FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Determining the Effect of the Removal of the Amino Acid Arginine on Viral Replication in *E.coli*

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Introduction

- Currently, there is a worldwide antibiotic resistance crisis in which bacteria that cause human infections can no longer be treated using traditional medicines because the bacteria have developed resistance to them.
- Bacteriophages are viruses that infect bacteria, not humans, and rely on amino acids like arginine for replication and protein synthesis (Jain, 2009). The viral replication process is shown in the figure below.

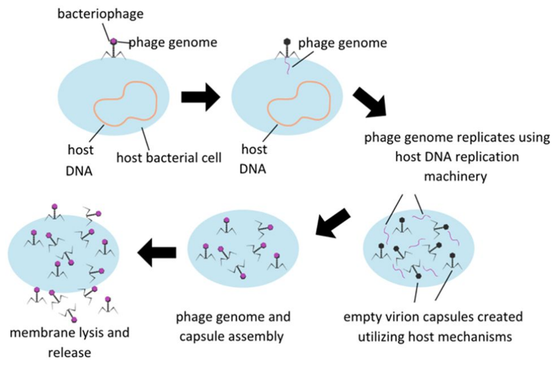


Figure from Durr et al.(2023)

- Our research project investigates how the removal of the amino acid arginine affects bacteriophage replication in E. coli, with a focus on the argA gene, responsible for the first step in arginine production
- By studying what happens when argA is absent, we aim to understand how bacteriophages interact with their bacterial hosts, which could lead to new treatments for bacterial infections
- Our findings may support the development of phage therapy, potentially incorporating arginine to enhance bacteriophage effectiveness against antibiotic-resistant infections.

Project Objectives

- Determine how the removal of arginine through the *ΔargA* knockout strain affect viral replication in *E.coli*.
- We hypothesize that the removal of *ΔargA* will decrease viral replication due to a deficiency in arginine.

Results

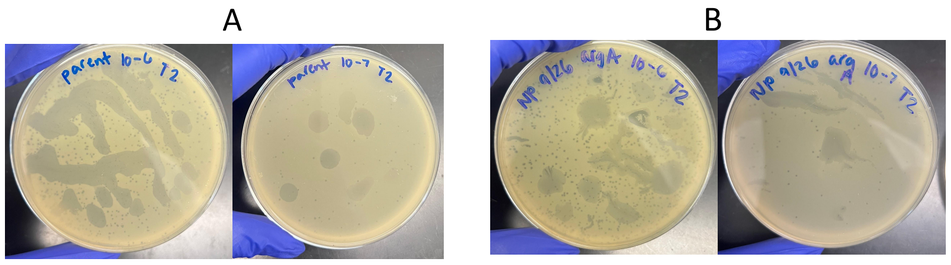


Figure 1 - Plaque Assay Plates Done by Double-Agar Overlay Method: Holes depict the presence of phage in each dilution (10^{-6},10^{-7}) of each strain (parent vs. *ΔargA* knockout)
A) assay conducted using dilutions of *E.coli* parent strain with T2 phage
B) asay conducted using dilutions of *E.coli ΔargA* knockout strain with T2 phage

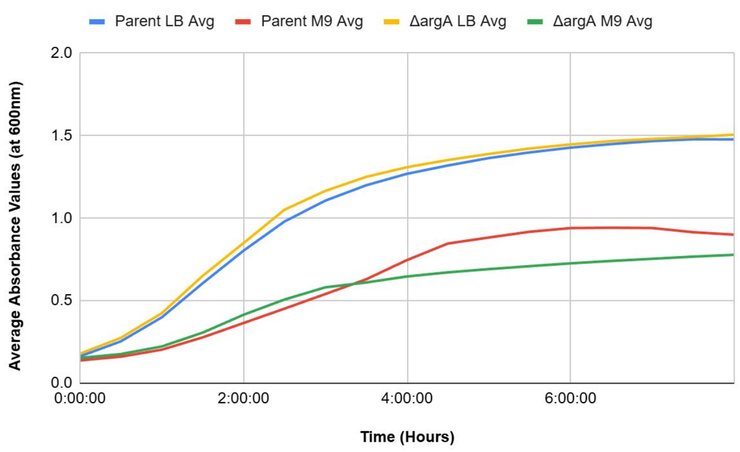


Figure 2 - Removal of *ΔargA* Gene Results in Decreased Cell Growth in M9 Minimal Media: Growth curves collected using plate reader. Parent and knockout strains were grown in LB media and M9 minimal media to determine any differences in growth between strains and within each strain. Bacteria was grown with shaking at 37℃.

