

IMPACT ON REMOVAL OF THE *fabF* & *fabH* GENES ON BACTERIOPHAGE REPLICATION IN *ESCHERICHIA COLI*

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INTRODUCTION:

- Fatty acid synthesis is an essential component of cell survival because it significantly creates the membrane that divides the interior from the exterior, creates a barrier for substances to enter or exit the cell, and maintains gradients or environments necessary for many functions.
- Bacteria have a type II fatty acid synthesis (FAS II) pathway associated with the Fab enzymes that are unique to them and not found in humans. This makes it a reasonable target for antibiotics because it can hinder the development of the bacterial membrane, but not the human.
- When bacteriophage infect *E. coli*, they hijack metabolic pathways, such as fatty acid synthesis (Volkova, et al., 2014). Therefore, alterations to these pathways, such as the knockout of the *fabF* gene, should result in affected replication patterns for the bacteriophage.
- Platensimycin, an antibiotic that inhibits bacterial fatty acid synthesis, presents a possible novel therapeutic strategy in humans infected with bacteria.

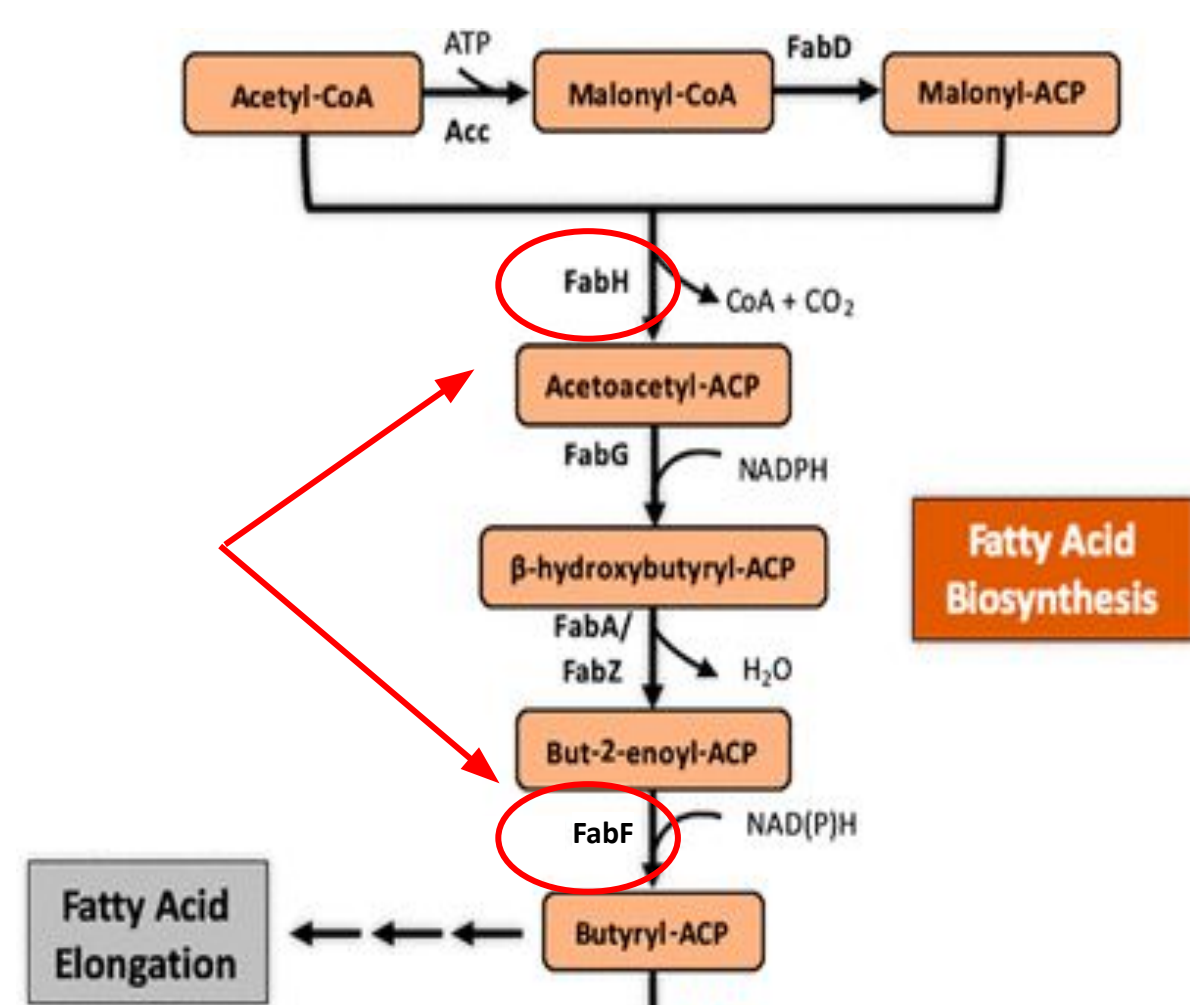


Figure 1. Location of *fabF* and *fabH* activity in Fatty Acid Synthesis II Pathway in *E. coli*.

