FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

The Effect of The *E.coli lysA* Gene on Viral Replication

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Objective: To determine the effects of removing the *lysA* gene on *E.coli* growth and the replication of T4r to advance methods for fighting viral infections.

Background on Viral Replication:

- The *lysA* gene in *E.coli* codes for the enzyme that catalyzes the last step of L-lysine synthesis.
- Viruses such as bacteriophage take advantage of a cell's materials such as lysine to replicate.
- Lysine, specifically, is important to phage replication as lysine residues play a key role in building phage DNA (Lee & Richardson, 2001).
- HIV has also been shown to be impacted by the presence of lysine as it may increase the viral load in patients (Butorov, 2015)
- Understanding the role of lysine in a virus's ability to replicate can improve methods of treatment for many viral illnesses such as HIV/AIDS.

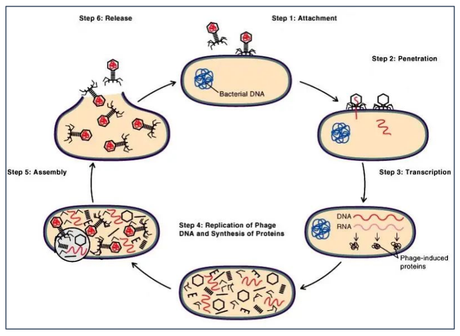

Figure 1. Bacteriophage lytic replication cycle

Materials:

- Parent Strain *E.coli* and *ΔlysA* strain *E.coli* from the keio knockout collection (Baba et al., 2006)
- LB 0.006 M lysine media, M9 minimal media, LB 0.06 lysine media, M9 0.006 M lysine media, M9 0.06 lysine media
- T4r bacteriophage stock
- LB plates, LB + kanamycin plates, top agar

Methods:

We will conduct the following procedures to assess how *E.coli* growth and phage replication respond to the absence of the *lysA* gene:

- Grow *lysA* knockout and parent strains in media with normal, high, and low L-lysine concentrations
- Measure growth rates using spectrophotometry and generate growth and lysis curves
- Perform plaque assays with serial dilutions and double agar overlay to evaluate bacteriophage replication under different media conditions
- Compare plaque formation between strains across media conditions

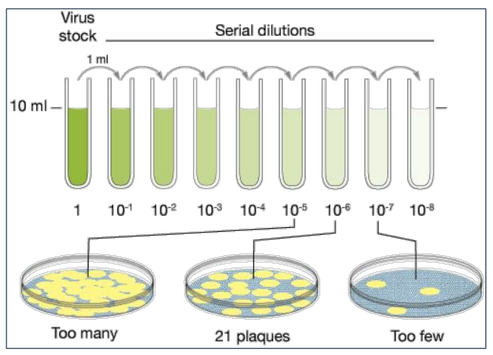

Figure 2. Diagram of serial dilution use for plaque assays

Discussion & Future Directions:

- Both the parent strain and *ΔlysA* strain grew similarly in LB media, indicating that the *lysA* gene is non-essential
- The parent strain exhibited steady growth in M9 minimal media, whereas the *ΔlysA* strain struggled to grow as seen in Figure 3. Additionally, when lysine was added back to M9 media in Figure 9, the *ΔlysA* was able to grow more steadily than in the M9 with no lysine. These findings highlight that *E.coli* need a source of lysine in order to grow and survive.
- Our two time-point experiment demonstrated reduced phage replication in the *ΔlysA* strain compared to the parent strain which supports the notion that lysine availability is important for efficient phage replication.
- Figure 8 further supports this hypothesis as *ΔlysA E.coli* conditions with more lysine lysed more quickly.
- Figure 11 may suggest that parent strain *E.coli* do not follow the same trend with media conditions. The parent strain showed slower lysing in the media with the most lysine. This may be due to the T4r's ability to use the host cell's metabolic pathways to synthesize lysine, rendering the lysine in media unnecessary for replication.
- These findings propose potential parallels in eukaryotic viral infections, such as HIV, where higher lysine levels have been associated with increased viral loads. Therapeutic applications should be explored.
- Exploring the impact of reduced lysine conditions on other viral strains can be done to determine the validity of our findings

Results:

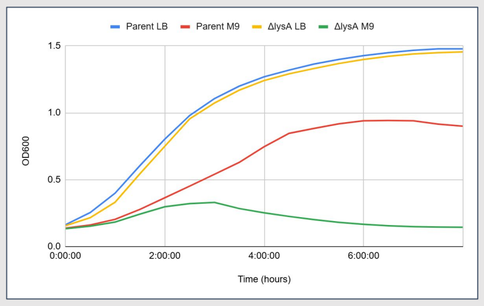

Figure 3. Parent Strain vs. ΔlysA Strain Growth Curve. There is similar growth of both parent strain and *ΔlysA* strain in LB Media. In M9 minimal media, the parent grew less than it did in the LB media, and the *ΔlysA* strain struggled significantly to grow at all.

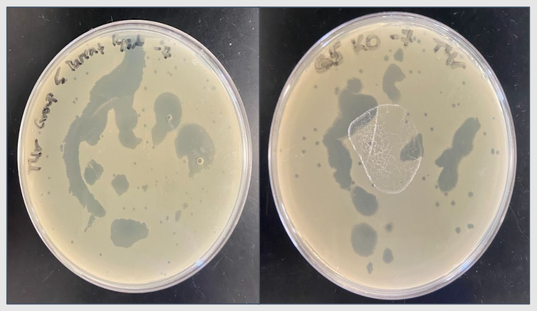

Figure 4. Plaque Assays Performed with T4r Phage. On the left, plaque assay of parent strain. On the right, plaque assay of *ΔlysA* strain. The parent strain plaque assay has a higher amount of plaques than the *ΔlysA* assay.

