FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Implications of the TCA Cycle on *Escherichia coli* Growth and Phage Replication

Eesha Bokil, Marissa Chuck, Carlos Koudouovoh, Aiswarya Suriaprakash

BACTERIA, PHAGE, & THE TCA CYCLE

- The constant use of antibiotics has resulted in recent increases in antibiotic resistant bacteria (Nguyen et al., 2005). An alternative treatment is phage therapy.
- This study aims to increase the understanding of the relationship between bacteria and phage. We are studying the TCA cycle and its implications on the relationship between *Escherichia coli* and T4 phage.
  - The TCA cycle is involved in the production of ATP within bacterial cells which T4 phage require for replication (Mahmoudabadi et al., 2017).

$$\text{Isocitrate} \xRightarrow{icd} \alpha\text{-ketoglutarate} \Rightarrow \text{Normal ATP}$$

$$\text{Isocitrate} \not\xRightarrow{\cancel{icd}} \cancel{\alpha\text{-ketoglutarate}} \Rightarrow \text{Decreased ATP}$$

Figure 1. Isocitrate Dehydrogenase Reaction in Krebs Cycle
This study researches this specific reaction. The removal of the *icd* gene hinders the catalyzation of this reaction, resulting in no product and decreased ATP production (Kabir & Shimizu, 2004).

EXPERIMENTAL APPROACH

- Bacterial Growth Curves: Allows for comparison of bacterial growth between strains and media.
- Cell Lysis Curves by T4 and T4r Phage: Allows for comparison of phage efficacy between strains and type of phage.
- Plaque Assays: Allows for comparison in plaque formation, phage efficacy, and lysis characteristics between strains and type of phage.

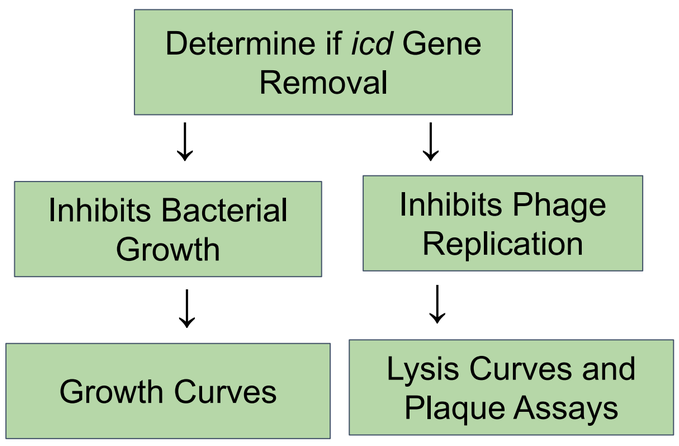


Figure 2. Flow Chart of Experimental Approach
This is the experimental approach of the study to determine the role of the *icd* gene in bacterial growth and phage replication.

DISCUSSION & FUTURE DIRECTIONS

Discussion

- The *Δicd* strain experienced less growth than the parent strain, supporting findings from previous research (Kabir and Shimizu, 2004).
- The *Δicd* strain experienced slower lysis by T4 and T4r phage than the parent strain. Formed plaques are similar meaning lysis of both strain cells is similar. Both are novel findings.
- Viral infection increases the amount of TCA cycle metabolites, supporting the findings from previous research (Munger et al., 2006).

Future Directions

- Measure the amount of phage present at different time points during cell lysis by T4 and T4r phage. This would allow further understanding of phage replication.
- Measure ATP levels during phage replication to better understand amount of ATP usage by phage at different times during replication.

REFERENCES & ACKNOWLEDGEMENTS

- Kabir, M.M., and Shimizu, K. (2004). Metabolic regulation analysis of icd-gene knockout Escherichia coli based on 2D electrophoresis with MALDI-TOF mass spectrometry and enzyme activity measurements. Appl Microbiol Biotechnol *65*, 84–96.
- Mahmoudabadi, G., Milo, R., and Phillips, R. (2017). Energetic cost of building a virus. PNAS *114*, E4324–E4333.
- Munger, J., Bajad, S.U., Coller, H.A., Shenk, T., and Rabinowitz, J.D. (2006). Dynamics of the Cellular Metabolome during Human Cytomegalovirus Infection. PLoS Pathog *2*, e132.
- Nguyen, T.V., Le, P.V., Le, C.H., and Weintraub, A. (2005). Antibiotic Resistance in Diarrheagenic Escherichia coli and Shigella Strains Isolated from Children in Hanoi, Vietnam. Antimicrobial Agents and Chemotherapy *49*, 816–819.

Financial support for this project was provided by the First-Year Innovation and Research Experience (FIRE) at the University of Maryland, College Park.
Research support for this project was provided by the Host-Pathogen Interactions (HPI) Peer Mentors of FIRE and Dr. John Purdy and Lisa Wise of the Purdy lab of the Immunobiology Department of the University of Arizona BIO5 Institute.

EXPERIMENTAL RESULTS

Project Objective: Determine if removal of the *icd* gene decreases *E. coli* bacterial growth and/or inhibits T4 phage replication.

