

BACKGROUND

- With the rise of antibiotic resistance, finding new ways to target harmful bacteria, including through the use of bacteriophage, is critical.
- Phages are viruses that can infect and kill bacteria, relying on the host's metabolic resources to replicate.
- To determine the role of bacterial energy metabolism in phage replication, we will conduct research using *E. coli atpB* and *atpE* knockout strains. These strains lack the nonessential *atpB* or *atpE* gene (Goodall et al. 2018) which codes for a proton channel involved in *E. coli* ATP production (Aksimentiev et al. 2004).

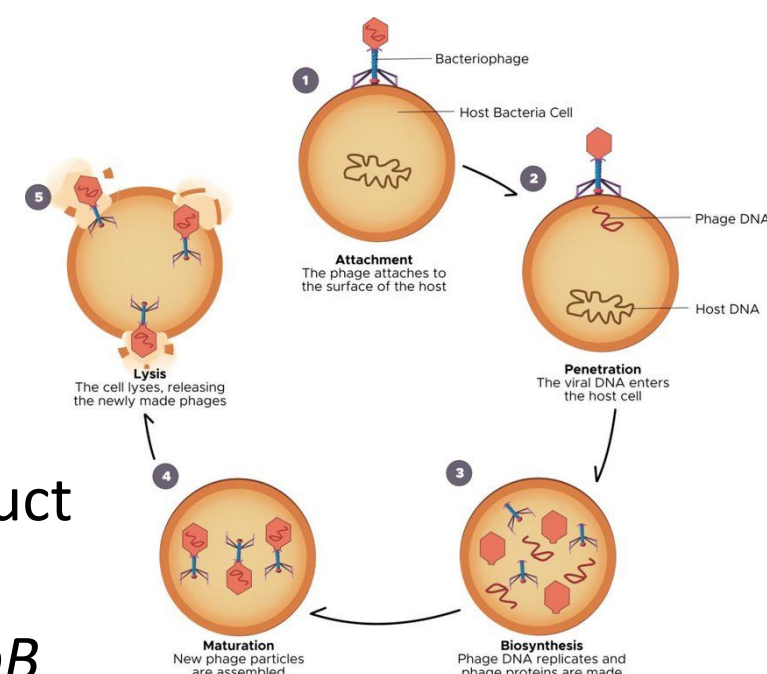


Figure 1: Viral Replication Cycle
Figure from Adesanya et al. (2020)

Objectives

- To determine if the removal of the *atpB* or *atpE* gene disrupts *E. coli* and bacteriophage growth and replication
- We hypothesize that the removal of either the *atpB* or *atpE* gene will slow or inhibit bacterial and phage replication.

METHODS

Materials

- *E. coli* parent strain
- *E. coli atpB* and *atpE* knockout strains (Baba et al. 2006)
- LB media, LB + Kan media, M9 media
- T2, T4, and T4r Bacteriophage
- Plating and overnight culture materials

Methods

Aim 1: Characterize Bacterial Fitness. We compared the growth curves of parent, $\Delta atpB$, and $\Delta atpE$ *E. coli* in LB and M9 media using spectrophotometry, with absorbance measured every 30 minutes for 8 hours to assess the impact of the gene deletions.

Aim 2: Quantify Phage Replication. All three strains were infected with T-series bacteriophages (T2 and T4). Plaque assays were performed to measure viral yield (PFU/mL) and generate lysis curves. A two-timepoint phage titer experiment was also conducted for the parent and $\Delta atpB$ strains using T4.

