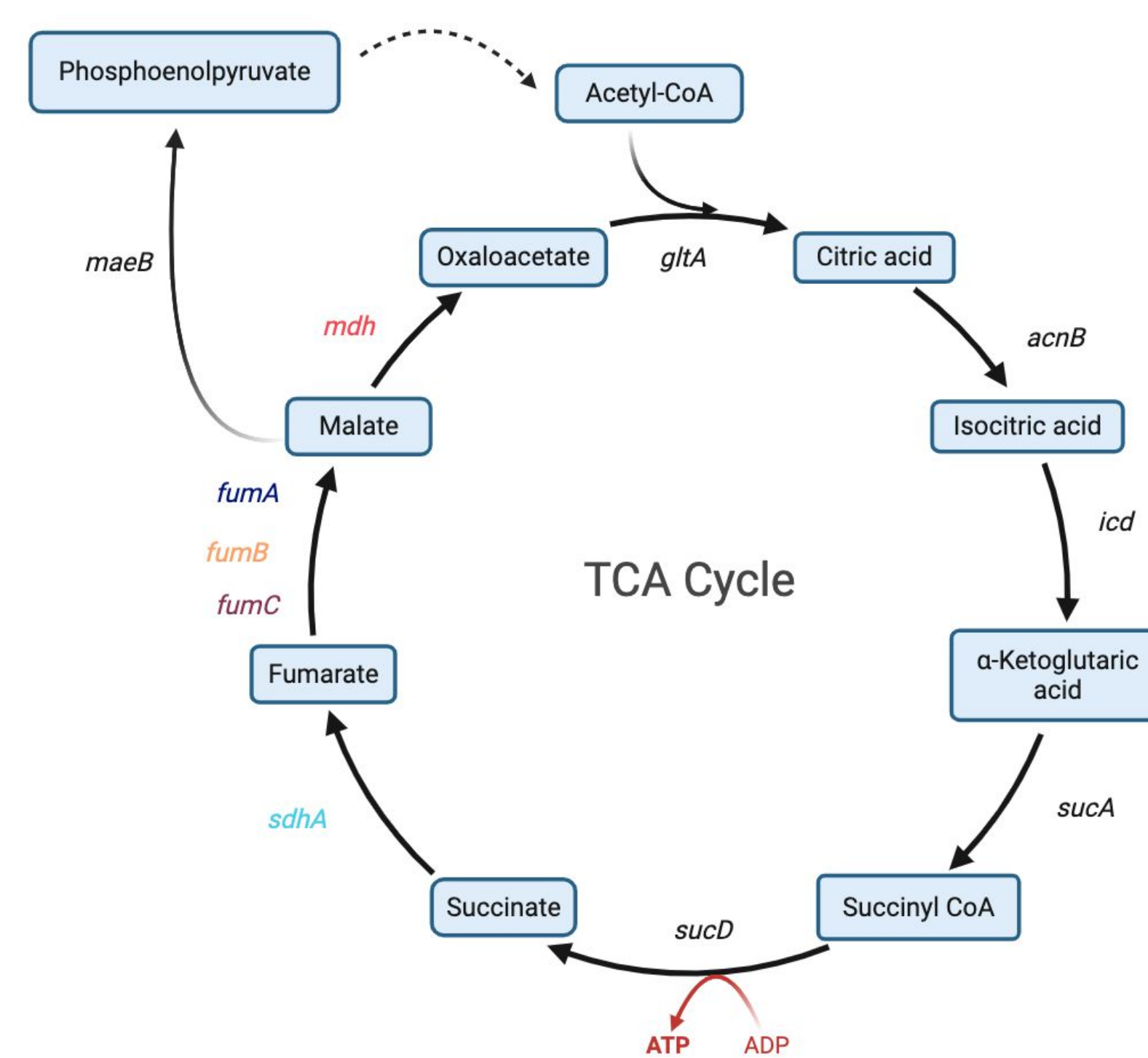


Background:

- The Tricarboxylic Acid (TCA) Cycle is a multi-step aerobic enzyme-catalyzed pathway that is responsible for generating electron carrier molecules, NADH and FADH₂, which are crucial for generating ATP for *Escherichia coli* cells during the process of cellular respiration. The *E. coli* genes, *sdhA*, *fumC*, *fumB*, *fumA* and *mdh* encode enzymes that catalyze necessary reactions in the TCA cycle.
- fumC*, *fumB*, and *fumA* encode three different Fumarase isoenzymes. *fumA* is most active under anaerobic conditions, *fumB* is most active under aerobic conditions, and *fumC* is active when *fumA/fumB* are inactive.
- 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 1. Simplified Schematic of The Citric Acid Cycle: Made using BioRender



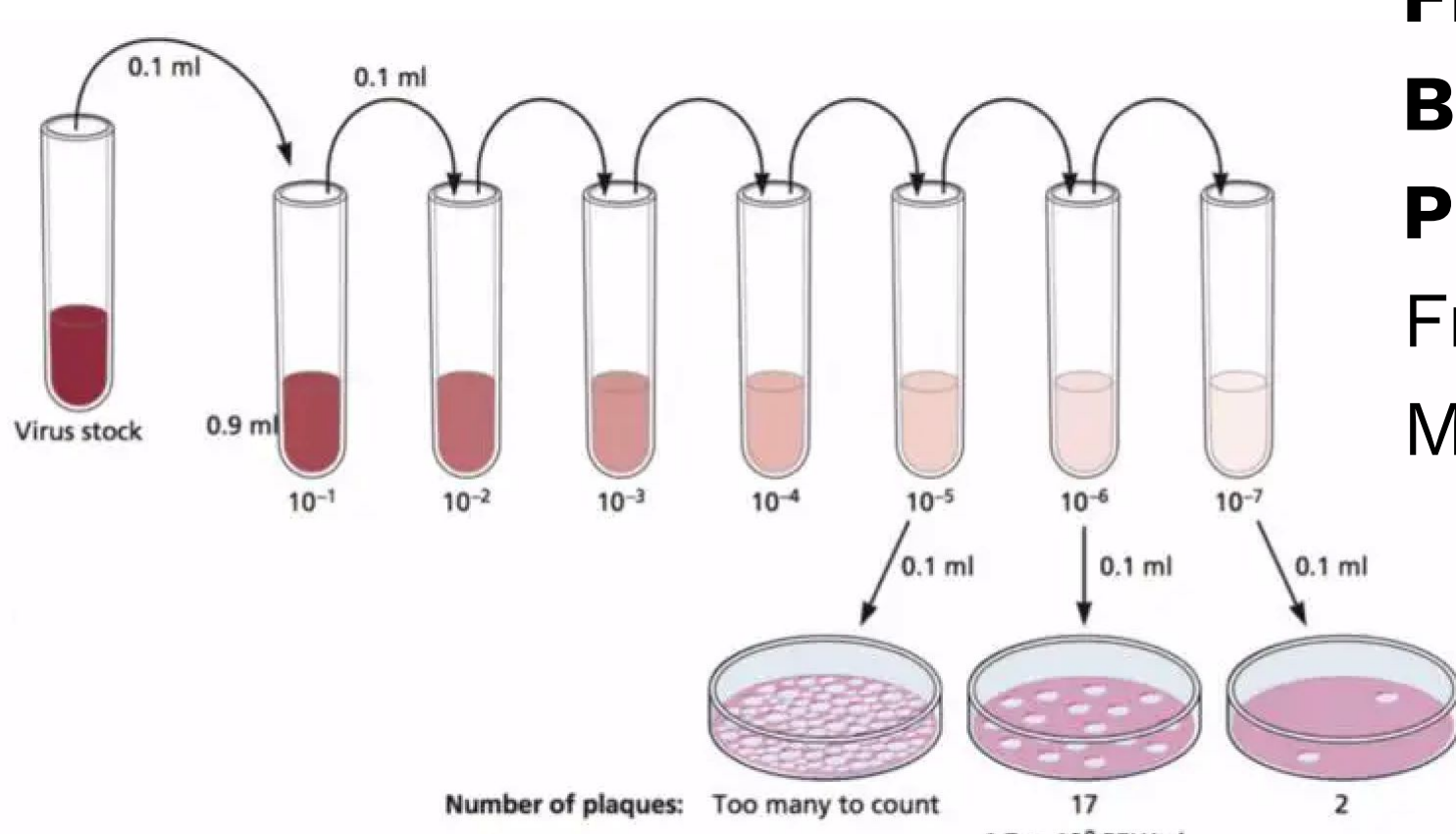
Hypothesis:

- The individual removal of the *sdhA*, *fumC*, *fumB*, *fumA*, and *mdh* genes would negatively impact the growth rate and ATP levels of *E. coli* and, as a result, inhibit or slow the replication of bacteriophage.