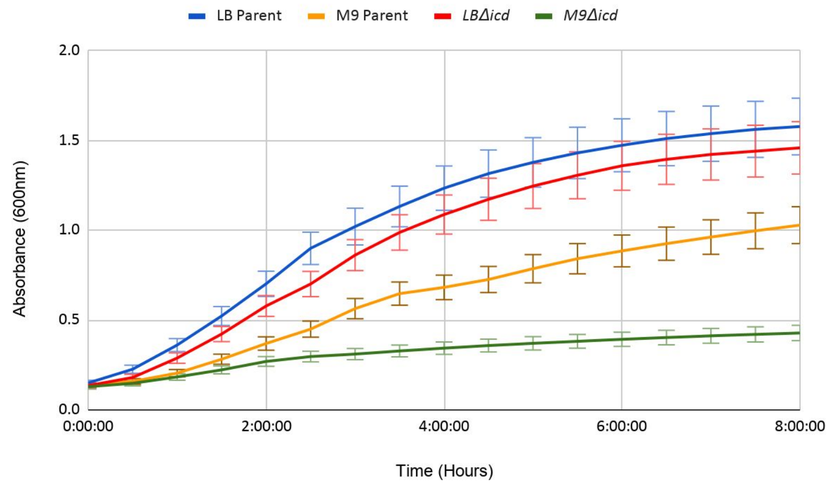
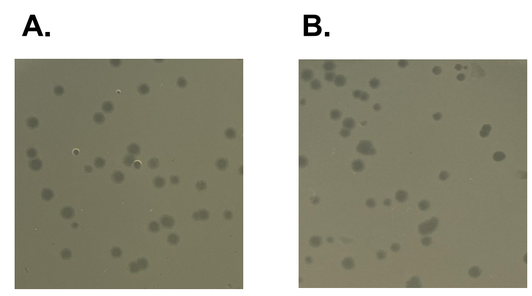


Figure 3. Removal of *icd* Gene Decreases Growth
The growth of the parent and *Δicd E. coli* strains in LB media and M9 media was observed over eight hours by measuring absorbance at 600 nm. In M9 and LB media, the knockout strain experienced less growth than the parent strain.

Figure 4. A. Plaque assay of *Δicd* strain with T4 phage (10^{-6} dilution). B. Plaque assay of parent strain with T4 phage (10^{-6} dilution).
Plaque assays were made using a double agar overlay technique. The plaque assays of the parent strain and *Δicd* strain with T4 phage feature plaques of similar size and shape, indicating similar lysis of bacterial cells by phage.



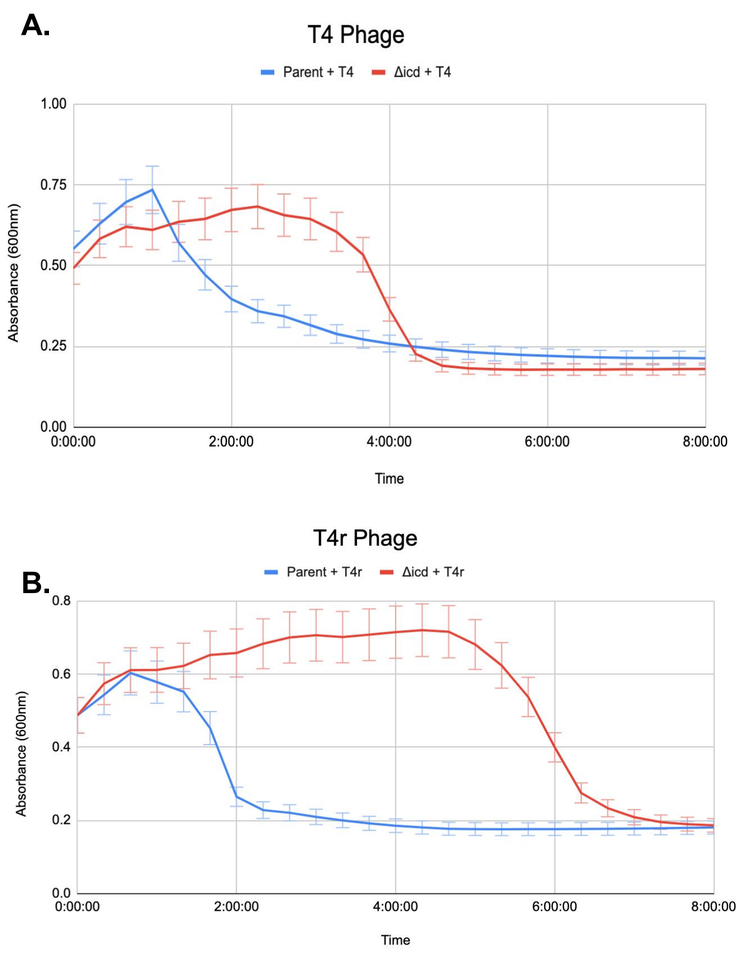


Figure 5. A. Removal of *icd* Gene Slows Lysis by T4 Phage. B. Removal of *icd* Gene Slows Lysis by T4r phage. Lysis of the parent and *Δicd* strain cells with phage was observed over eight hours by measuring absorbance at 600nm. The *Δicd* strain experienced slower lysis by phage than the parent strain.

Figure 6. HCMV Infection Increases TCA Cycle Metabolite Levels
Various TCA cycle metabolite levels were observed at 48 and 72 hours post infection in human fibroblast cells during human cytomegalovirus (HCMV) infection and mock infections. This figure shows the fold change (HCMV infected/mock infected) of metabolite levels, and data is displayed on a log2 scale. All metabolites except malate increased in amount over time. Malate did experience an increase in amount after infection, but this increase lessened over time.