FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

The Effect of the *pykA* gene on *Escherichia coli* Growth and Bacteriophage Replication

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INTRODUCTION

Background

Viruses hijack host cells in order to replicate. The use of antibiotics to treat these infections is becoming less effective and many bacteria are becoming resistant due to overuse. Therefore, bacteriophage therapy is a helpful alternative way to fighting bacterial infections.

About *pykA*:

- The *pykA* gene is key in glycolysis as it catalyzes one of the substrate level phosphorylation steps that generate ATP
- This gene converts ADP to ATP which is essential for all bodily functions (Schormann et al., 2019)

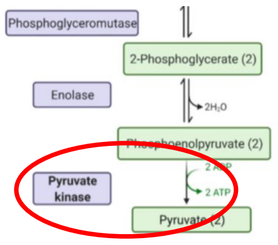

Figure 1: the function of *pykA* in glycolysis (Grant, 2021)

Objectives

- Determine if removing *pykA* gene impacts *E. coli* replication in the presence and absence of T4 bacteriophage
- Hypothesis: the removal of gene will result in an increase in bacteriophage replication due to lack of negative regulation on glycolysis, as seen in other experiments (Rodríguez-Sánchez, I., & Munger, J., 2019)

METHODS/MATERIALS

Research Aim

Within this experiment we will analyze if bacteriophage can replicate in *E. coli* cells where the *pykA* gene is absent.

***E. coli* Replication**

- Create overnight cultures for both the *pykA* gene and parent strain
- Complete serial dilutions of the bacteria and measure absorbances every 30 minutes in both LB and M9 media
- Create growth curves of both strains in each media to determine which conditions are best for replication
- We will also plate our dilutions every 30 minutes to further explore how the removal of *pykA* will affect viral replication

Bacteriophage Replication

- Quantify T4 bacteriophage growth in both the parent and *pykA* strains in LB media through plaque assay and lysis curves
- We will perform serial dilutions to measure their absorbances and formulate growth curve

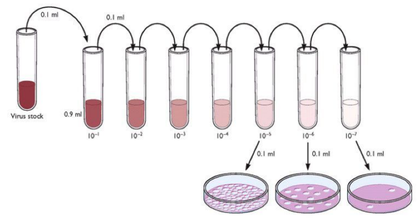

Figure 2: Depicting the conduction of plaque assays (Racaniello, 2009)

FIGURES & RESULTS

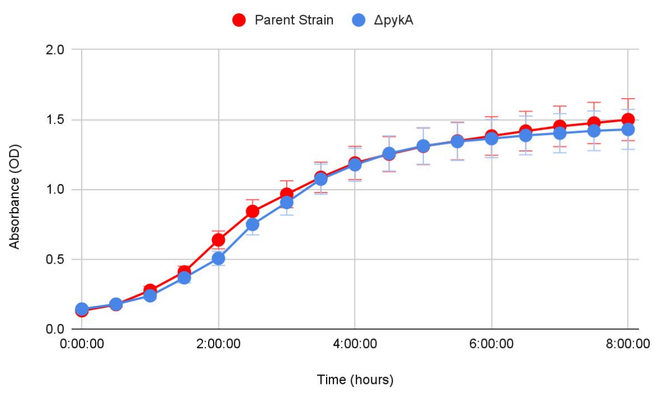

Figure 1: Insignificant difference in growth of Parent and *pykA* strains in LB media over time: The data was collected by using a plate reader for 8 hours containing the parent and *pykA* strain in LB media which is rich in nutrients. The graph above shows a comparative growth of both strains over time.

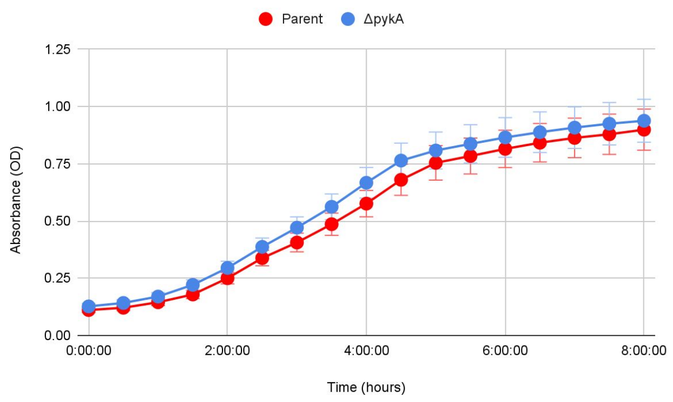

Figure 2: Slight difference in growth of of Parent and *pykA* strains in M9 media over time: The data was collected by using a plate reader for 8 hours containing the parent and *pykA* strain in M9 minimal media which is limited in nutrients. The graph above shows a comparative growth of both strains over time.