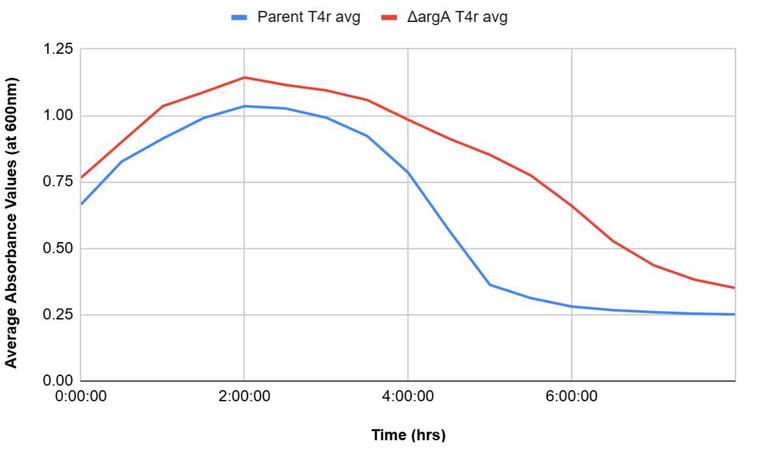


Figure 3 - Removal of *ΔargA* Gene Results in Dleayed Lysis: Lysis curves collected using plate reader. Parent and knockout strains were grown after adding T2 phage to determine how bacteriophage lyse the two strains.

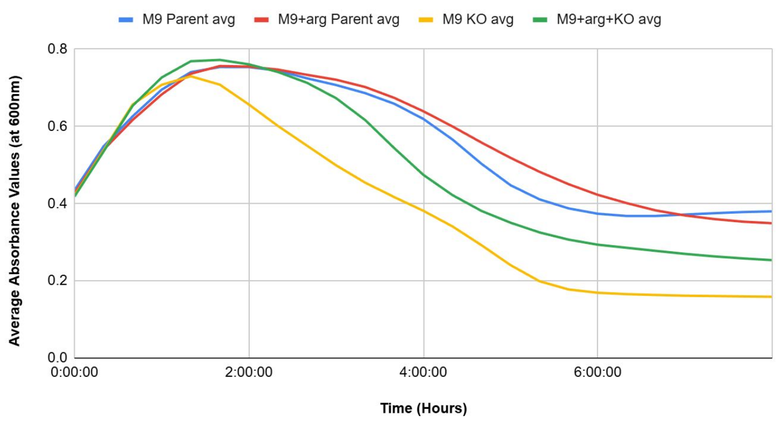


Figure 4 - Addition of Arginine in M9 Minimal Media Results in Decreased Lysis of Knockout Strain: Lysis curves collected using plate reader. Parent and knockout strains were grown in original M9 minimal media and M9 media with added arginine.

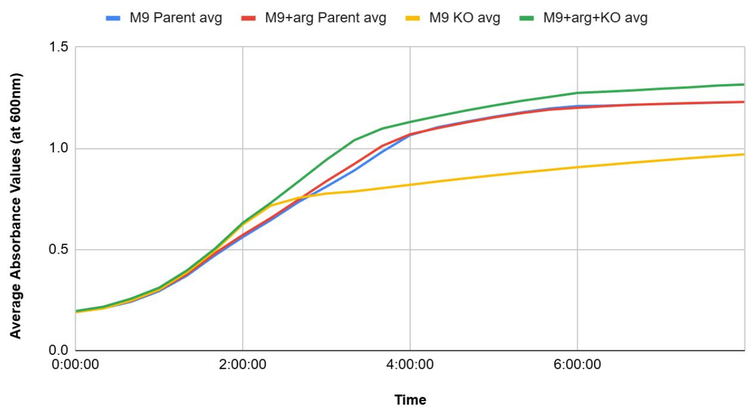
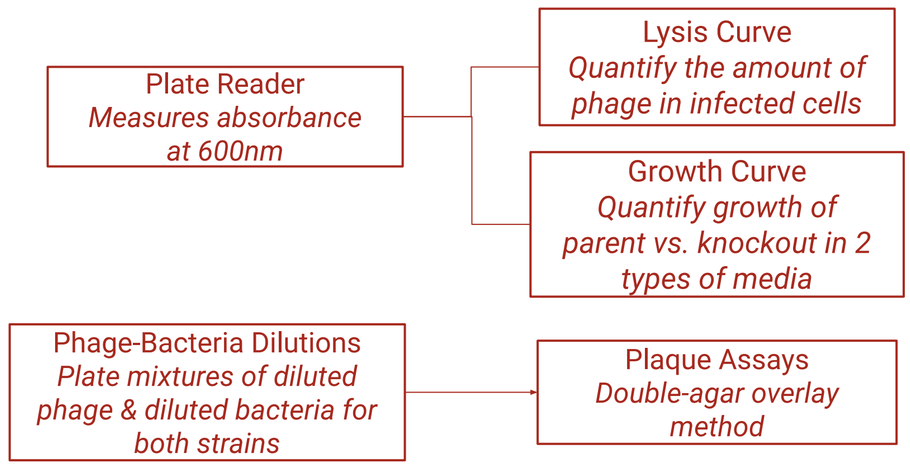