Figure adapted from Yazdani et al. (2020)

Abbreviations: Acc, acetyl-CoA carboxylase; *FabA/FabZ*, β-hydroxyacyl-ACP dehydratase; *FabD*, malonyl CoA-ACP transacylase; *FabG*, β-ketoacyl-ACP reductase; *FabH*, β-ketoacyl-ACP synthase III; 3-oxoacyl-[acyl carrier protein] synthase 2

OBJECTIVES:

- We want to determine whether impairing fatty acid metabolism in the host cell has an effect on phage replication.
- We want to determine whether this result could slow down viral replication in the big picture.
- We aim to hypothesize the effects of platensimycin on phage growth.

REFERENCES:

Wang Y, Wang Y, Wu Y, et al. (2023). Using the inner membrane of *Escherichia coli* as a scaffold to anchor enzymes for metabolic flux enhancement. *Eng Life Sci.* ; 23:e2200034.

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Ecocyc.org/gene?orgid=ECOLI&id=EG12606. Accessed 20 Nov. 2024.
Biocyc.org/gene?orgid=ECOLI&id=EG10277. Accessed 20 Nov. 2024.

Knockout strains of *E. coli* were obtained through the Keio Collection:

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., & Mori, H. (2006). Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular systems biology*, 2, 2006.0008. <https://doi.org/10.1038/msb4100050>

MATERIALS & METHODS:

- Prepare overnight cultures (parent *E. coli*, *fabF* and *fabH* knockout genes)
- Isolate colonies through streak plating
- Perform plaque assays (parent and *fabF* knockout strains)
- Record growth and lysis curves (parent and both knockout strains)
- Observe Lysogeny Broth and minimal media growth
- Compare results from each strain
- Determine if *fabF* or *fabH* has a significant effect on phage replication in *E. coli*