Objectives & Methods:

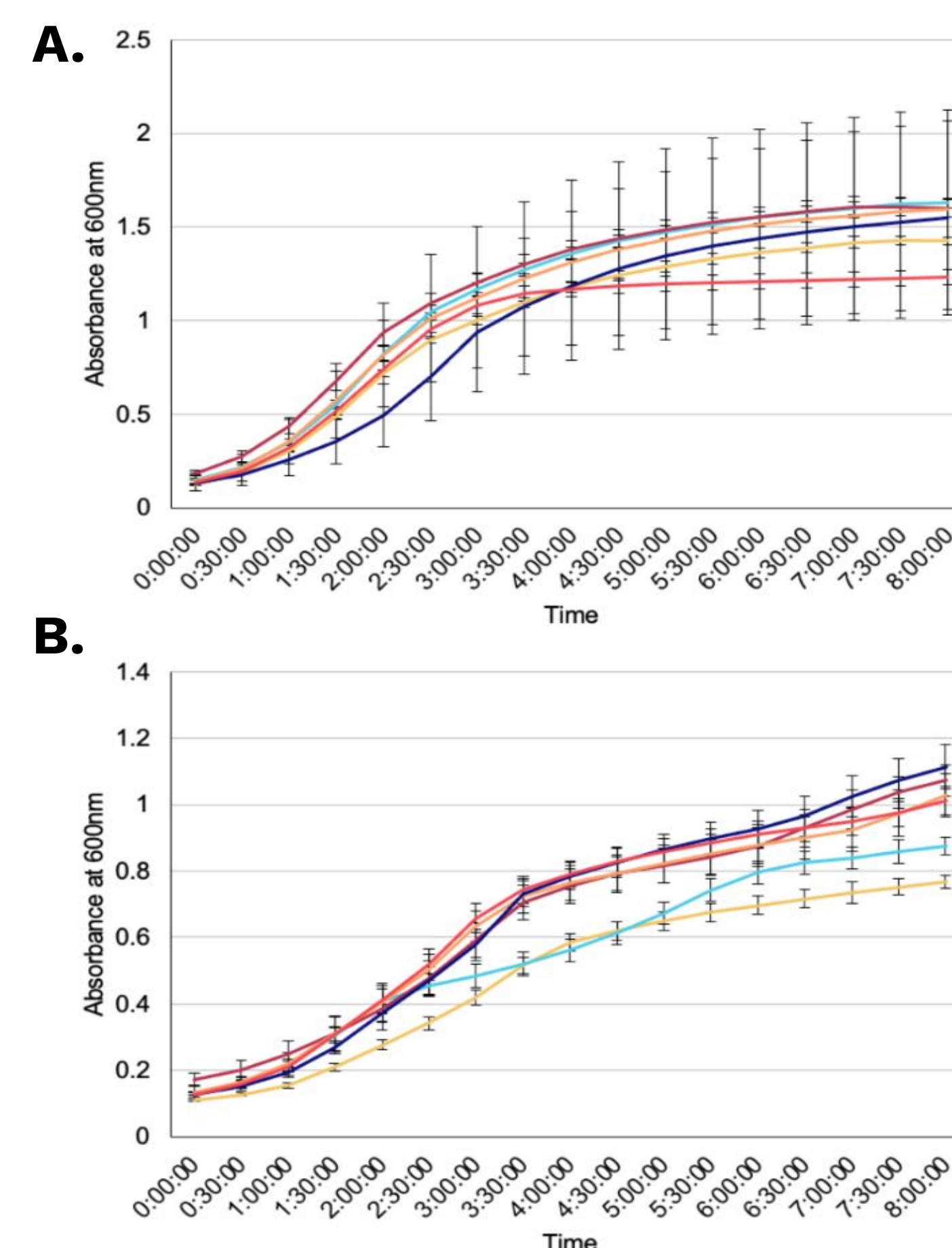
- To compare and analyze growth and lysis curves between the parent, Δ *sdhA*, Δ *fumC*, Δ *fumB*, Δ *fumA*, and Δ *mdh* knockout strains of *E. coli* (Baba et al., 2006).
- To quantify bacteriophage replication in parent and knockout strains by performing double agar overlay plaque assays. This involves 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.

