FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Effects of the *aceE* Gene on *E. coli* Growth and Bacteriophage Replication

Celia Lippert, Julie Anne Lynskey, James Lee, Ruben Dasgupta

BACKGROUND

Background:

- Phage therapy uses bacteriophage viruses to kill bacteria and combat antibiotic-resistant bacterial infections (Schmidt, 2019).
- Viruses can alter host cellular metabolism to induce replication (Sanchez & Lagunoff, 2015).
- The Tricarboxylic Acid (TCA) cycle creates reduced electron transport carriers that can be used for multiple cellular processes in *E. coli* (Sanchez & Lagunoff, 2015)
- The *aceE* gene is a critical regulator of the TCA cycle in *E. coli* (Sanchez and Lagunoff, 2015). Without it, the TCA cycle would become far less efficient.

Objective:

- Determine the effects of removing the *aceE* gene from *E. coli* on bacterial growth and viral replication.

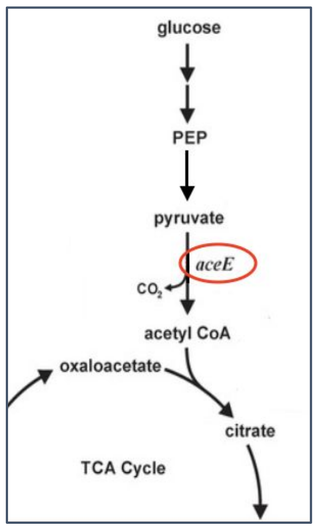

Figure 1: *aceE's* role as a subunit of Pyruvate, a critical regulator of the TCA cycle. Figure adapted from Moxley et al. (2023)

METHODS

Materials:

- LB media and M9 media
- Bacteriophage (T2, T4, T4r)
- Top agar, LB plates, and LB + Kanamycin plates
- Parent *E. coli* strain and *aceE* knockout strain (from Keio KO collection - Baba. et al.)

Methods:

- Create serial dilutions from overnight cultures of parent strain and *aceE* knockout strain → use spectrophotometr to compare growth.
- Perform phage quantification using the double agar overlay method.
- Create growth and lysis curves.
- Measure and compare phage titer in each strain at two time points (45 minutes and 90 minutes post-log phase).
- Conduct ATP Quantification Assays.

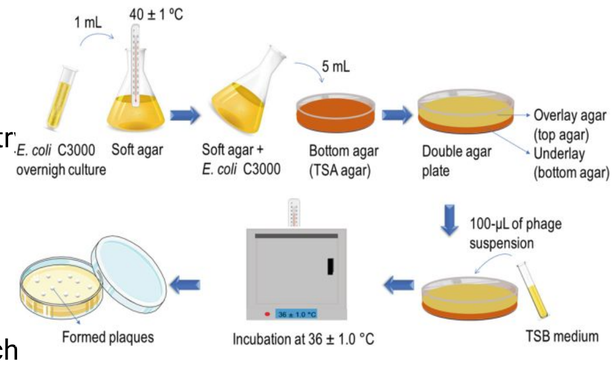

Figure 2: Phage Quantification using the Double Agar Overlay Method
Figure from da Silva et al. (2021)

RESULTS

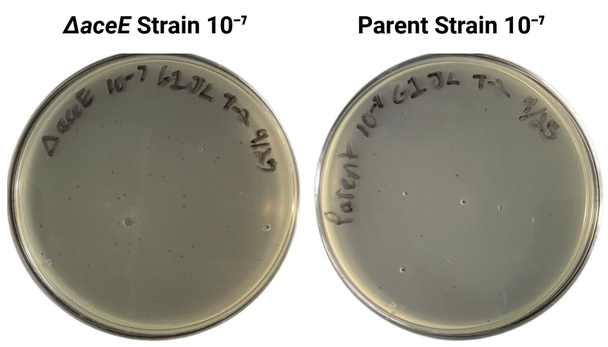

Figure 3: Plaque Assays of Parent *E. coli* and *ΔaceE* Strains using T2 Bacteriophage. T2 bacteriophage dilutions of 10^{-7} are shown for the Parent *E. coli* strain and *ΔaceE* strain. Plaque assays were made using the double agar overlay method. Each plaque represents an area where the bacterial lawn was lysed by bacteriophage. These assays depict a larger number of plaques in the *ΔaceE* strain compared to the parent strain.

