FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Investigating the Effects of *tyrB* on *Escherichia coli* and Bacteriophage Replication

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INTRODUCTION

Background:

- Aspartate is an essential amino acid for protein synthesis and other cellular processes for organisms. By exploring more about its effect on cell replication, this will help develop a better understanding of phage behavior when it does not have as much access to certain cell materials.
- Phage replication first makes contact with a host cell, and rewires its metabolic pathways to siphon resources from the host cell, which it will then use to fuel phage replication.
- The bacterial cell produces aspartate by using catalytic enzymes encoding from both *tyrB* and *aspC*. Recent literature has identified aspartate aminotransferase (aspC) as the primary enzyme for aspartate biosynthesis. Though *tyrB* is a nonessential gene, it is essential to the production of aspartate, as it is a secondary catalyst in the metabolic pathway.
- One pathway that will be significant to our study will be aspartate role in building the bacterial cell membrane. If aspartate synthesis were hindered, a deficiency may cause weaknesses in the cytoplasmic membrane due to a lack of peptidoglycans (Zhao et al, 2018).

Project Objectives:

- Determine the significance of *ΔtyrB* and *ΔaspC* in phage resistance and bacterial growth in M9 and LB media
- Explore the effect of change in aspartate production to measure T2 bacteriophage replication

We hypothesize that the Δ*tyrB* strain in *E. coli* will lead to a decreased growth rate and decreased phage resistance.

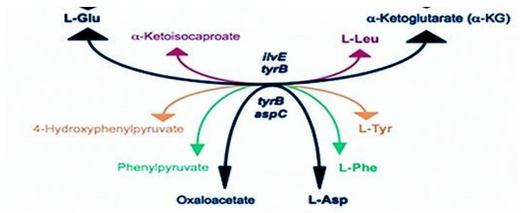


Figure 1: Toshio Iwasaki Group (2024)
Required genes in the production of essential amino acids, describing the necessity of *tyrB* as a secondary gene and *aspC* as a primary gene for amino acid production, and aspartate in particular.

MATERIALS/METHODS

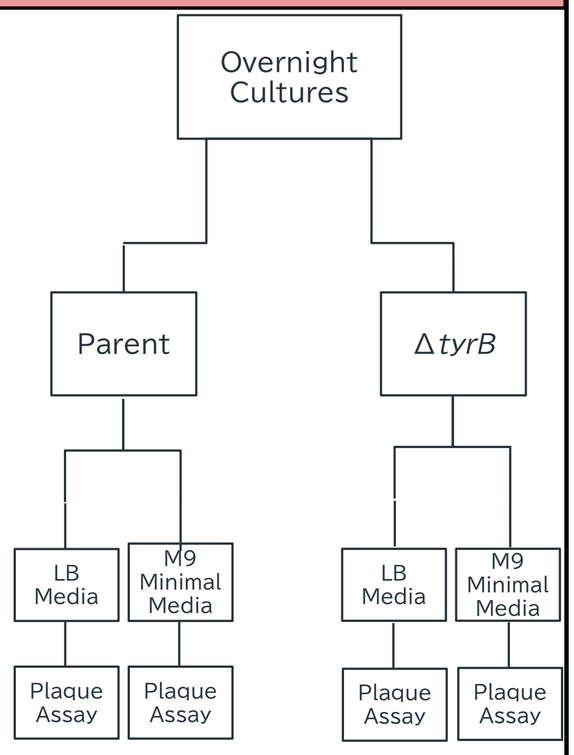
Materials:

- *ΔtyrB E. coli* knockout strain
- T2 Bacteriophage
- LB Top agar, LB, and LB + Kanamycin plates
- LB Media & M9 Media

Methods:

Creating overnight cultures of the Δ*tyrB* and parent strain in order to:

- Create streak plates comparing overall growth of strains in M9 and regular LB media
- Pair with serial dilutions of T2 bacteriophage to create plaque assays and measure *E. coli* growth
- Create growth and lysis curves of the parent strain and the Δ*tyrB* strain and compare



RESULTS

Figure 2: Comparative Growth of Parent E. coli and ΔtyrB Strains Show Minimal Difference