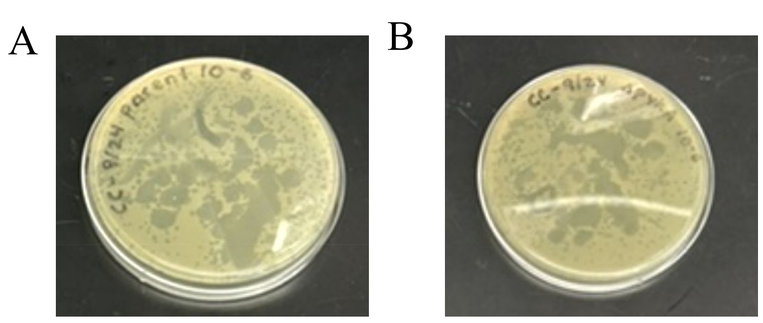

Figure 3*:* Comparative plaque assays of parent and *pykA* strains show similar growth: Overnight cultures of the parent (A) and *pykA* (B) strains with with T2 bacteriophage were diluted to 10^{-8}. The E. coli and bacteriophage were vortexed and poured onto agar plates in the completion of a double agar overlay and incubated at 37 degrees celsius.

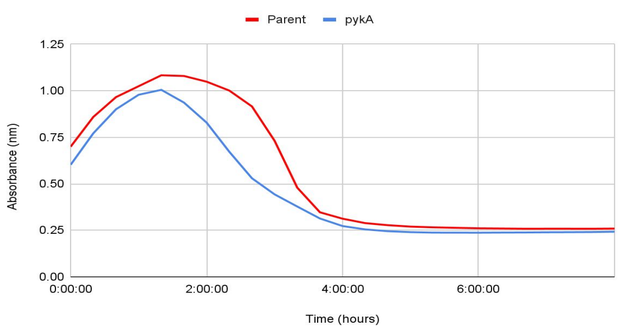

Figure 4: Slight difference of lysis within Parent and *pykA* with slower lysis in the parent strain: This figure represents the lysis curves of the infected parent and knockout strains from the T4r bacteriophage. The data was collected using a plate reader for 8 hours. The average absorbance of the bacteria in LB media with T4r bacteriophage were similar.

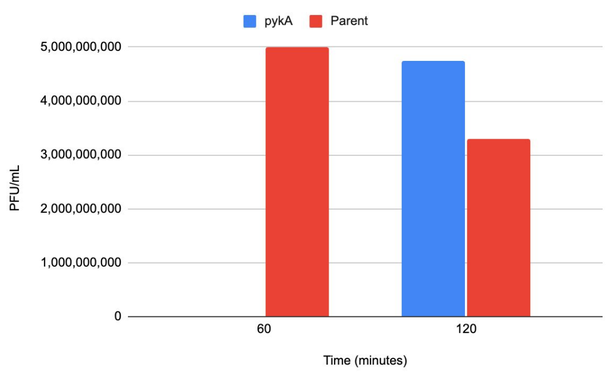

Figure 5: T4 concentration in parent strain and *pykA* strain at 0, 60, and 120 minutes: Samples were taken from the parent and the *pykA* strains at 1 hour intervals to measure the growth of T4 phage in each sample. The T4 phage was added at time 0 once the *E. coli* strains were in log phase with an absorbance around 0.45. The *pykA* strain had a lower PFU/mL than the parent at time point 1. At time point 2, the *pykA* strain had a higher PFU/mL than then parent strain. This indicates that phage replicate faster in the *pykA* strain.

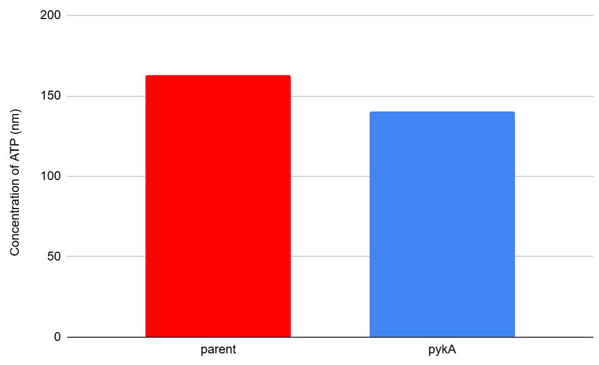

Figure 6: Difference ATP concentration between parent and pykA strain. Due to a focus on the glycolysis cycle, the amount produced by both strains was measured through this assay. It was found that the parent strain produces more ATP than the pykA knockout.

DISCUSSION & FUTURE DIRECTIONS

Discussion

- We saw growth in both the parent and *pykA E.coli* strains. This indicates that the removal of the *pykA* knockout strain is not essential for the growth of *E. coli* (Figure 1)
- We saw slightly less growth in the parent strain in M9 media than the *pykA* knockout strain (Figure 2)
- As shown in Figure 3, bacteriophage replication was similar in both the parent and *pykA* knockout strains
- Figure 4 indicates that the *pykA* strain lysed faster than the parent strain. This indicates that the *pykA* strain was broken down faster from the T4r bacteriophage
- Figure 5 indicates that phage replicate faster within the *pykA* strain than the parent
- Figure 6 indicates that the there is an inhibition in the glycolysis cycle that decreases the production of ATP in the pykA strain showing that there is a difference in the glycolysis cycle of these strains

Future Directions

- We can investigate with different forms of bacteriophage to see how that affects the removal of *pykA*
- We can examine the effect of the *pykF* knockout strain which is another gene that encodes for pyruvate kinases
- Perform more replications of our plaque assays and growth curves in order to strengthen our data

REFERENCES & ACKNOWLEDGEMENTS

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