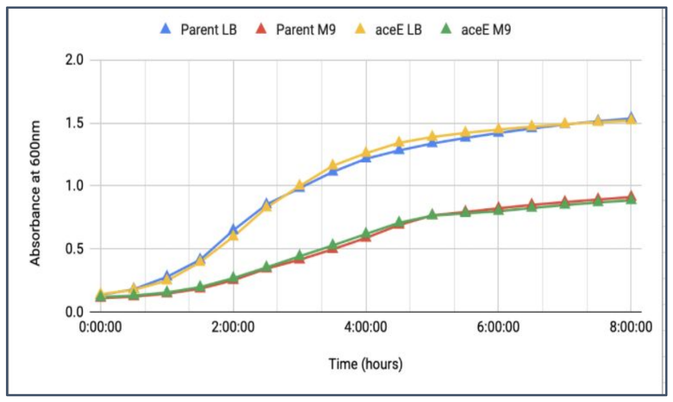

Figure 4: Parent *E. coli* and *ΔaceE* Strain Grow at Similar Rates, M9 Media Limits Growth in Both Strains. There is no discernible difference in growth between the Parent *E. coli* strain and *ΔaceE* strain in LB media. Cultures of the *E. coli* and *ΔaceE* strains were grown at 37℃ overnight in a shaking incubator. The growth curve was created by taking absorbance at 600nm using a microplate reader. Absorbance was taken for the Parent strain and the *ΔaceE* strain in LB and M9 minimal media every 30 minutes over a period of 8 hours.

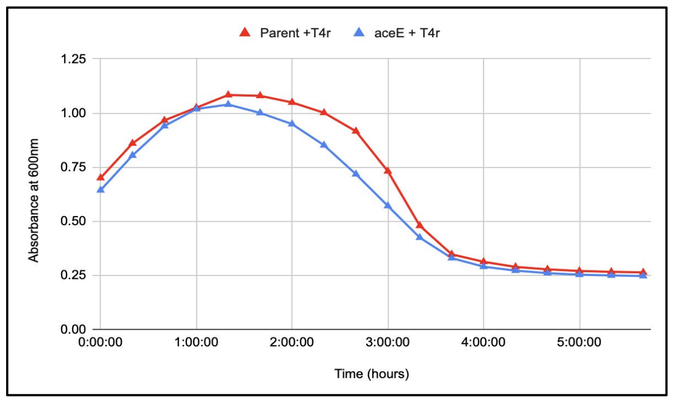

Figure 5: Lysis of *E. coli* Bacteria by T4r Bacteriophage Occurs at Similar Rates in the Parent and *ΔaceE* Strains. Cultures of the *E. coli* and *ΔaceE* strains were grown at 37℃ overnight in a shaking incubator.T4r bacteriophage was added to the overnight cultures. The absorbance at 600nm was taken using a microplate reader for the Parent strain and the *ΔaceE* strain in LB minimal media every 30 minutes over a period of 8 hours. The lysis curve shows no discernible difference in lysis between the strains.

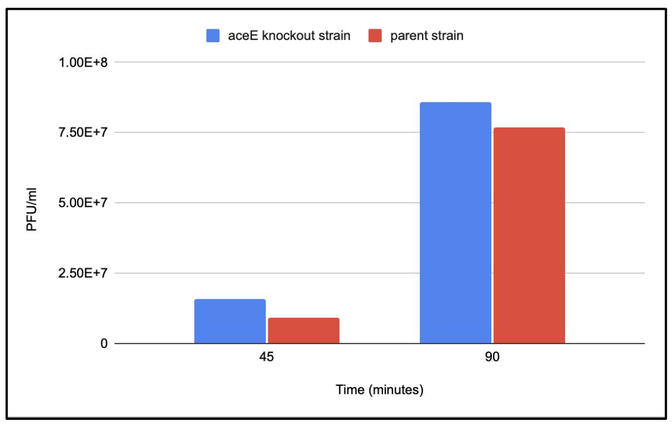

Figure 6: Greater Bacteriophage Replication in *ΔaceE* vs. Parent Strain at Both 45 and 90 Minutes. Cultures of the *E. coli* and *ΔaceE* strains were grown at 37℃ overnight in a shaking incubator. T4r bacteriophage was added to the overnight cultures. The cultures were allowed to grow at two 45 minute intervals in a 37℃ shaking incubator before being plated at both time points using the double agar overlay method. The graph of this two time point phage titer shows greater bacteriophage replication in the *ΔaceE* strain at both time points.