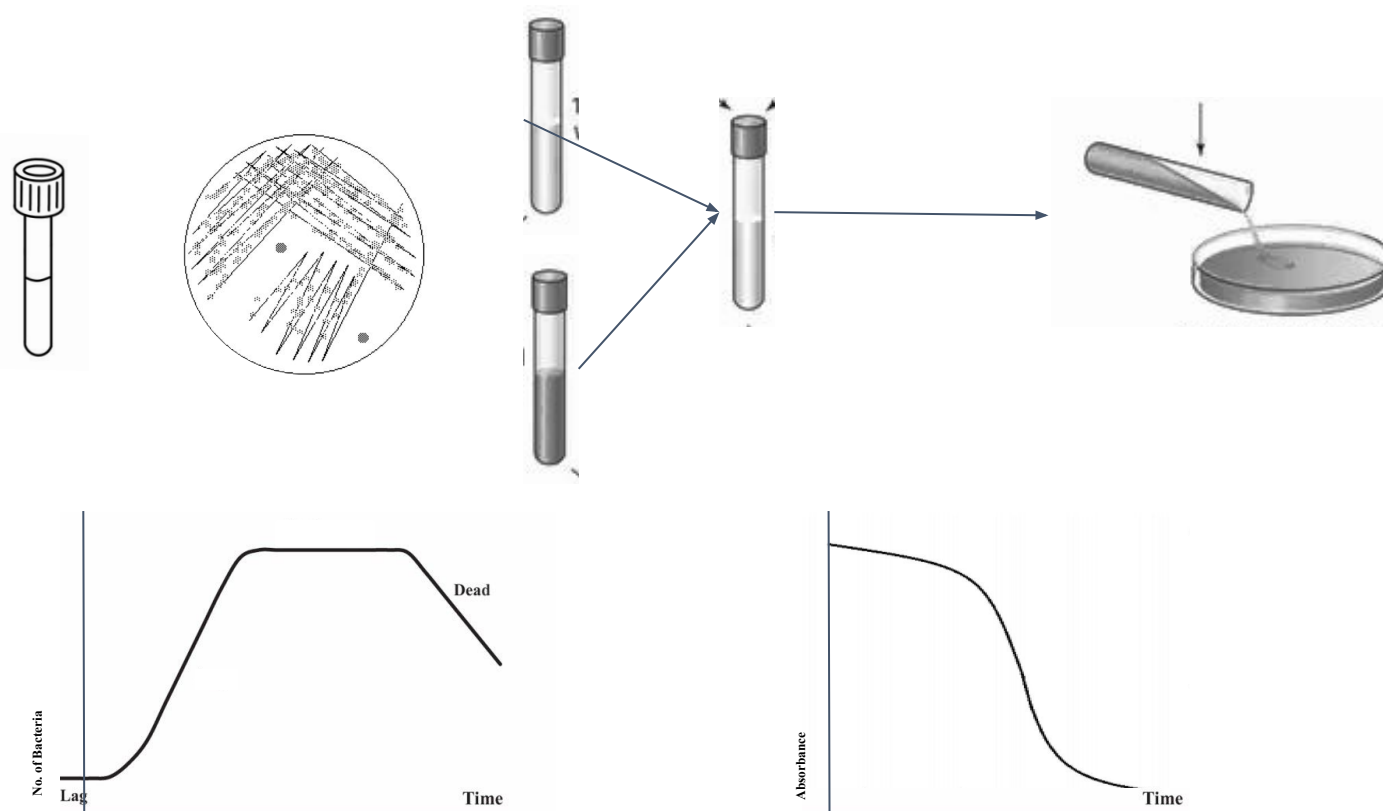


Figure 2. Methods of quantifying phage, growth/lysis curve examples.

Images adapted from BioRender

RESULTS:

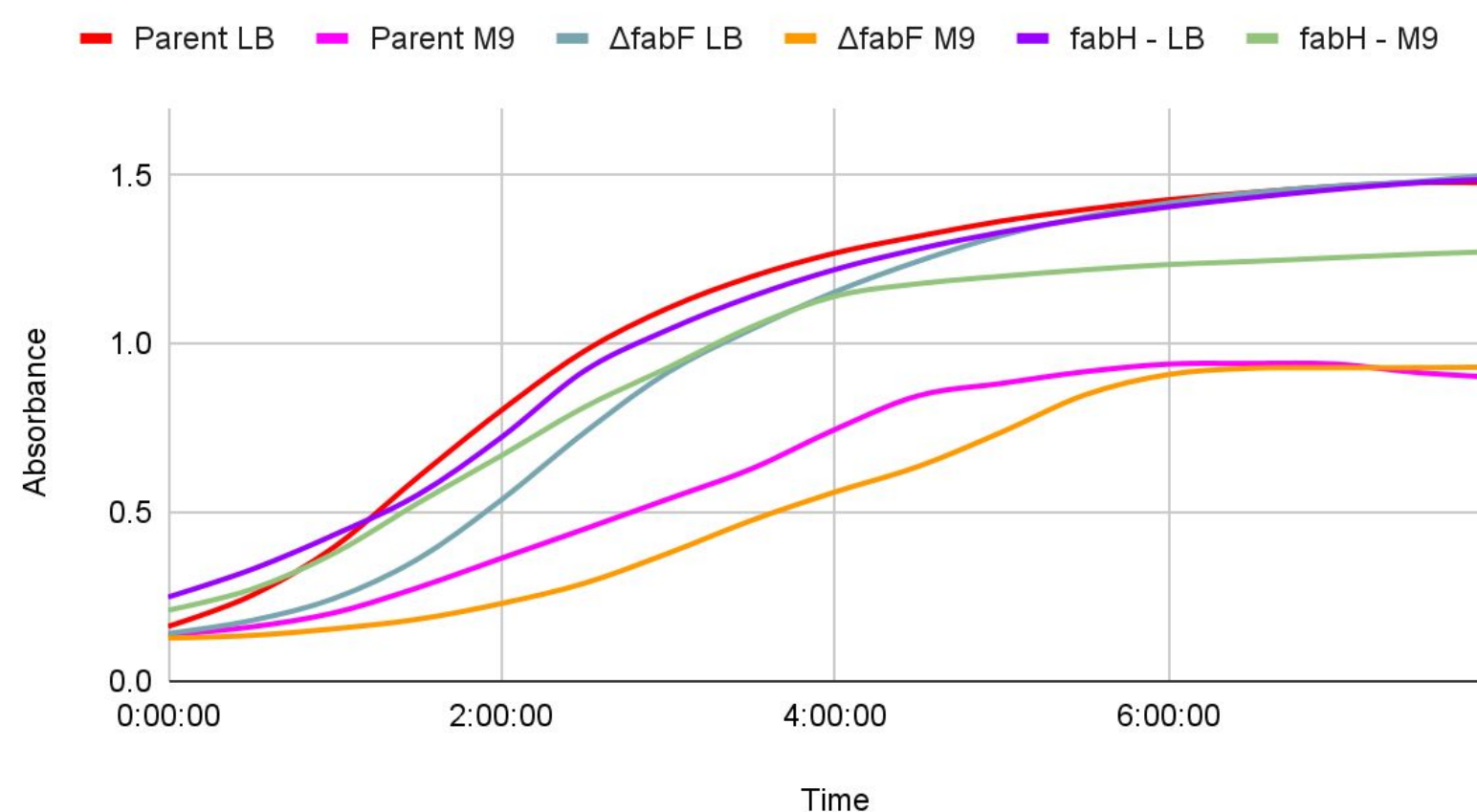


Figure 3. Growth Curves for Parent *E. coli*, *fabF* and *fabH* knockouts in LB media and M9 media

Cultures of Parent and Knockout strains were grown in lysogeny broth (LB) and minimal media (M9). These cultures were grown in a 96-well plate at 37°C with continuous agitation over a period of 8 hours. Absorbance was recorded for each well at 600nm in 30 minute intervals.

