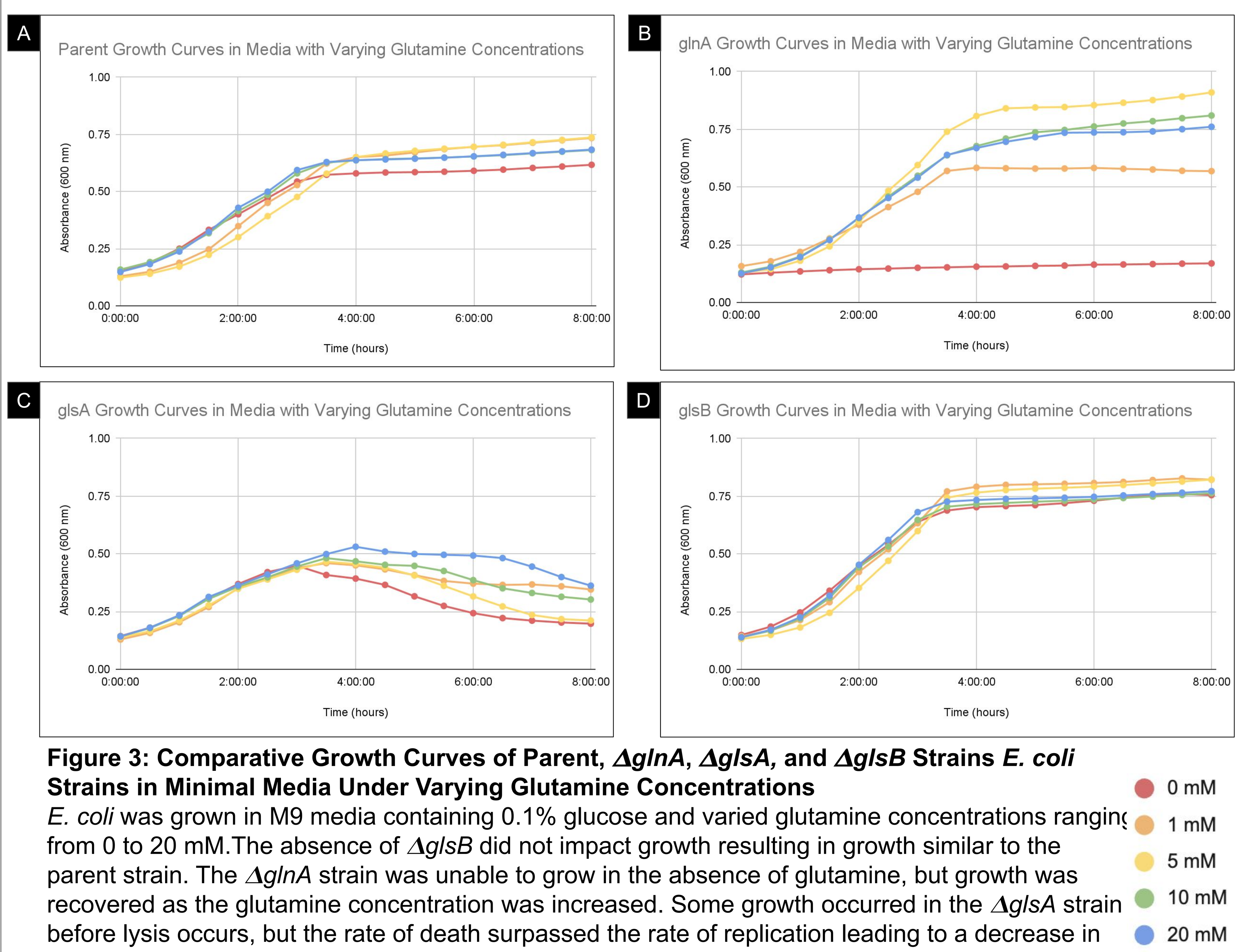

Figure 3: Comparative Growth Curves of Parent, *ΔglnA*, *ΔglsA*, and *ΔglsB* Strains *E. coli* Strains in Minimal Media Under Varying Glutamine Concentrations
E. coli was grown in M9 media containing 0.1% glucose and varied glutamine concentrations ranging from 0 to 20 mM.The absence of *ΔglsB* did not impact growth resulting in growth similar to the parent strain. The *ΔglnA* strain was unable to grow in the absence of glutamine, but growth was recovered as the glutamine concentration was increased. Some growth occurred in the *ΔglsA* strain before lysis occurs, but the rate of death surpassed the rate of replication leading to a decrease in absorbance and increased glutamine concentration slightly increased max absorbance.

