FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE
HOST-PATHOGEN INTERACTIONS

Disrupting Glutamate Metabolism: Exploring the Role of gdhA in E. Coli Growth and Bacteriophage Replication

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Background

- Glutamate is a key metabolite that links carbon and nitrogen metabolism and supports bacterial survival under stress conditions such as acid, oxidative, and osmotic stress (Feehily & Karatzas, 2013).
- One enzyme that regulates this pathway is glutamate dehydrogenase (GDH), encoded by the gdhA gene, which converts glutamate to α-ketoglutarate, a critical energy intermediate in the TCA cycle (Riba et al., 1988).
- While glutamate metabolism is well-studied in stress resistance and energy balance, its influence on bacteriophage replication remains largely unknown.
- Because phages rely on host metabolism for replication, altering glutamate pathways could impact both bacterial growth and viral productivity (Miller & Brenchley, 1984; Janes et al., 2001).

Project Objectives

- Determine how deletion of the gdhA gene in E. coli affects bacterial growth and phage replication.
- Assess whether supplementing the knockout strain with glutamate or α-ketoglutarate can restore growth and/or phage replication.

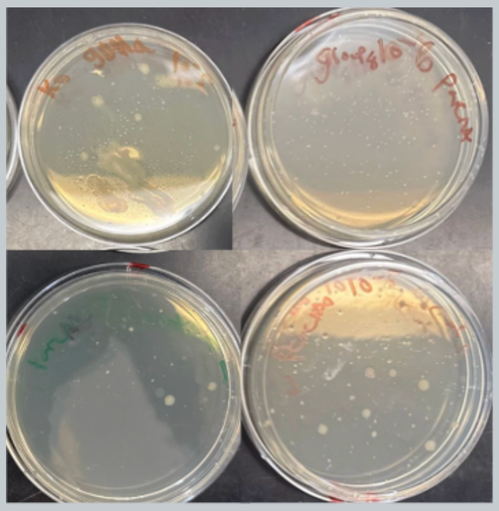


Figure 2. Plaque Assays of gdhA Strain and Parent E.coli With T4 Phage

The figure represents the plaque assays for both the parent E.Coli strain and the gdhA knockout strain. We used T4 phage in both strains, growing the parent in regular LB media, and the gdhA strain in LB kanamycin. In the gdhA 10^{-5}, gdhA 10^{-6}, parent 10^{-5}, and parent 10^{-6}, there is observable growth. The parent strain had slightly more bacterial lawns compared to the gdhA strain.

Discussion

- We can confirm that in M9 media, the gdhA gene grows significantly slower than the parent strain
- Additionally, we can confirm that in rich LB media, the gdhA and knockout strain grew at a relatively similar rate
- Additionally, we can report that the lysis of gdhA is slightly slower than that of the parent strain.
- Both strains grew more robustly in rich (LB) media, confirming that external nutrient availability can compensate for loss of gdhA.
- These results suggest that glutamate metabolism, via GDH, plays a role not only in bacterial energy production but also in supporting optimal phage infection and propagation.

Future Directions

- The next steps for the experiment will include running a two-time point phage titer experiment
- Redoing both the lysis and growth curves to verify the accuracy of the first run
- Re-running the plaque formation experiment would be beneficial in the future for more accurate data and results.
- We would like to continue research on the TCA cycle and how intermediates are affected by the gdhA gene and what products are altered in the TCA cycle

Materials and Methods

- Observe the growth rate of the ghdA knockout strain compared to the parent strain using a spectrophotometer.
- Complete serial dilutions of each strain to measure absorbance values, allowing us to plot growth curves.
- Track the growth effects of gdhA deletion. Measure OD_{600} growth curves of parent vs. gdhA E. coli in growth media. Since gdhA encodes glutamate dehydrogenase, its loss may limit α-ketoglutarate supply for the TCA cycle, impairing energy production and growth.
- Perform dilutions with phage to analyze plaque assays in both strains to determine if the removal of the knockout strain affects viral replication
- Significance: Glutamate metabolism links to stress responses (acid, oxidative, osmotic), this work may reveal how central metabolism shapes host–phage interactions and identify new vulnerabilities in microbial defense.

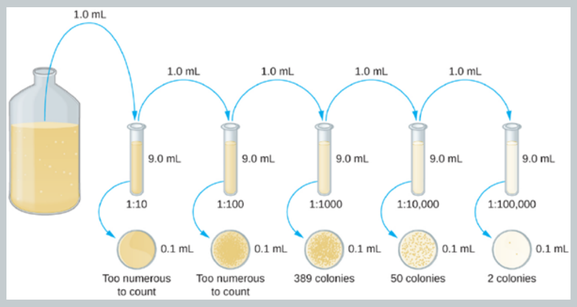
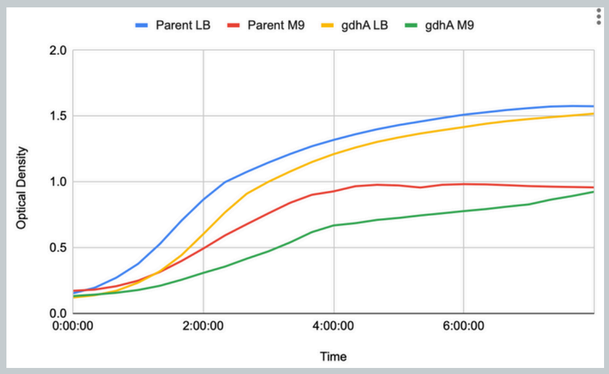


Figure 1. Demonstration of How to Perform Serial Dilutions

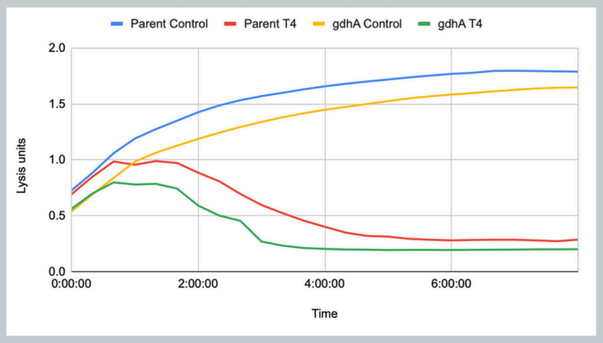
This image shows how we perform serial dilutions for various experiments to test our hypothesis and measure growth patterns with/without phage and plot for our growth curves.

Figure 3. Growth of Parent Strain Is Higher in LB and M9 Media Compared to Growth of gdhA



Both strains grew faster and to higher levels in LB than in M9, showing LB supports stronger growth. In M9, growth was slower and lower overall, with the gdhA knockout reaching a slightly smaller maximum, suggesting a mild growth disadvantage only in nutrient-limited conditions.

Figure 4. gdhA Lysed at a Slower Rate compared to parent E.coli When Infected with T4 Phage



The parent strain lysed more quickly and extensively after T4 infection, while the gdhA knockout showed slower and weaker lysis. This suggests the gdhA mutant is less susceptible to T4-induced lysis. Controls without phage showed steady growth with no lysis.

References

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