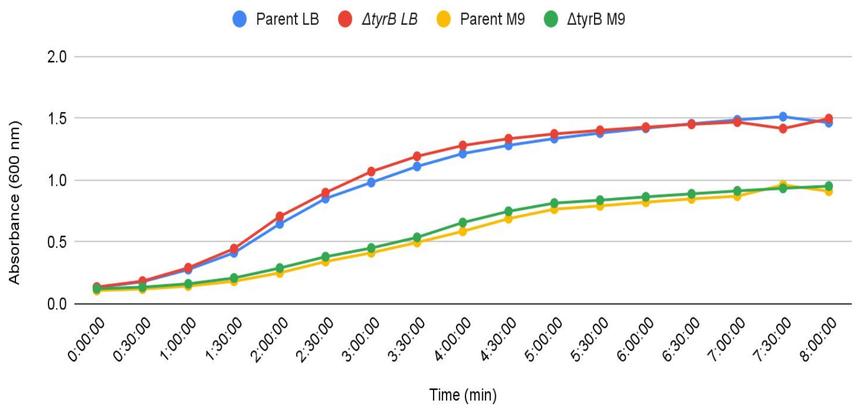


Figure 2: Demonstration of the growth of the parent and *tyrB* knockout strain in LB media and M9 minimal media. Each strain was exposed to both LB Media and M9 minimal media (w/ 0.4% glucose) for a period of 8 hours, measuring absorbance at 600nm (OD_{600}) to determine growth over time. Both parent and Δ*tyrB* strains have similarly efficient growth in regular LB media, while both have a reduced rate of growth in M9 media. There is no distinguishable difference between the growth of the parent strain and Δ*tyrB*.

Figure 3: Comparative Lysis of Parent and ΔtyrB Strains Shows Partial Difference

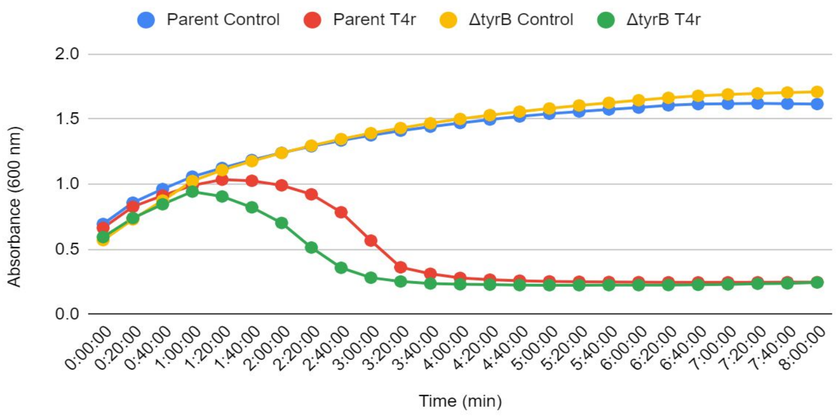


Figure 3: Comparative Lysis Curve of Parent *E. coli* and *ΔtyrB* Strains
Each strain was exposed to T4r bacteriophage for a period of 8 hours, measuring absorbance at 600nm (OD_{600}) to determine lysis over time. Data shows statistical differences between strains and their response to phage. Both control strains have shown successful growth in LB, while the *ΔtyrB* strain exposed to T4r lyses quicker than the parent *E. coli* strain.

Figure 4: T4r Phage Replication in Parent and ΔtyrB Strains

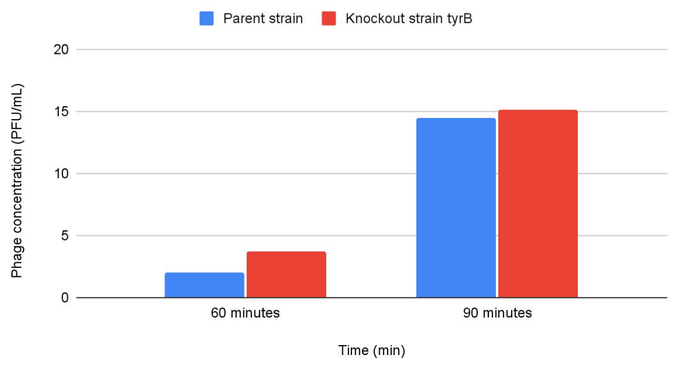


Figure 4: A representation of the results of T4r phage introduction to parent and *tyrB* knockout *E. coli* after hitting log phase. Phage efficacy was quantified by plating results and counting plaques to calculate PFU/mL. In both the 60 and 90 minute samples, the parent strain has a slightly lower amount of plaques as compared to the knockout strain.