Dynamics in Viral Replication

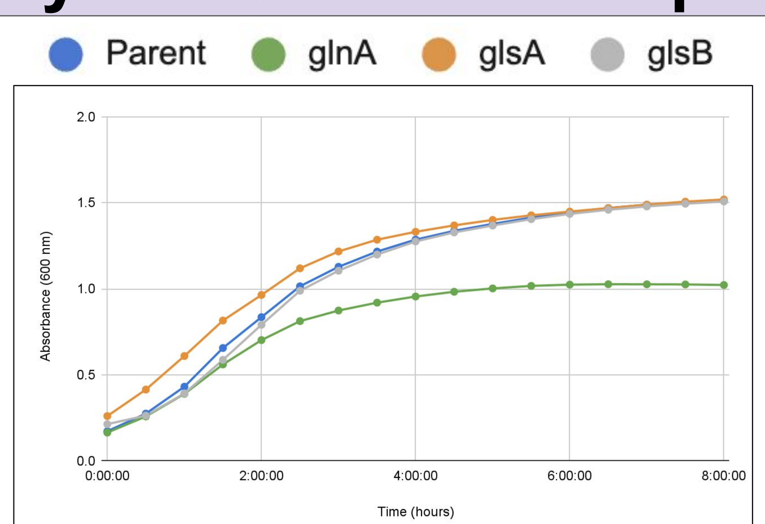

Figure 4: Comparative Growth Curve of Parent, *ΔglnA*, *ΔglsA*, and *ΔglsB E. coli* Strains in LB Media
ΔglsB grew less in LB media compared to the other strains. The *ΔglsA* strain initially grew faster. All strains except *ΔglsB* reached the same max absorbance.

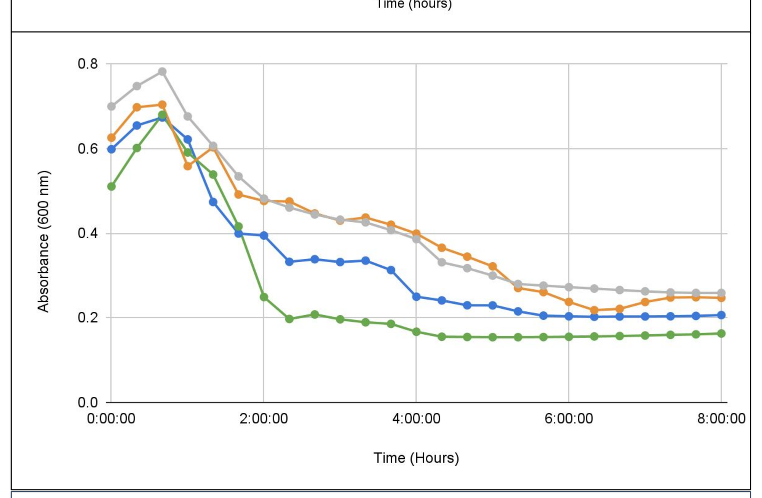

Figure 5: Comparative Lysis Curve of Parent, *ΔglnA*, *ΔglsA*, and *ΔglsB E. coli* Strains in LB Media
The difference in initial absorbance is attributed to the slow rate at which *ΔglnA* strain grows. Phage lysis was relatively the same between all strains.