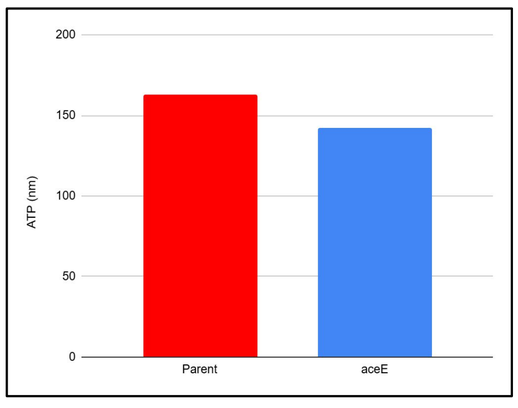

Figure 7: Lower ATP Production in *ΔaceE* vs. Parent Strain. Cultures of the *E. coli* and *ΔaceE* strains were grown at 37℃ overnight in a shaking incubator. Cultures were diluted then allowed to grow to OD_{600}= 0.25. Each culture was centrifuged to concentrate, resuspended in a lysis buffer, allowed to incubate for 15 min, and max centrifuged for 10 min. An ATP Detection Kit was used to prepare Standard and Blank wells. A reaction Mixture consisting of 1X ATP Detection Assay Buffer, D-Luciferin, and Luciferase was added to cell lysates. Incubation was allowed for 20 minutes, and luminescence was collected from a plate reader. ATP concentration was then calculated. The graph shows less ATP production in the *ΔaceE* strain compared to the Parent strain.

DISCUSSION & FUTURE DIRECTIONS

Discussion:

- As seen in Figure 4, removing the *ΔaceE* gene from *E. coli* has no effect on its overall growth.
  - Growing *E. coli* in M9 minimal media limits growth in both the Parent strain and *ΔaceE* strain.
- The plaque assays and phage titer show greater phage replication in the *ΔaceE* strain.
  - From this, it can be assumed that the *ΔaceE* gene being present makes it harder for bacteriophage to replicate in the host cell.

Future Directions:

- Examine strains of *E. coli* with the *aceE* gene overexpressed to see the effect on bacteriophage replication.
- Use other types of phage (besides T2) to see if *aceE* affects all bacteriophage replication.
- Study other genes within the pathway (like *aceF* or *lpd*) to see if they have the same effect on bacteriophage replication.

REFERENCES

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/msb4100050

da Silva, R. T., de Souza Grilo, M. M., Magnani, M., & de Souza Pedrosa, G. T. (2021). Double-Layer Plaque Assay Technique for Enumeration of Virus Surrogates. In M. Magnani (Ed.), Detection and Enumeration of Bacteria, Yeast, Viruses, and Protozoan in Foods and Freshwater (pp. 157–162). Springer US. https://doi.org/10.1007/978-1-0716-1932-2_14

Moxley, W. C., Brown, R. E., & Eiteman, M. A. (2023). Escherichia coli aceE variants coding pyruvate dehydrogenase improve the generation of pyruvate-derived acetoin. Engineering in Life Sciences, 23(3), e2200054.

Sanchez, E. L., & Lagunoff, M. (2015). Viral activation of cellular metabolism. Virology, 479–480, 609–618. https://doi.org/10.1016/j.virol.2015.02.038

Schmidt, C. (2019). *Phage Therapy Could Beat Drug-Resistant Illnesses*. Scientific American. https://www.scientificamerican.com/article/phage-therapy-could-beat-drug-resistant-illnesses/

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

We'd like to thank Dr. O'Hara and the Host-Pathogen Interactions Lab for their support and advising.