FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Impact of 2-isopropylmalate synthase (*LeuA*) Knockout on *Escherichia coli* Growth and Bacteriophage Replication

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Introduction

Background

- The overuse of antibiotics has resulted in antibiotic resistant strains of pathogenic bacteria (Lu & Koeris, 2011;) (Khawbung et al., 2021)
- Bacteriophage therapy is a promising alternative treatment strategy
- Bacteriophages are viruses that infect bacteria by hijacking its machinery to replicate and eventually cause cell lysis (Hibstu et al., 2022)
- We must further research the host-pathogen interactions between bacteriophage and their hosts
- *LeuA*, our gene of interest, plays a key role in the synthesis of the amino acid leucine (Ren et al., 2025)
- We aim to add to the library of host-pathogen interactions between *E. coli* and bacteriophage

Hypothesis

- The knockout of the ΔleuA gene will negatively affect *E. coli* growth and bacteriophage replication

Glucose ⟶ 2-ketoisovalerate $\xrightarrow{leuA}$ 2-isopropylmalate ⟶ L-Leucine

Figure 1: l-leucine biosynthesis pathway of Escherichia coli
Adapted from Hao, et. al (2023). Construction of a plasmid-free L-leucine

Materials and Methods

Growth Curves

- Grow overnights of the ΔleuA knockout strain and parent strain in LB Media
- Use spectrophotometry to create a general growth curve
- We hypothesize that the knockout strain will show reduced growth in a low-Leucine and low-nutrient environment

Lysis Curves

- Grow overnight culture of both strains in each LB media, and expose each to T4 phage
- Use spectroscopy to compare phage lysis
- We predict that phage replication will show reduced growth in a low-Leucine and low-nutrient environment

Two-time point phage titer

- Parent and knockout strains were grown in LB media till log phase, after which phages were introduced
- Phage was extracted and quantified after 45 and 90 min time intervals

Conclusions and Future Directions

- Broadly found no noticeable impact of the Δ*leuA* knockout gene
  - Our growth and lysis curves showed no significant difference, with the knockout even growing slightly better in the parent strain
- However, the two point phage tier showed several times more Bacteriophage in the parent strain than the Δ*leuA* strain
  - This may suggest a role leucine has in phage replication
- Repeating the growth and lysis curves with media containing differing levels of leucine could help further understand the impact of leucine on bacteriophage metabolism
- Using other gene knockouts in the leucine biosynthesis pathway would further validate whether or not ΔleuA's results are unique
- Other amino acid knockouts would help understand the impact other amino acids have on bacteriophage replication

Results

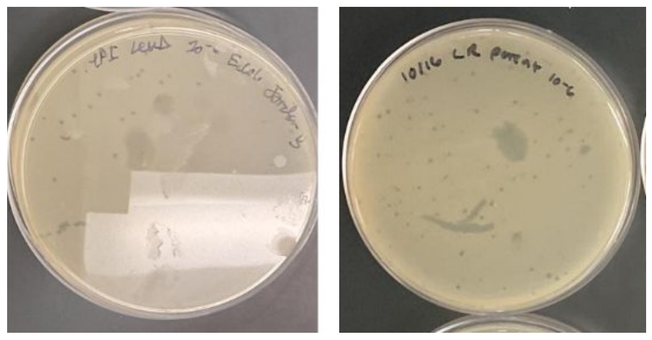

Figure 2: Comparative plaque assays show no noticable difference between the two strains. Plaque Assays were made using the double agar plating method with *E. coli* and T4 bacteriophage. On the right, the parent *E. coli* strain was used, on the left, the ΔleuA strain was used; both were using a 10^{-6} phage dilution. Both have a comparable number of plaques, indicating bacteriophage replication is not significantly affected in the knockout.