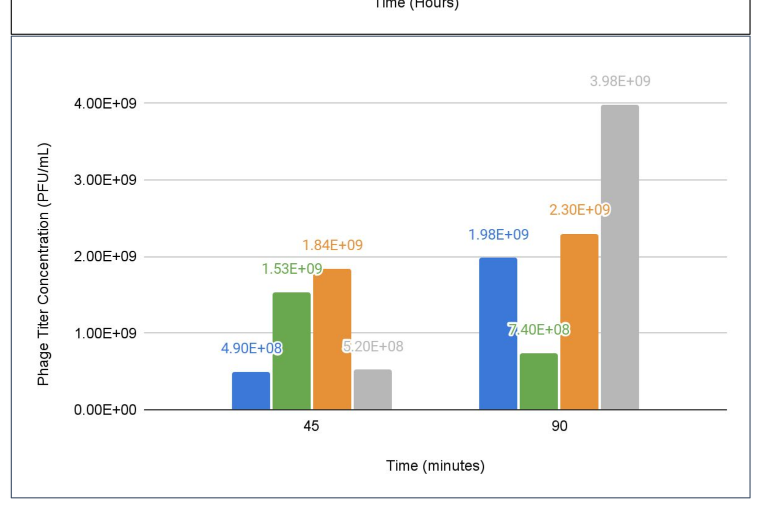

Figure 6: Two-Time Point Phage Titer of Parent, *ΔglnA*, *ΔglsA*, and *ΔglsB E. coli* Strains
Comparison of the concentration of T4 phage at 45 and 90 minute time points between each strain. The *ΔglsB* demonstrated the highest concentration of phage. Phage concentration did not change in *ΔglsA*. The *ΔglnA* decreased in phage concentration.

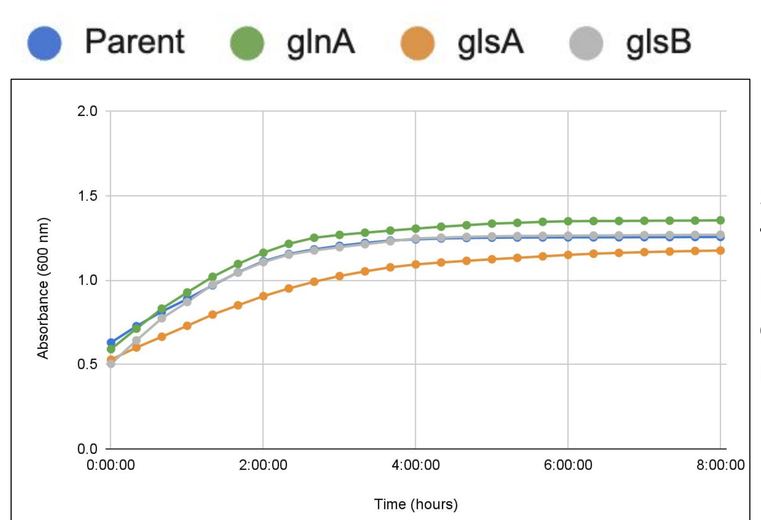

Figure 7: Comparative Growth Curves of Parent *ΔglnA*, *ΔglsA*, *and ΔglsB* E. coli Strains in M9 Media
The bacteria were grown in M9 media [containing 0.4% glucose and 20 mM of glutamine]. While *ΔglsA* grows at a slightly lower rate, the strains exhibited similar growth