Figure 2. Bacteriophage Plaque Assay: From MicrobeOnline



Comparative Growth Curves Show Nonuniform Growth in LB Media and Minimal 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°C and measured absorbance at 600 nm every 30 minutes for 8 hours. **A.** The Δ *mdh* strain plateaued earlier than the other strains when grown in the LB media. **B.** Each knockout with the exception of Δ *sdhA* grew to a higher absorbance than the parent strain.



Comparative Lysis Curves Show Delayed Lysis in Δ *fumC* Compared to Other Knockout Strains:

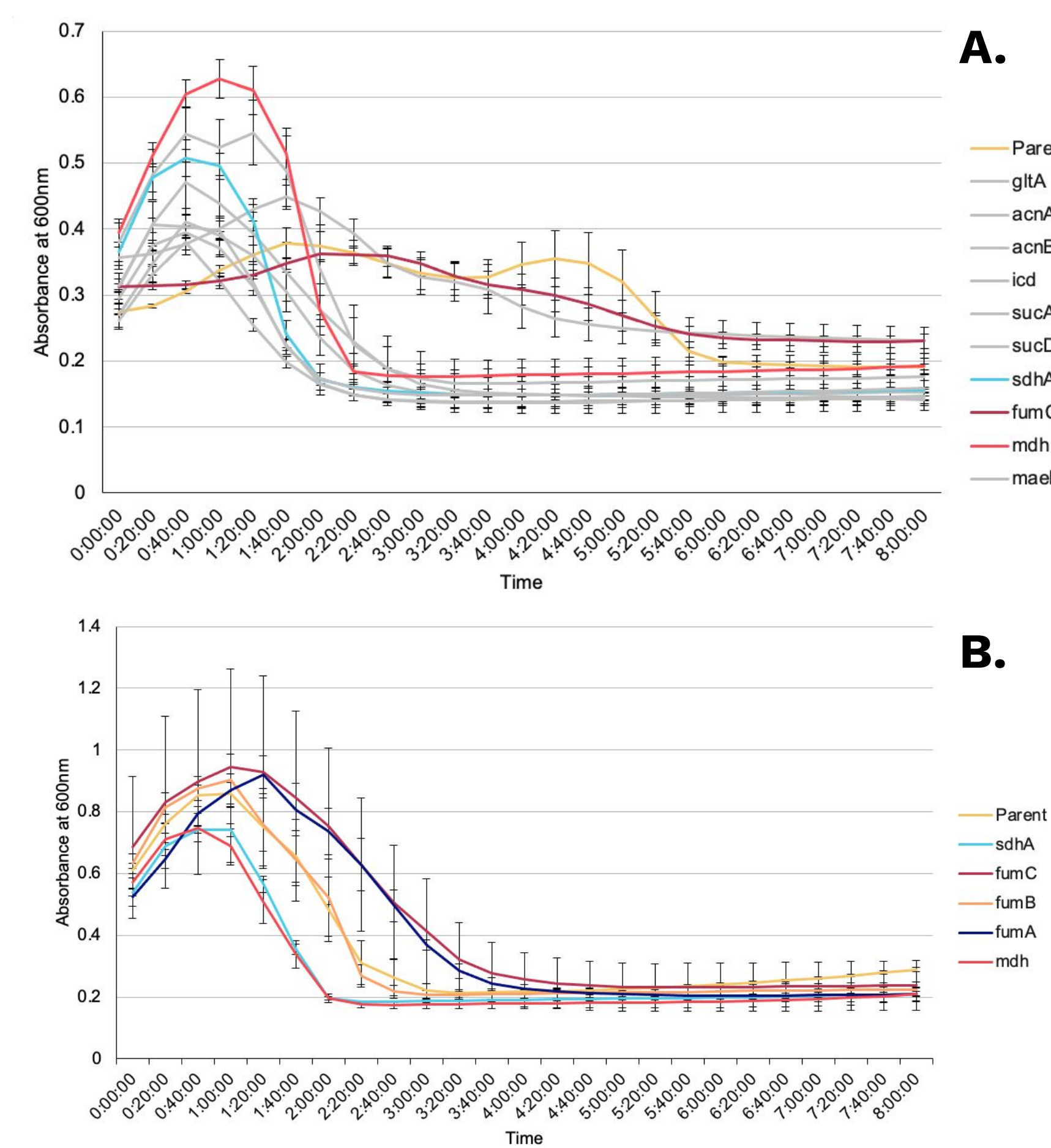