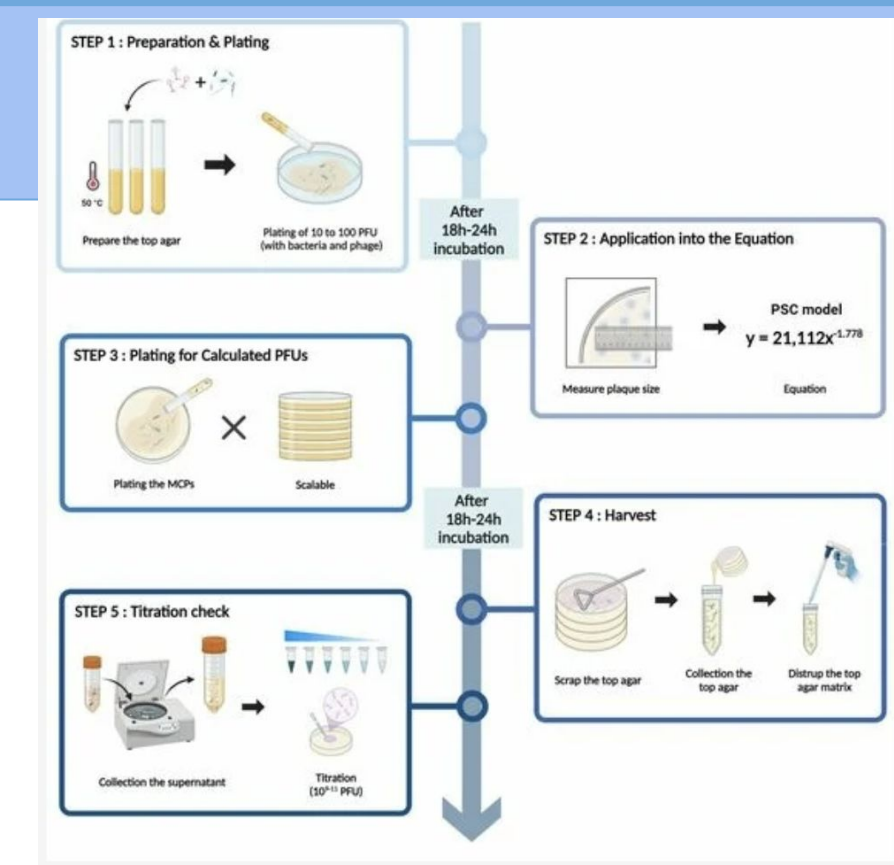


Figure 2: Bacteriophage Quantification Workflow
Figure from Jo et al. (2025)

EXPERIMENTAL RESULTS

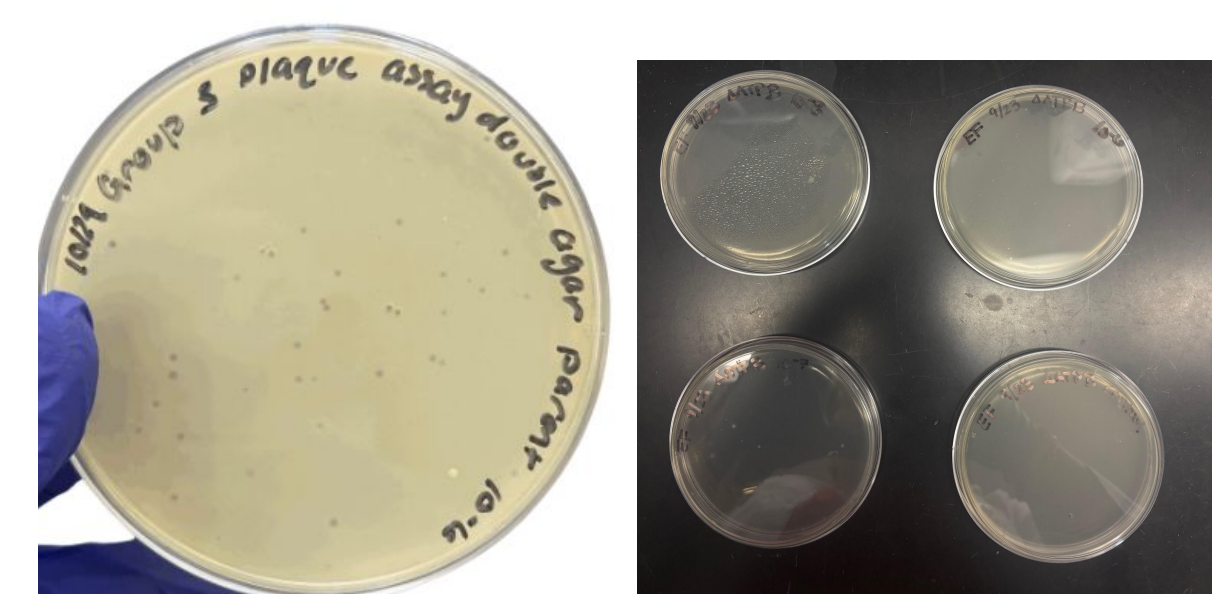


Figure 4. $\Delta atpB$ Knockout Prevents T4 Phage Plaque Formation in *E. coli*. Assays were conducted using the double agar overlay method with LB media. Parent 10⁻⁶ dilution shown on left, *atpB* knockout control, 10⁻⁶, 10⁻⁷, and 10⁻⁸ dilutions on right. No visible plaques were observed on the plated *atpB* dilutions.

