FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Investigating Effects of *sucA* Gene Knockout on *E. coli* Growth & Phage Replication

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Role of *sucA* in *E.coli* Metabolic Pathway

Background:

- The growing use of antibiotics has led to the emergence of antibiotic-resistant bacteria that no longer respond to anti-bacterial medications, making it difficult to treat infections caused by them. Bacteriophage therapy, which uses bacteriophage or viruses targeting specific bacteria is being investigated as a potential solution for bacterial infections (Hibstu et al., 2022).
- Our experiment explores the impact of the removal of a specific gene (*sucA*) on viral replication and the TCA cycle responsible for ATP synthesis. Knockout gene obtained from: Baba et al., 2006

Objective:

- We hypothesize that removal of the *sucA* gene in *E. coli* will inhibit growth and negatively impact viral replication.
- To determine the necessity of the Δ*sucA* gene in phage replication, and explore future directions for antibiotic treatments in humans.

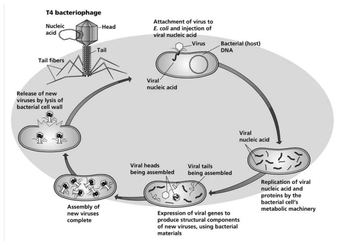


Figure 1.Lytic Cycle of Viral Replication: This figure shows how viruses manipulate the host cell's metabolic pathways to utilize resources for its own replication. (Figure from Townsend, 2023)

Materials and Methods

- Plaque assays compare parent and knockout strains phage growth.
- Growth and lysis curves compare the progression of cell growth and lysis in nutrient varying environments
- Two time-point experiment compares bacteriophage density. Phage concentrations were determined via serial dilutions and the DAO method.
- ATP quantification assay measures ATP levels in the parent and Δ*sucA* gene strains

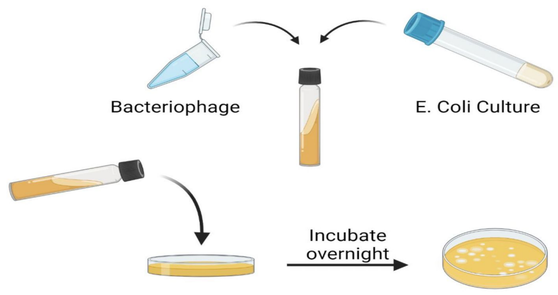
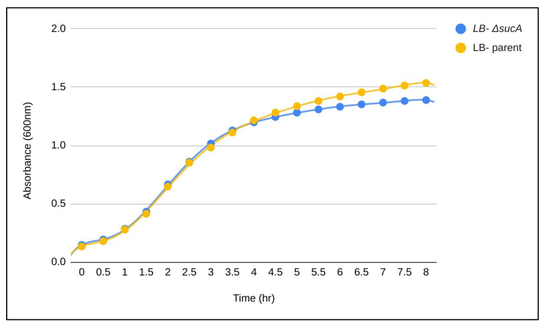


Figure 2.Quantification via Double Agar Overlay (DAO):

The plaque assays and two time point experiments conducted required the growth of overnight cultures to prepare DAO plates. DAO plates were prepared with overnight *E. coli* cultures and bacteriophage diluted samples to observe the growth of plaques or colonies.

Experimental Results

(A)



(B)

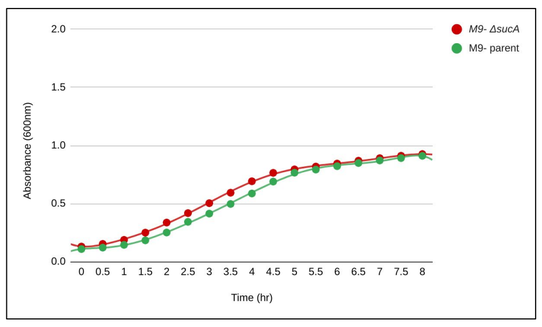
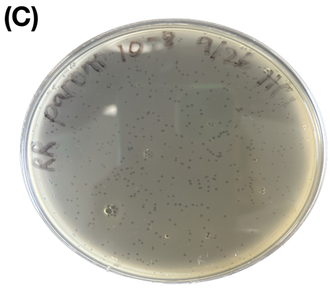


Figure 3.Comparative Growth Curves: Measuring the absorbance of the Parent strain and Δ*sucA* gene strain in *E. coli* separately, using (A) LB Media and B) M9 media. Growth was monitored using a plate reader, which performed continuous shaking in incubation at 37°C, measuring optical density at 600 nm for 8 hours.

(C)



(D)

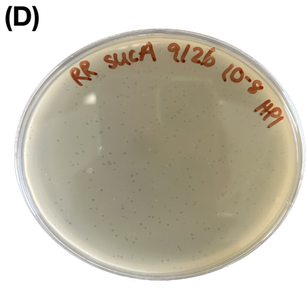


Figure 4. Plaque assays for Δ*sucA* strains show less T2 bacteriophage replication compared to parent strain.
The plaque assays show the results of double agar plating with *E. coli* bacteria and T2 bacteriophage. The (C) 10^{-8} dilution with the parent strain *E. coli* shows the most bacteriophage replication. Plaques for (D) 10^{-8} dilutions of the Δ*sucA* strains show substantially less bacteriophage replication. This shows that the knockout strain does have an effect due to growth of bacteriophage.