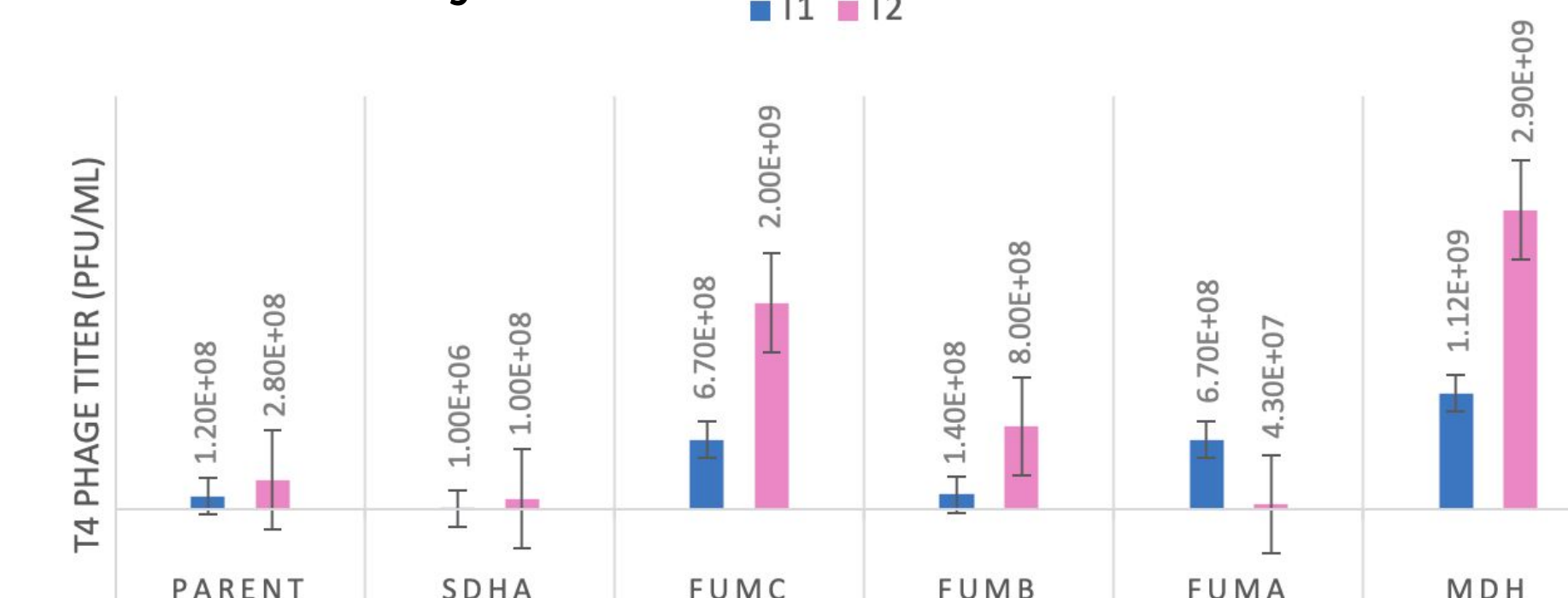


Figure 4. Comparative Lysis Curves: The *E. coli* strains were separately inoculated in LB media and T4 bacteriophage was added at time 0. Then, a plate reader measured absorbance at 600 nm every 20 minutes for 8 hours. **A.** Δ *fumC* shows delayed lysis compared to Δ *mdh* and Δ *sdhA*. **B.** Δ *fumC* and Δ *fumA* show similar lysis patterns, Δ *fumB* and parent show similar lysis patterns. Δ *mdh* and Δ *sdhA* show similar lysis patterns and are the slowest to lyse compared to the other strains.