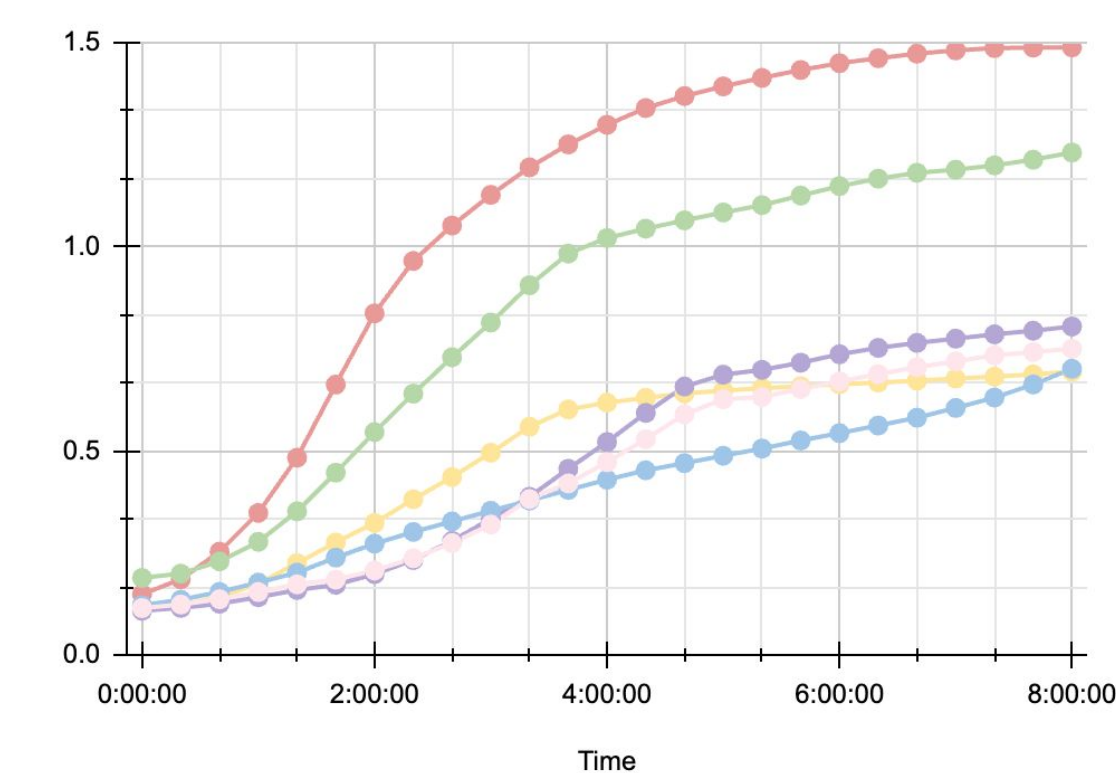


Figure 3. $\Delta atpB$ and $\Delta atpE$ Knockout Reduce Growth Rate in Both Rich and Minimal Media compared to Parent Strain. Each strain was grown in rich Luria Broth (LB) media and minimal M9 media with 0.4% glucose for 8 hours, with absorbance values taken every 30 minutes by a plate reader. The two knockout strains were used to determine the how genes related to ATP production affected growth compared to each other and a parent strain. The *atpB* knockout strain was significantly slower growing in both medias than the parent strain. The *atpE* strain was also slower growing than the parent in both medias but not as significantly.

