FIRE: THE FIRST-YEAR
INNOVATION & RESEARCH
EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Nutrient Uptake Shifts During T4 Phage Replication in *Escherichia coli*

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Introduction

- *Escherichia coli* (*E. coli*) bacteria utilizes metabolic pathways like the TCA cycle to generate energy and vital intermediates.
- Bacteriophage are viruses that target bacteria and alter host metabolisms to support their replication. (Sanchez & Lagunoff, 2015)
- Glucose is a well-known carbon source that yields high energy, and can enter glycolysis and produces molecules that can enter the TCA cycle.
- Glutamine is a substrate that, through a series of reactions, can form alpha-Ketoglutarate and anaplerotically replenish the TCA cycle.
- Some viruses, such as Human cytomegalovirus and Vaccinia Virus, were found to mainly utilize glutamine for replication over glucose (Fontaine et al., 2014).

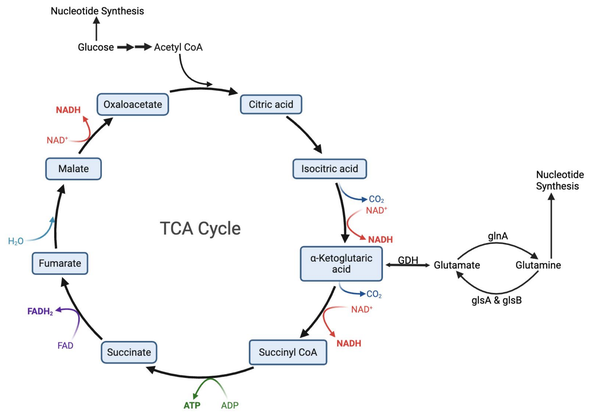


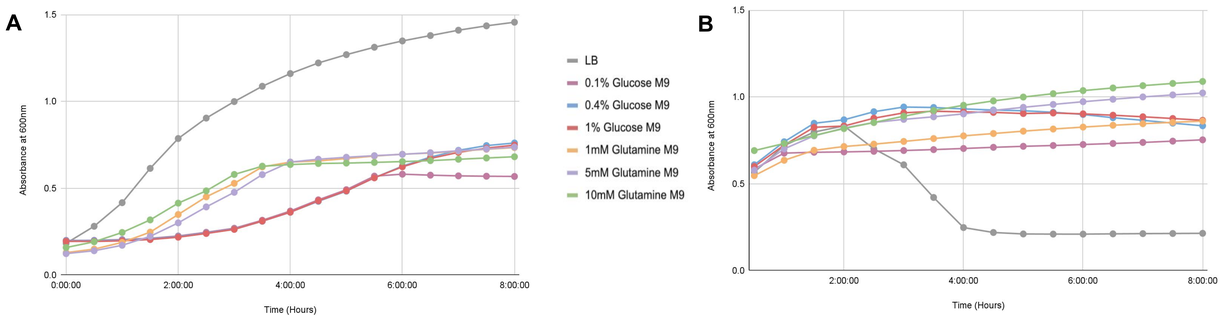
Figure 1: Diagram of the TCA Cycle and Glutamine Metabolism. Figure was made with BioRender.

Hypothesis

If T4-bacteriophage replication in *E. coli* depends on glucose and glutamine, then *E. coli* cells infected with T4 phage will exhibit significantly higher uptake of these nutrients compared to uninfected cells.

Background

Figure 2: Comparative Growth and Lysis Curves: Parent *E. coli* was cultured in LB rich media and minimal M9 media, with varying glucose concentration or with 0.1% glucose and varying glutamine concentrations. **(A)** Cells grown in LB rich media exhibited rapid growth, but growth was reduced in cells cultured with M9 media. The addition of glutamine helped expedite growth in cells grown in M9 media containing only glucose. **(B)** Due to varying bacterial growth rates in M9 media, trials were run separately, and T4 phage was added when absorbance at 600nm equals 0.4 to ensure uniformity. Significant lysis was observed in cells grown in M9 media, slight lysis was observed in M9 media containing 0.4% and 1% glucose, and no lysis was observed in M9 media containing varied glutamine concentrations.



Discussion & Future Directions

- The increase in glucose and glutamine uptake in T4-infected cells indicate their importance in propagating viral replication.
- Both one-time point phage titer and uptake experiments confirmed that both glucose and glutamine are essential for optimal phage replication.
- Conducting both uptake assay on T4-infected and uninfected *glsA*, *glsB*, and *glnA* knockout strains may reveal how the cell utilizes each nutrient.
- Perform the ATP assay in the parent strain and three knockout genes to compare changes in ATP production in phage-infected cells and uninfected cells.