Figure 5: Double Agar Plaque Assays for Parent *E. coli* strain + Δ*tyrB* strain

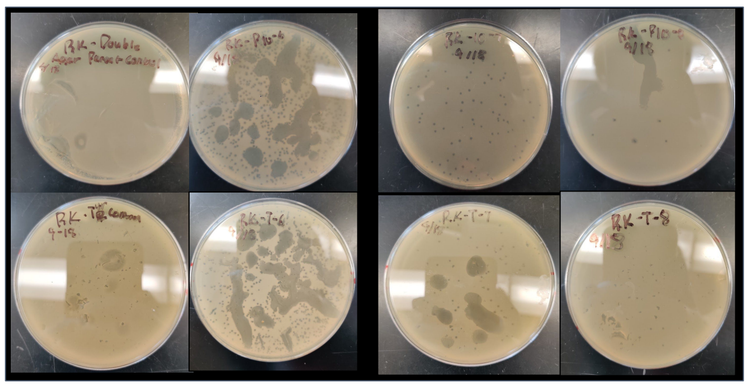


Figure 5: Demonstrating plaque morphology and measuring PFU/mL. Plates produced from the double agar plaque assay, with T2 bacteriophage in dilutions of 10^{-6}, 10^{-7}, 10^{-8}, introduced to *tyrB* knockout *E. coli*, as well as parent strain and *tyrB* knockout parent control plates. The relative plates show more significant phage lysis in the Δ*tyrB* strain than the parent strain.

Figure 7: Comparative Growth of Parent E and ΔaspC Strains Show Difference

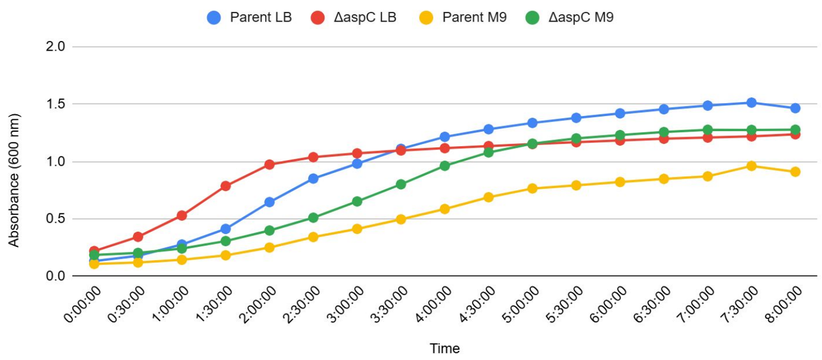


Figure 6: Demonstration of the growth for the parent and *aspC* knockout strain in LB media and M9 minimal media. The Δ*aspC* strain shows unusually high amounts of cell proliferation in LB and M9 media as compared to the parent strains.

Figure 7: Comparative Lysis Curve of Parent E. coli and ΔaspC Strains

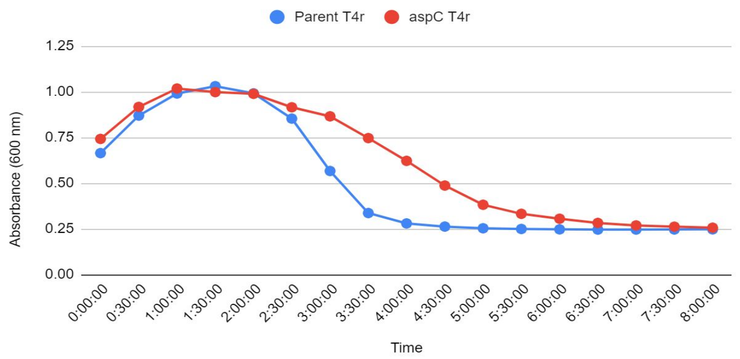


Figure 7: Description of the lysis of the parent and *ΔaspC E. coli* strains in LB media exposed to T4r bacteriophage. Both control strains have relatively successful growth in LB, while the *ΔaspC* strain exposed to T4r bacteriophage has a slower rate of lysis as compared to the parent strain

DISCUSSION/FUTURE DIRECTIONS

Discussion:

There is little to no significant effect on bacterial growth when the *tyrB* gene is removed in *E. coli*, as shown in Figure 2, which demonstrates growth in M9 minimal media and LB media, and similar growth occurs for both the parent strain and the *tyrB* knockout strain. The lysis results indicated in Figure 4 show that *ΔtyrB* lyses faster when exposed to T4r bacteriophage in comparison to parent strain *E. coli* after hitting log phase. This novel finding is not mentioned in recent literature, and most likely indicates that the *tyrB* gene has a certain level of importance to the parent cell that prevents it from being lysed by bacteriophage.

Future Directions:

Doing further research on genes that have more significant impacts on amino acid synthesis, such as those having higher control over leucine or cystine, as shown to be more heavily involved in bacteriophage replication (Daugavet et al). Testing different environments of growth and lysis under bacteriophage by introducing different amino acids into M9 minimal media, to test the efficacy of *tyrB* in various amino acid biosynthetic pathways.

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