Figure 5 - Addition of Arginine in M9 Minimal Media Results in Increased Cell Growth of Knockout Strain: Growth curves collected using plate reader. Parent and knockout strains were grown in original M9 minimal media and M9 media with added arginine. Bacteria was grown with shaking at 37℃.

Materials and Methods

- To examine how the removal of the *ΔargA* gene affects the growth of *E. coli* and to measure the dynamics of viral infection in *E.coli*:
  - Construct growth curves and lysis curves of parent strain vs. *ΔargA* knockout strain by measuring absorbances via plate reader.
  - Construct growth curves and lysis curves of both strains in different types of media which contain additional arginine
- To quantify phage replication in *E.coli*:
  - Perform plaque assays so we can visually determine the quantity of phage present in parent strain vs. *ΔargA* knockout strain.



Discussion

- We found that the knockout grows in LB media the same as the parent, meaning *ΔargA* is non-essential for growth in LB. (Figure 2)
  - This aligns with previous literature on the essentialness of *argA.* (EcoCyc)
- We found that the knockout has a slightly delayed lysis time compared to the parent strain. (Figure 3)
  - This could mean that the phage had a difficult time replicating inside the *E.coli* cells due to a reduction in the amount of arginine present.
- We found that there is not a significant difference between the amount of phage present in the knockout vs. the parent. (Figure 1)
  - This could mean that despite a reduction in arginine production in the *E.coli* cells, the phage were still able to replicate.
  - Since this data is quantified based on what is seen with the eye, it is difficult to truly determine how much less phage there was.
  - This is a novel finding in that no previous literature has quantified the amount of phage present in the *ΔargA* knockout strain of *E.coli.*
- We found that the knockout strain grew significantly more in M9 media with added arginine than in original M9 media. (Figure 5). The M9+arginine media also resulted in less lysis of the knockout strain compared to lysis using original M9 media (Figure 4).
  - The growth data aligns with expectations, as the knockout strain is unable to synthesize arginine intracellularly; therefore, its growth rate is higher in media supplemented with arginine compared to media lacking this amino acid.
  - The lysis data aligns with the growth data above; the higher cell density in M9+arginine media limited phage lysis, leading to higher absorbance values compared to M9 media which had lower cell density.
  - These are novel findings in that no previous literature has examined lysis and growth curves using M9+arginine media and the *ΔargA* knockout strain of *E.coli.*

Future Directions

- Perform two-time point phage titer experiment to quanitfy phage concentration at different time intervals, which allows us to determine if there are differences in rates of replication between the parent and knockout strains.

References:

- Durr, H.A.; Leipzig, N.D. (2023) Advancements in bacteriophage therapies and delivery for bacterial infection. *Mater Adv*, 4, 1249-1257
- Jain, R., & Srivastava, R. (2009). Metabolic investigation of host/pathogen interaction using MS2-infected Escherichia coli. *BMC Systems Biology*, 3(1)
- Hvid, U., Mitarai, N. (2024) Competitive advantages of T-even phage lysis inhibition in response to secondary infection. *PLoS Comput Biol* 20(7)

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