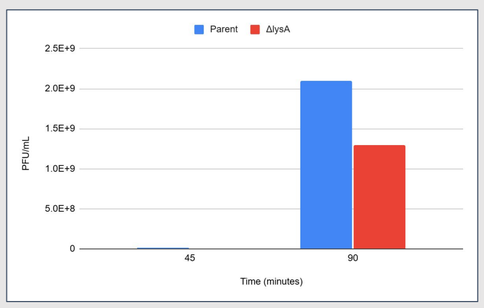

Figure 5. Greater Plaque Formation in Parent Strain. Plaque assays were performed at two 45 min intervals after adding T4r phage to cultures. At each of these, plaque formation was greater in the parent strain.

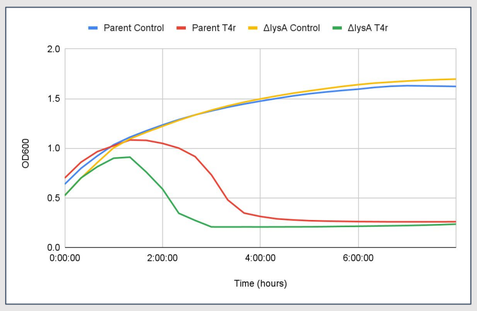

Figure 6. Parent Strain vs. ΔlysA Strain Lysis Curve. After adding T4r to the parent and *ΔlysA E.coli*, the *ΔlysA* strain displays quicker lysing. The controls were not treated with T4r phage.

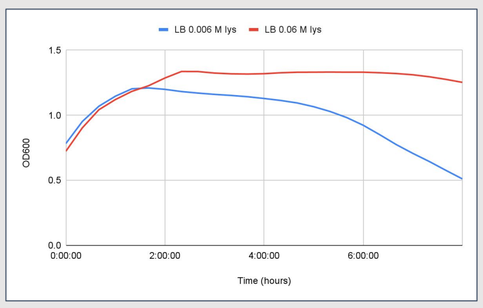

Figure 7. Lysis of *ΔlysA* Strain Under Various LB Media Lysine Conditions. The *E.coli* lysed faster in the LB media conditions that have a lower concentration of lysine.hi

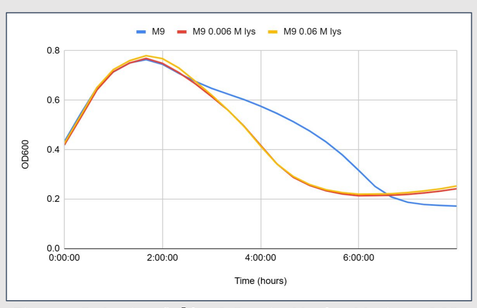

Figure 8. Lysis of *ΔlysA* Strain Under Various M9 Media lysine Conditions. The *E.coli* lysed at similar rates in the M9 conditions that had lysine added, and lysed more slowly in the M9 conditions with no lysine.

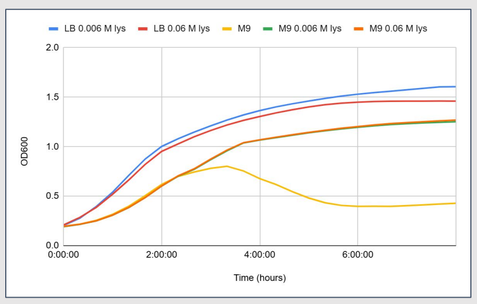

Figure 9. Growth of *ΔlysA* Strain Under Various Conditions. The *E.coli* grew similarly in both LB conditions and grew the most out of all conditions. They grew less than this in both M9 conditions, but not by a large amount. They showed significant struggle to grow in M9 conditions with no lysine.

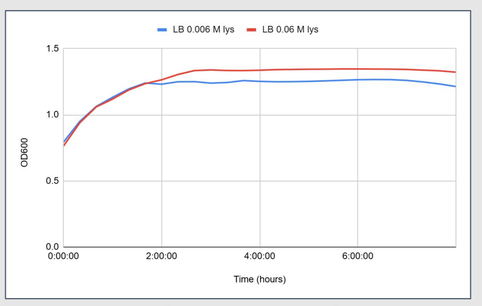

Figure 10. Lysis of Parent Strain Under Various LB Media Lysine Conditions. The parent strain showed minimal lysing in both conditions of LB media. However, the parent strain in the lower concentration of lysine, lysed slightly faster.

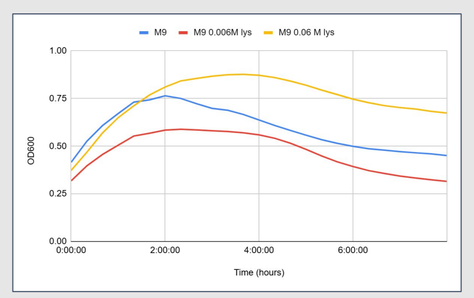

Figure 11. Lysis of Parent Strain Under Various M9 Media Lysine Conditions. The parent strain showed the slowest lysing in the M9 conditions with the high concentration of lysine. The M9 conditions with no lysine seemed to lyse at a slightly faster rate than the conditions with a normal amount of lysine.

Acknowledgements:

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