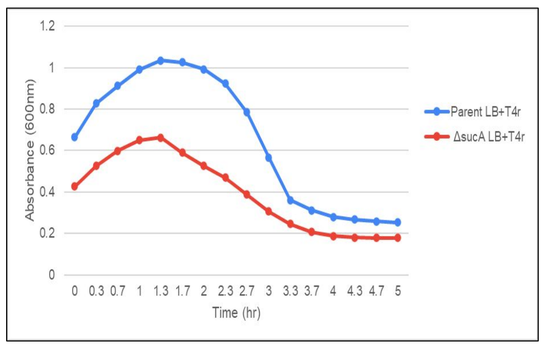


Figure 5.Lysis Curves:
Parent and Δ*sucA* strain of *E. coli* were grown in LB media with and without T4r phage. Growth was monitored using a plate reader, which performed continuous shaking at 37°C and measured optical density at 600 nm every 20 minutes for 8 hours (only 5 hours shown here).

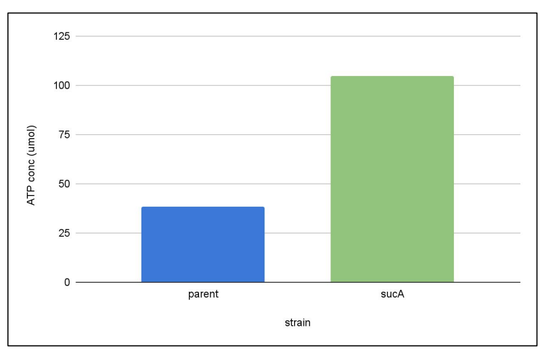
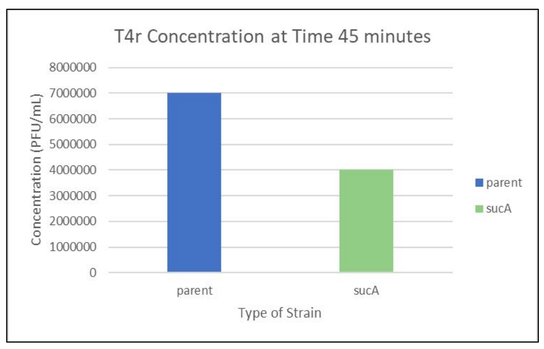


Figure 7. ATP Quantification Assay:
The bar graphs depict the ATP levels in E. coli samples lacking our specific genes, allowing for a constructive analysis of the potential impact of these genes on ATP production in the E. coli samples. The *sucA* strain showed a greater quantity of ATP produced, the opposite of what was expected, suggesting that perhaps the *sucA* gene itself plays an inhibitory role in the TCA cycle, such that its removal increases ATP production.

(E)



(F)

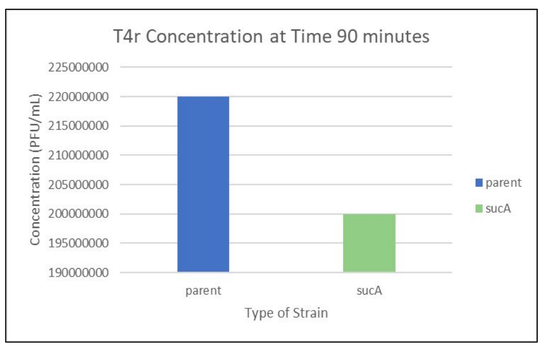


Figure 6. Two time phage Titer shows higher plaque forming units in Δ*sucA E. coli* strain compared to parent strain:
T4r phage was added to growing bacterial cultures and time points were taken at (E) 45 minutes and (F) 90 minutes. Phage concentrations were then determined via serial dilutions and double agar overlay which showed that T4r phage grew with lower concentrations of plaque forming units for the Δ*sucA* strain at both time points measured.

Discussion & Future Directions

❖ Parent strains formed more plaques than KO strains, indicating reduced viral efficiency in the absence of TCA genes

❖ KO strains exhibited similar growth and lysis to parental strains in LB media, and minimal growth and lysis in M9 media, suggesting reduced efficiency in reduced nutrient environments.

❖ Results from our lysis curves show that the Δ*sucA* strain had lower absorbance values than the Parent strain.

❖ Phage continued to replicate in the absence of the target genes, suggesting they are non-essential in host metabolism, but have a beneficial role in viral replication efficiency.

❖ Our results point to targets for future antibiotic strategies. Future studies could focus on exploring metabolic interventions in target host's to enhance efficacy against antibiotic-resistant bacteria and reduce viral replication

References & Acknowledgements

- Hibstu, Z., Belew, H., Akelew, Y., & Mengist, H. M. (2022). Phage Therapy: A Different Approach to Fight Bacterial Infections. Biologics : targets & therapy, 16, 173–186. https://doi.org/10.2147/BTT.S381237
- Townsend, M. (2023, June 16). Lytic Cycle of Viral Replication. EduBirdie. https://edubirdie.com/docs/harrisburg-university-of-science-and-tec/biol-370-molecular-biology/54871-lytic-cycle-of-viral-replication
- Mayer, K. A., Stöckl, J., Zlabinger, G. J., & Gualdoni, G. A. (2019). Hijacking the Supplies: Metabolism as a Novel Facet of Virus-Host Interaction. Frontiers in immunology, 10, 1533. https://doi.org/10.3389/fimmu.2019.01533
- 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

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