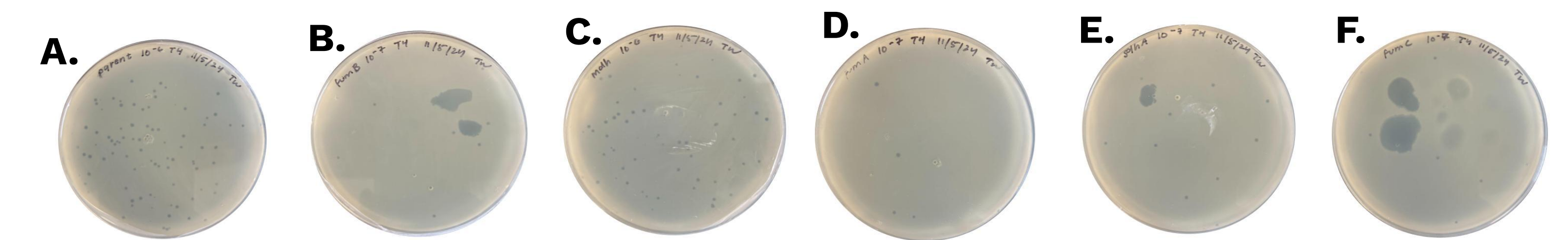
Two Time Point Titer Shows Δ *fumC* Has Less Resistance to Bacteriophage Lysis Compared to Δ *fumA* and Δ *fumB* :

Figure 5. Two Time Point Phage Titer: Overnight cultures of the three strains were grown, and cultures were then incubated at 37°C in separate flasks containing LB, CaCl₂, and T4 bacteriophage. Time points were taken at 45 and 90 minutes. The samples were diluted then plated with a double agar overlay. From there the PFU/mL was calculated based on the 10E-6, 10E-7, and 10E-8 dilutions and the plaques formed in each overlay.