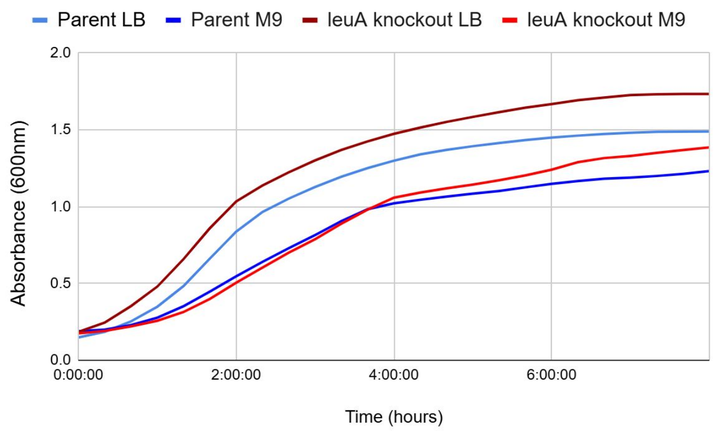

Figure 3: Comparative Growth Curves show slight increase in growth of the knockout strain in LB media and no significant difference in M9 media. Growth Curves of the parent and ΔleuA strain were generated in M9 media and LB media. A plate reader was used to monitor growth, which performed continuous shaking at 37 C and measured optical density at 600 nm every 20 min for 8 hours. Growth was linear with M9 curves resulting in lower growth patterns than that of the LB growth curve.

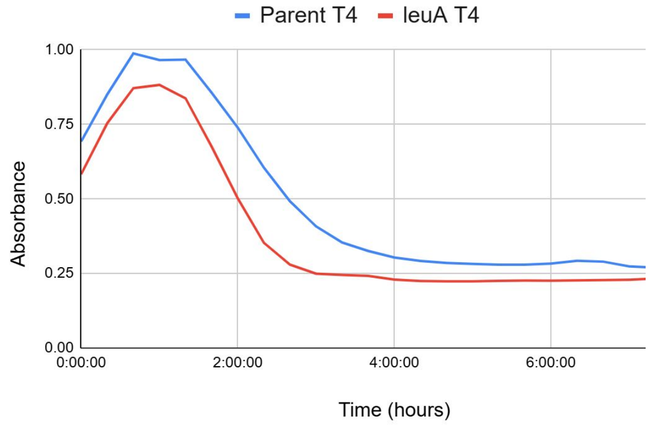

Figure 4: Comparative Lysis Curves show no difference between the parent and knockout strain. Lysis Curves of the parent and ΔleuA strain were generated using T4 phage. A plate reader was used to monitor growth, which performed continuous shaking at 37 C and measured optical density at 600 nm every 20 min for 8 hours. Bacterial growth saw linear decrease until the 3 hour time point where it stayed stagnant.

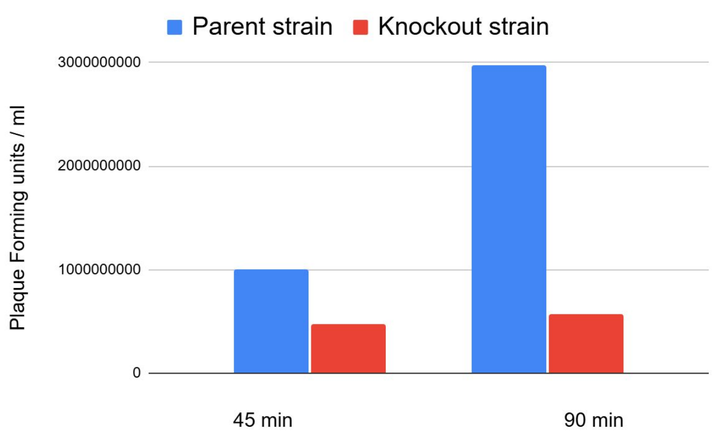

Figure 5: Two Point Phage Titer shows higher concentrations of phage in the parent culture. A bacterial culture was allowed to grow to log phase, and T4 phage was introduced. At 45 and 90 min time intervals, a sample of the colony was extracted and phage was isolated and plated using the double agar overlay method and plaques were counted.

References

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