References & Acknowledgements

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- Fontaine, K., Camarda, R., & Lagunoff, M. (2014). Vaccinia virus requires glutamine but not glucose for efficient replication. Journal of Virology, 88(8), 4366–4374. doi.org/10.1128/JVI.03134-13
- Sanchez, E. L., & Lagunoff, M. (2015). Viral activation of cellular metabolism. Virology, 479–480, 609–618. doi.org/10.1016/j.virol.2015.02.038

Glucose Uptake Increases in T4 Phage-Infected *E. coli* Cells

Figure 3. Glucose Uptake Assay: The Promega Glucose Uptake-Glo™ Assay kit was adapted for use in Bacterial cells. The schematic depicts how the assay detects glucose uptake. Cells were incubated in LB media until absorbance at 600nm equals 0.4 and were washed and resuspended in M9 media. A portion of the cells received T4 phage. Mechanical lysis was used to break open the cell wall instead of the provided lysis buffer.

Figure 4. Glucose Uptake Schematic. From Uptake-Glo™ Protocol

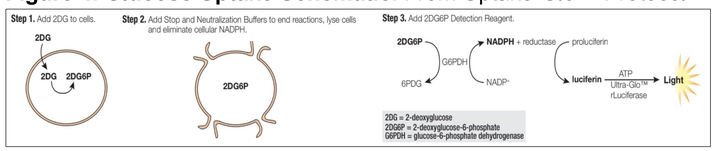
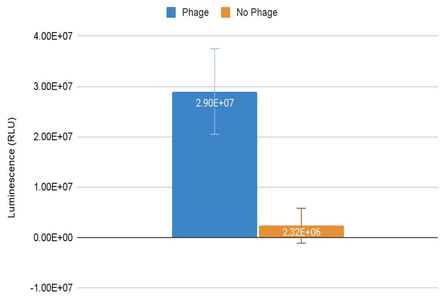
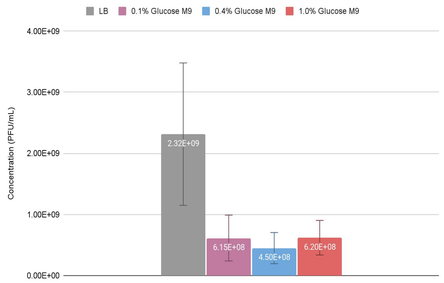


Figure 5. One-Time Point Phage Titer: Quantification of T4 phage plaque forming units (PFU) was carried out in the parent *E. coli* strain in LB rich media and M9 media with varying glucose concentrations (0.1%, 0.4%, or 1%). Cells were incubated in flasks in each media, and when each one reached an absorbance of 0.4 at 600nm, indicating log phase, T4 phage was added. Samples were taken 90 minutes after each flask received phage. A higher concentration of phage was observed under LB nutrient rich conditions, and lower phage concentration was observed in the minimal media conditions with no significant differences among the varying glucose concentrations. Error bars represent the average of triplicate plating.





Increased Glutamine Uptake in T4 Phage-Infected *E. coli* Cells

Figure 6. Glutamine Uptake Assay: The schematic below depicts how the Promega Glutamine Uptake-Glo™ Assay kit, which was adapted for use in Bacterial cells assay, works. *E. coli* cells were incubated in LB until absorbance at 600nm reached 0.4. Cells were washed and resuspended in M9 media. A portion of the cells were treated with phage, and all cells either received the glutaminase enzyme or didn't. Mechanical lysis was used to break open the cell wall. To quantify the amount of glutamine, we subtracted the luminescence values for the enzyme treated cells by the non-enzyme treated cells.

Figure 7. Glutamine Uptake Schematic. From Uptake-Glo™ Protocol

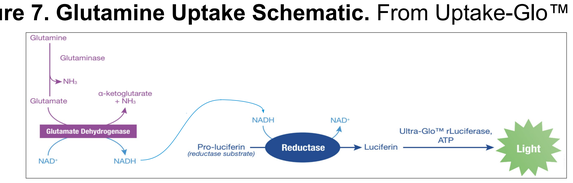


Figure 8. One-Time Point Phage Titer: Quantification of T4 phage plaque forming units (PFU) was carried out in the parent *E. coli* strain in LB rich media and M9 media with 0.4% glucose and varying glutamine concentrations (1mM, 5mM, or 10 mM). Parent *E. coli* cells were grown in separate flasks containing LB media or M9 media. When each flask reached an absorbance of 0.4 at 600nm, T4 phage was added and incubated for 90 minutes. At the end of the time, a sample from each flask was used to create plaque assays to quantify the PFU present in each media. Each sample was plated once.