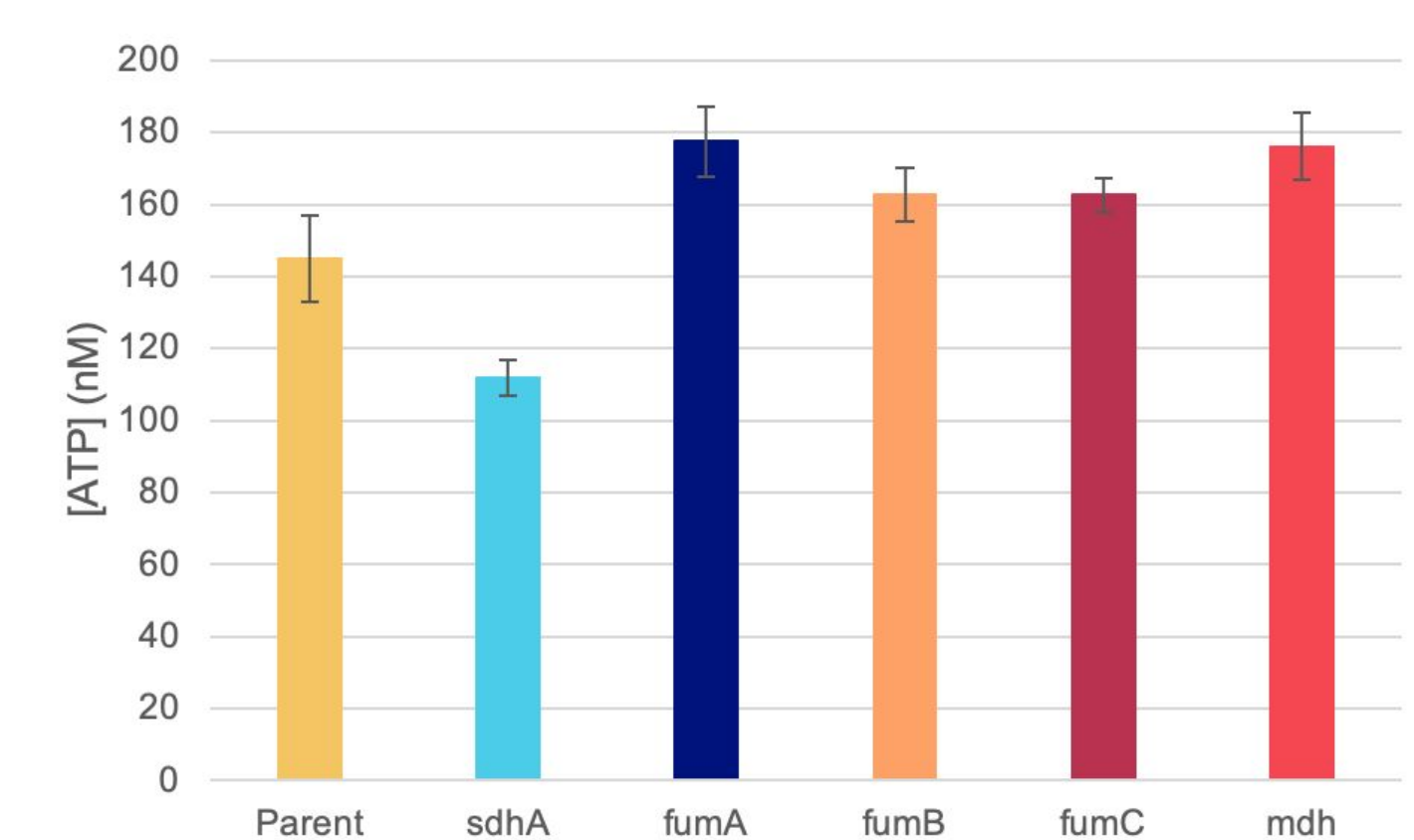
Plaque Assays Revealed Δ *fumA* had Greater Resistance to Bacteriophage Lysis Compared to Δ *fumB* and Δ *fumC* :

Figure 6. Comparative Plaque Assays: These plates represent the resistance the parent, Δ *sdhA*, Δ *fumC*, Δ *fumB*, Δ *fumA*, and Δ *mdh* knockout strains of *E. coli* had for the T4 bacteriophage replication at a concentration of 10E-6, and 10E-7. **A./B.** Parent and Δ *fumB* showed similar resistance to lysis (8.5x10⁸ PFU/mL and 9x10⁸ PFU/mL, respectively). **C./F.** Δ *mdh* and Δ *fumC* showed similar and slightly more resistance to lysis (5.7x10⁸ PFU/mL and 6x10⁸ PFU/mL, respectively) **D.** Δ *fumA* showed the most resistance to lysis (4x10⁸ PFU/mL) **E.** Δ *sdhA* showed the least resistance to lysis (11x10⁸ PFU/mL).



Comparative ATP Concentrations in Parent and Knockout Strains:

Figure 7. Comparative ATP Assays: *E. coli* strains were grown to OD₆₀₀ = 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. Δ *sdhA* exhibited the lowest ATP concentration, suggesting an upstream disturbance to the TCA cycle.



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.
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Discussion & Future Directions:

- Comparative plaque assays revealed that Δ *fumA* consistently showed greater resistance to lysis than Δ *fumC* and Δ *fumB* in both the Comparative Plaque Assays and the Two Time Point Phage Titer, possibly indicating that the presence of *fumA* supports bacteriophage replication more than *fumB* or *fumC*.
- [ATP] did not significantly decrease in Δ *fumA*, compared to the parent strain, suggesting that deletion of *fumA* does not directly impact [ATP]. This could mean that the deletion of *fumA* does not directly impede the TCA Cycle.
- Redo Lysis Curves and LB Growth curve to obtain more statistically significant data. Redo ATP Assay to obtain replicable data.
- Further investigate the interaction between the Fumarase isoenzymes by using double knockout strains, and experimenting in different aerobic conditions.