FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

INVESTIGATING THE ROLE OF *E. COLI* TCA CYCLE METABOLISM IN BACTERIOPHAGE REPLICATION

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Background:

- The Tricarboxylic Acid (TCA) Cycle is a multi-step aerobic enzyme-catalyzed pathway that is responsible for generating electron carrier molecules, NADH and $FADH_2$, which are crucial for generating ATP for *Escherichia coli* cells during the process of cellular respiration. The *E. coli* genes, *acnA* and *acnB* encode enzymes that catalyze necessary reactions in the TCA cycle.
- Bacteriophages are viruses that specifically infect and hijack *E. coli's* metabolic processes in order to proliferate, ultimately destroying the host cell in the process
- The TCA cycle not only provides an energy source for *E. coli,* but for bacteriophage that take over the host cell and use the metabolic resources to drive their own replication

Figure 2. Comparative Lysis Curves: The *E. coli* strains were separately inoculated in LB media and T4 bacteriophage was added at time 0. Then, a plate reader measured absorbance at 600 nm every 20 minutes for 8 hours.

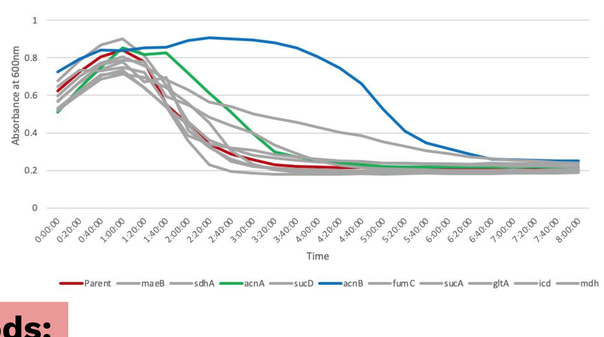
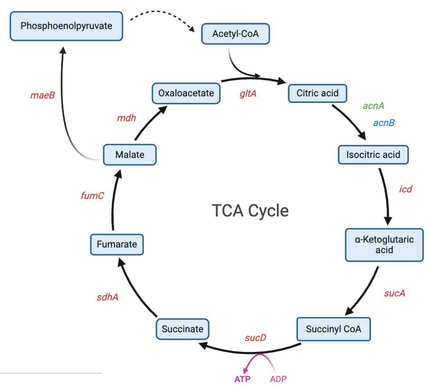


Figure 1. Simplified Schematic of The Citric Acid Cycle: Made using BioRender



Objectives & Methods:

- To compare and analyze growth and lysis curves between the parent, *ΔacnA*, and, *ΔacnB* knockout strains of *E. coli* (Baba et al., 2006).
- To quantify bacteriophage replication in parent and knockout strains by performing double agar overlay plaque assays. This involves treating the parent and knockout strains of *E. coli* with varying concentrations of T4 bacteriophage. The plaque forming units (PFU) were then quantified for each serial dilution of the phage.
- To compare ATP concentrations among different knockout strains of *E. coli* using Cayman Chemical ATP Detection Assay Kit.

Hypothesis:

- The removal of the *acnA and acnB* genes would negatively impact the growth rate and ATP levels of *E. coli* and, as a result, inhibit or slow the replication of bacteriophage.

Discussion & Future Directions:

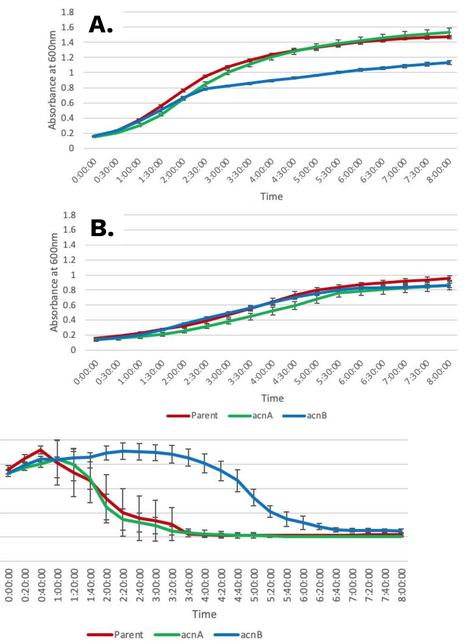
- The *ΔacnB* strain showed significantly less growth in LB media compared to the *ΔacnA* and parent strains.
- Comparative plaque assays revealed that the *ΔacnA* strain formed more plaques in the presence of T4 bacteriophage than both the *ΔacnB* and parent strains.
- Redo the One-Step Growth Curve protocol to calculate burst size as previous results were inconclusive.
- Performing the Two Time Point Titer again to further investigate the differences between plaque formation and bacteriophage replication between *ΔacnA* and *ΔacnB*.
- Redo the ATP Detection Assay Kit because *ΔacnB* should have lower [ATP] compared to parent and *ΔacnA*
- Examine *ΔgltA and Δicd* due to their slower growth rates in M9 media would be an additional direction to take.