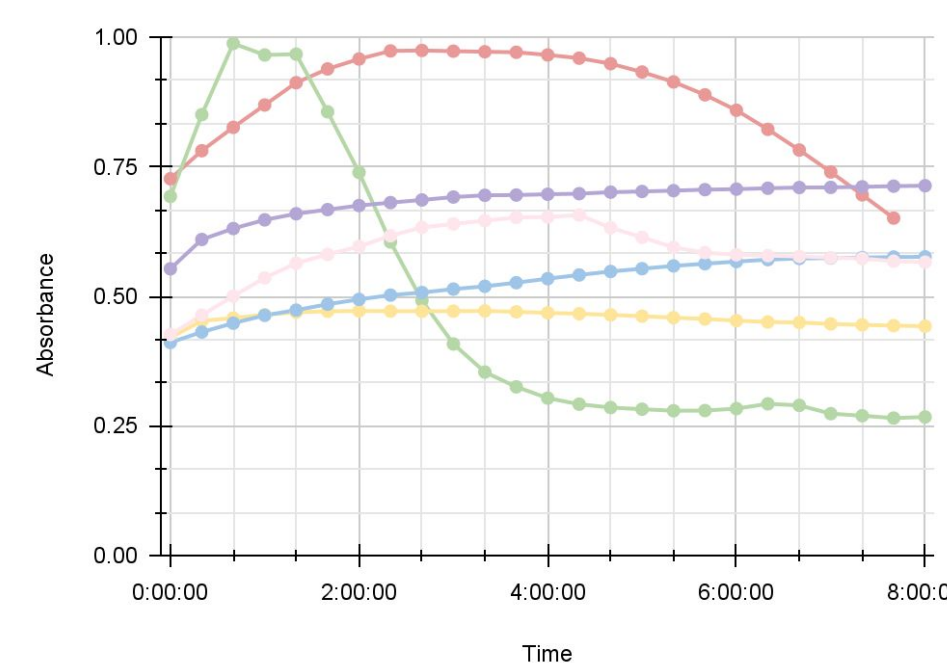


Figure 5. $\Delta atpB$ Knockout Prevents Phage-Induced Lysis, $\Delta atpE$ Knockout Partially Reduces Lysis.. The $\Delta atpB$ strain did not show any signs of cellular lysis, indicating that the knockout *atpB* gene has a great impact on the ability of phage to grow within *E. coli*. The *atpB* control also grew much slower than the parent and *atpE* control. Bacteriophage had trouble growing in the *atpE* knockout strain as well, indicated by the minimal decrease in absorbance over time.

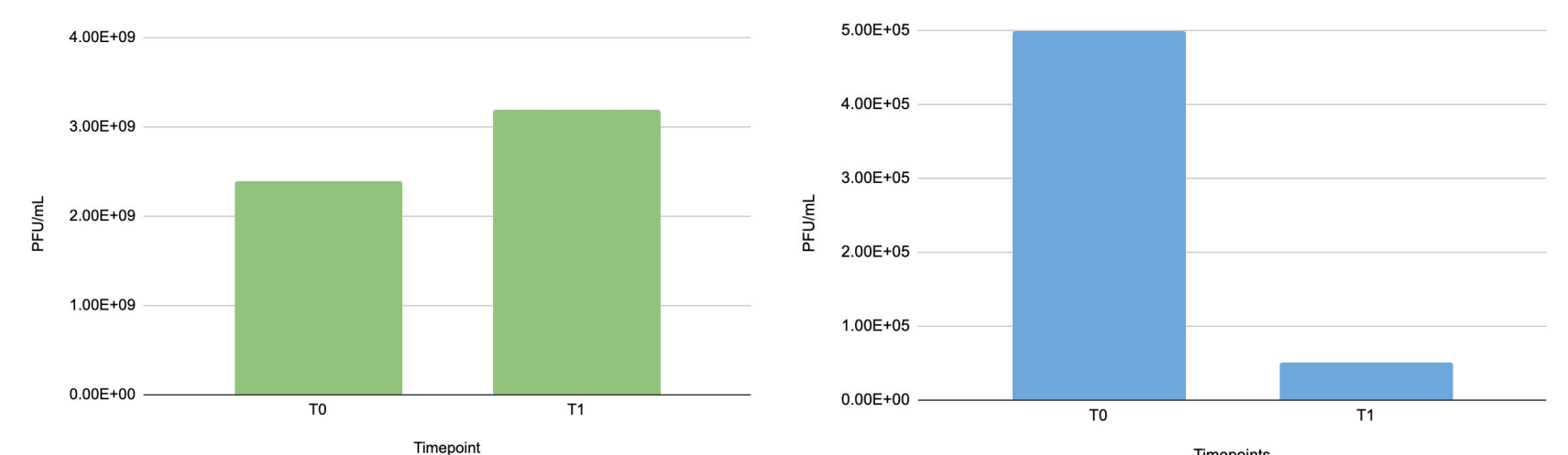


Figure 6. $\Delta atpB$ Knockout Significantly Reduces T4 Phage Replication. T4r phage was added to the bacterial strains. Phage concentrations at two timepoints were then quantified via double agar overlay plaque assays. Parent strain PFU/mL was determined at a dilution of 10⁻⁷, *atpB* PFU/mL was determined at a dilution of 10⁻³. The *atpB* strain showed significantly lower PFU/mL value over time, indicating difficulty in viral replication in the knockout strain compared to the parent.

DISCUSSION/ FUTURE DIRECTIONS

- ❖ The *atpB* knockout strain showed significantly slower growth than the parent in LB and M9 media, while the *atpE* knockout also grew slower, though less dramatically, suggesting that multiple ATP-related genes contribute to bacterial growth.
- ❖ Plaque assays showed that the parent strain formed more plaques than both $\Delta atpB$ and $\Delta atpE$ strains, indicating that deletion of these genes impairs host metabolism and reduces T4 phage replication.
- ❖ Loss of *atpB* greatly weakened phage-driven lysis, whereas *atpE* deletion caused a moderate reduction, highlighting the role of ATP biosynthesis genes in supporting both bacterial growth and phage replication.
- ❖ These findings support the hypothesis that ATP-related genes are essential for efficient phage propagation. Targeting genes like *atpB* or *atpE* could guide future antibiotic strategies, and further research could explore how manipulating ATP metabolism might control phage replication or improve therapeutic phage design.

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