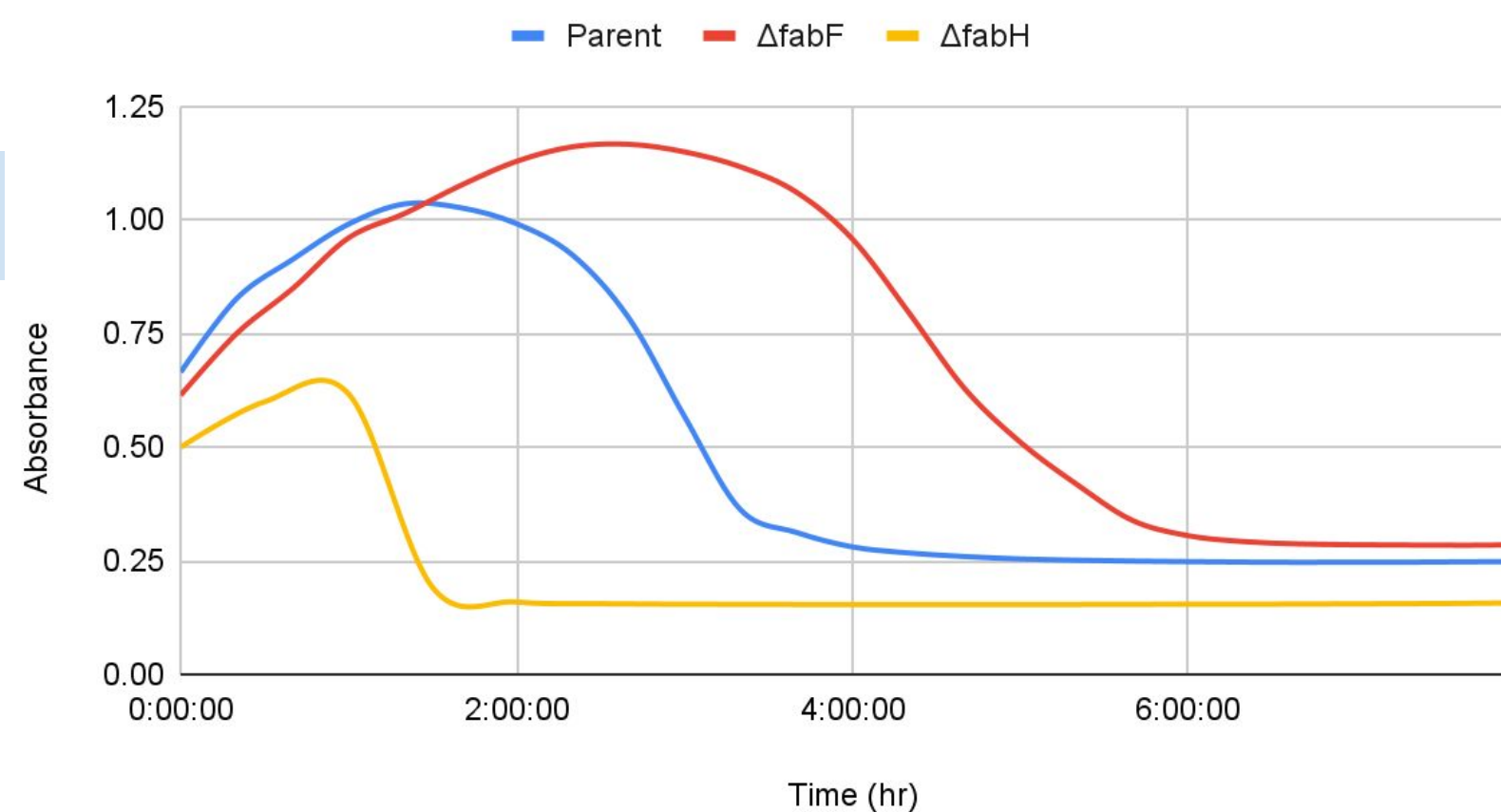


Figure 5. Lysis Curves for Parent *E. coli*, *fabF* and *fabH* knockout

Dilutions of T4 phage were added to cultures of Parent *E. coli*, *fabF* and *fabH* knockouts, grown in lysogeny broth (LB). These cultures were grown in a 96-well plate at 37°C with continuous agitation over a period of 8 hours. Absorbance was recorded for each well at 600nm in 30 minute intervals.

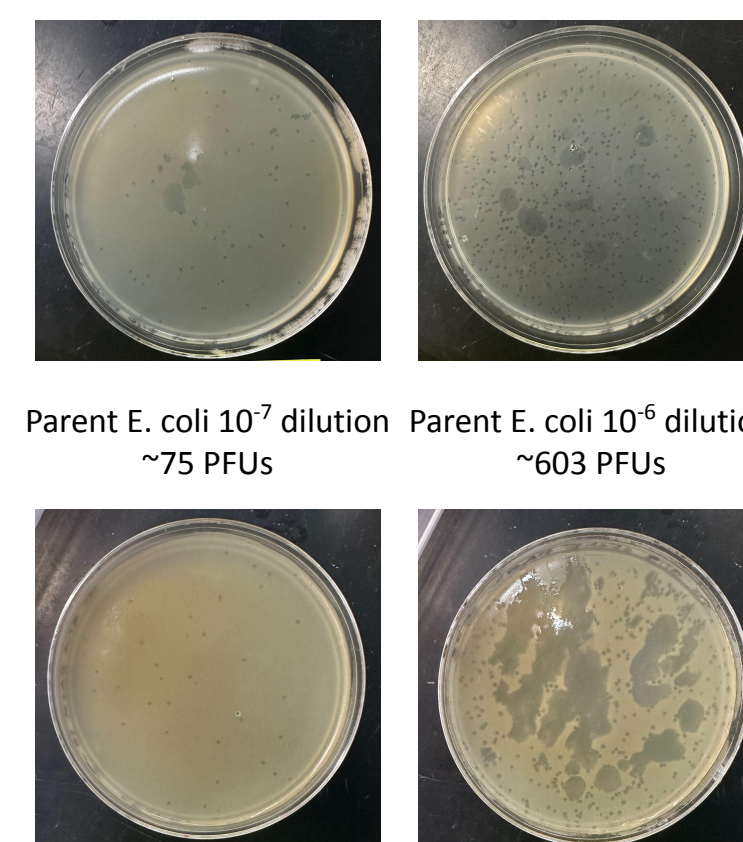


Figure 4. T2 Plaque Assays for Parent and *ΔfabF* Strains at 10⁻⁷ Dilution

Plaque assays performed using a double agar overlay in LB media. Plaques were grown overnight in the incubator. Plaque images are of the -6 and -7 dilution for both the parent and knockout strain.