Acknowledgements & References:

- Financial support for this project was provided by the First-Year Innovation and Research Experience (FIRE) at the University of Maryland, College Park.
- 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
- EcoCyc E. Coli Database. (2022). Retrieved October 9, 2022, from https://ecocyc.org/

Comparative Growth Curves Show Non-Uniform Growth in LB Media:

Figure 3. Comparative Growth Curves in LB and Minimal Media: The parent and knockout strains were grown in rich LB media and M9 media. Growth was measured using a plate reader, which performed continuous shaking at 37℃ and measured absorbance at 600 nm every 30 minutes for 8 hours. **A.** The *ΔacnB* strain grew slower and plateaued earlier than the other strains when grown in the LB. **B.** The knockouts grew nearly identically when grown in M9.

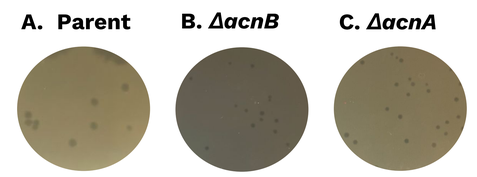


Comparative Lysis Curves Show Delayed Lysis in *ΔacnB*:

Figure 4. Comparative Lysis Curves: The *E. coli* strains were separately inoculated in LB media and T4 bacteriophage was added at time 0. Then, a plate reader measured absorbance at 600 nm every 20 minutes for 8 hours. As shown by the graph, the *ΔacnB* strain lysed much later than the other strains once bacteriophage was introduced.

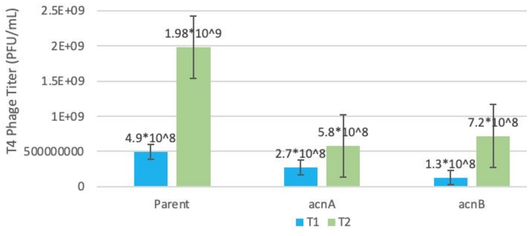
Comparative Plaque Assays Revealed *ΔacnB* had Greater Resistance to Bacteriophage Lysis than *ΔacnA*:

Figure 5. Comparative Plaque Assays: These plates represent the resistance the parent, *ΔacnA*, and *ΔacnB* knockout strains of *E. coli* had for the T4 bacteriophage replication at a concentration of 10E-6. At this dilution, **A./B.** Parent and *ΔacnB* showed similar resistance to lysis (N=51 and N=55, respectively). **C.** *ΔacnA* had the least amount of resistance (N=75).



Two Time Point Titer Revealed *ΔacnA and ΔacnB* Resistance to Bacteriophage Lysis:

Figure 6. Two Time Point Phage Titer: Overnight cultures of the three strains were grown, and cultures were then incubated at 37℃ in separate flasks containing LB, $CaCl_2$, and T4 bacteriophage. Time points were taken at 45 and 120 minutes. The samples were diluted then plated with a double agar overlay. From there the PFU/mL was calculated based on the 10E-6 dilution and the plaques formed in each overlay. As shown by the graph, *ΔacnB* and *ΔacnA* showed significantly lower PFU at both time points when compared to the parent strain.



Comparative ATP Concentrations show a decrease in [ATP] in Knockout Strains :

Figure 7. Comparative ATP Assays: E. *coli* strains were grown to OD_{600} = 0.25, lysed with E. *coli* lysis buffer, and their luminescence was measured with a plate reader. Using the linear regression equation from the ATP standard curve, ATP concentration values were calculated. Both knockout strains exhibited a decrease in ATP concentrations in comparison to the parent strain. Indicating a disturbance to the TCA cycle.