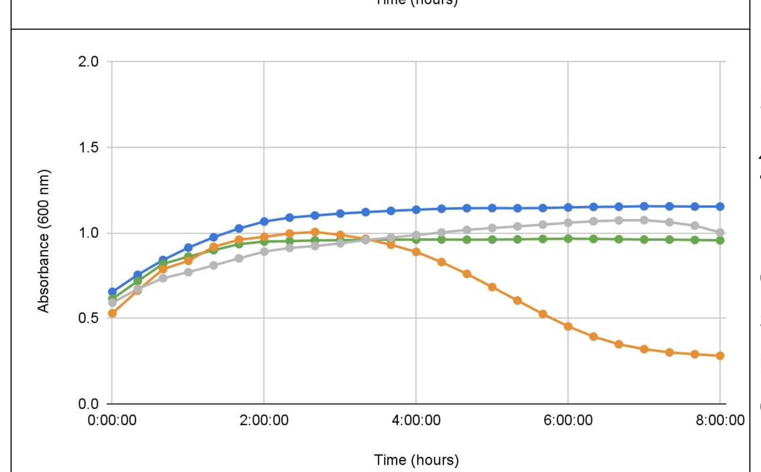

Figure 8: Comparative Lysis Curves of Parent Strain, and Knockout *ΔglnA*, *ΔglsA*, *and ΔglsB E. coli* Strains in M9 Media
The bacteria were grown in M9 media [containing 0.4% glucose and 20 mM of glutamine]. The *ΔglsB* strain exhibited the most significant lysis in the glucose conditions measured with T4 phage. Lysis was not observed in the other strains

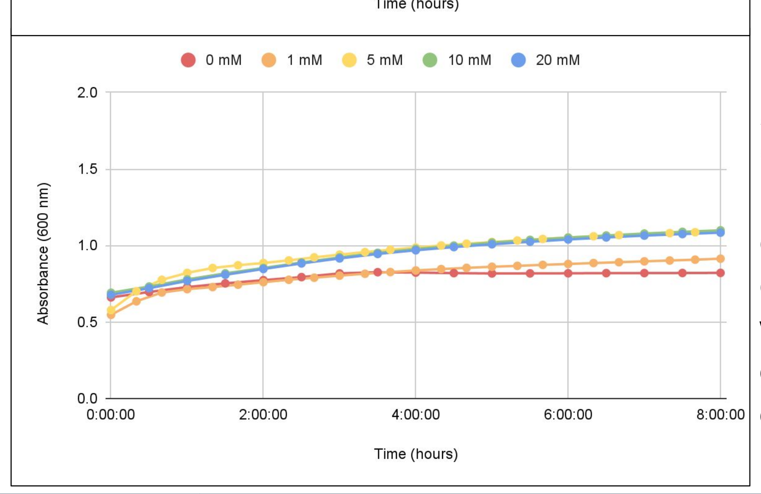

Figure 9: Comparative Lysis Curves of Parent *E. coli* Strain in Minimal Media Under Varying Glutamine Concentrations
Parent *E. coli* strain was grown in M9 media containing 0.1% glucose and varied glutamine concentrations ranging from 0 to 20 mM. Lysis with T4 phage was not observed. Bacterial growth of the parent strain increased slightly as glutamine increased.

DISCUSSION & FUTURE DIRECTIONS

Discussion:

- The parent, ΔglsA, and ΔglsB strains grew similarly in rich LB media conditions. The *ΔglnA* strain showed a slight decrease in growth.
- Despite glutamine metabolism being fully active in the parent strain (Fig. 3A), bacteriophage was unable to replicate (Fig. 9). This could indicate that other nutrients, such as additional glucose, are required for optimal replication
- When glucose concentration increased from 0.1% to 0.4% while still containing 20 mM glutamine, T4 phage was able to replicate and lyse the *ΔglsB* strain (Fig 7). T4 phage may have a similar replication method to HCMV.
- The *ΔglsA* enzyme contributes more to the glutaminolysis pathway than *ΔglsB* since it has greater enzymatic activity. The removal of the *glsB* gene did not impact bacterial growth under varying glutamine concentrations. This can indicate the bacteria preference for the *glsA* gene than the *glsB* gene.

Future Directions:

- Determination of optimal T4 phage replication conditions can be determined by adding an ample concentration of glutamine and varying glucose concentrations.
- Further characterization of viral replication can be found through burst size calculations.
- TCA intermediates such as alpha-ketoglutarate can be introduced into the knockout strains to see if bacterial growth can be recovered during lysis.

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