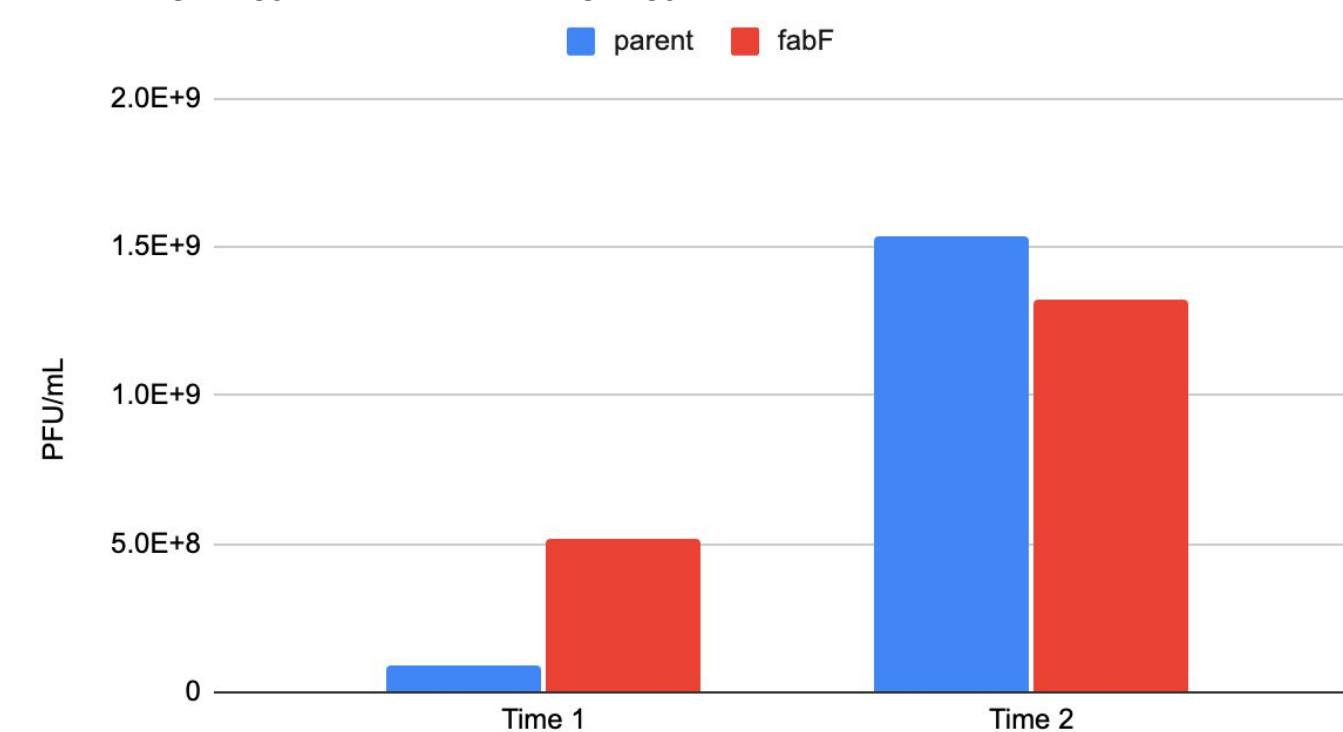


Figure 6. Two-Time Point Phage Titer of Parent and *fabF* Knockout (60 and 120 minutes)

Cultures of Parent and knockout *E. coli* were incubated. After log phase had passed (Time 1), a sample of the *E. coli* was removed and T4 phage was added. This solution was diluted to 10⁻⁶ and plated. After 60 minutes (Time 2), another sample was removed and T4 phage was added. This solution was diluted to 10⁻⁶ and plated.

DISCUSSION:

- Growth:** The *fabF* knockout strain exhibited slower initial growth than the parent strain in both rich (LB) and minimal media. However, growth rates equalized after six hours (Fig. 3). This contrasts with previous studies that did not link *fabF* to growth rate (Barve et al., 2010). The *fabH* knockout strain performed similarly in LB, and somewhat underperformed in M9. (Fig. 3).
- Lysis:** The *fabF* knockout strain displayed a delayed lysis time of at least 30 minutes compared to the parent strain (Fig. 5). This is a novel observation. The *fabH* knockout strain displayed a earlier lysis time of at least 30 minutes compared to the parent strain, contrasting *fabF* (Fig. 5).
- Phage Production:** Plaque assays revealed a lower PFU/mL for the *fabF* knockout strain compared to the parent strain (Fig. 4), corroborating earlier findings.
 - The Two-Time Point Phage Titer experiment showed an intriguing trend: a higher PFU/mL for the knockout at the first time point, followed by a lower PFU/mL at the second time point compared to the parent strain (Fig. 6). This is a novel finding that warrants further investigation.

FUTURE DIRECTIONS:

- All *fabF* experiments should be repeated with other fab cluster genes:

fabH - *fabD* - *fabG* - *acpP* - *fabF*

This will give a comprehensive picture of the relationship between phage replication and the fatty acid biosynthesis pathway.

- An additional variable of antibiotics can be added to the experiments. Fatty acid synthesis in humans differs from that in *E. coli*, so implementing some of the same experiments in media containing antibiotic will reveal an area to target in future medical research.